Nonsteroidal Cardiotonics. 1. 2-Pyridyl-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-ones, a Novel Class of Cardiotonic Agents[1]

A. Mertens,*† B. Müller-Beckmann,‡ W. Kampe,† J.-P. Hölck,† and W. von der Saal†

Departments of Chemistry and Pharmacology, Boehringer Mannheim GmbH, 6800 Mannheim, West Germany.
Received July 9, 1986

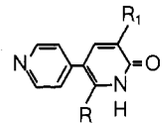
A series of substituted 2-pyridyl-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-ones 1–**24** were synthesized and evaluated for positive inotropic activity. In rats, cats, and dogs most of these tricyclic heterocycles produced a dose-related increase in myocardial contractility with little effect on heart rate and blood pressure. The increase in contractility was not mediated via stimulation of β-adrenergic receptors. Compound 1 (BM 14.478) was more potent than milrinone (**25**) and enoximone when administered intravenously to rats, cats, and dogs. After oral administration of 1 mg/kg, compound 1, milrinone, and pimobendan were equipotent. However, only 1 and pimobendan were still active after 6 h. The structural requirements necessary for optimal cardiotonic activity within this novel class of heterocycles were investigated.

As long ago as 200 years Withering discovered digitalis glycosides as drugs for the treatment of congestive heart failure.[2] Since then these drugs have been the principal agents in the treatment of the failing heart.[3,4] However, the narrow therapeutic range and arrhythmogenic liability of these cardiac glycosides has led to increasing efforts in the development of safer drugs. Sympathomimetic agents such as dobutamine[5] and dopamine[6] have also been used successfully in the treatment of congestive heart failure. However, the most significant limitations of these drugs are oral ineffectiveness and tachyphylaxis resulting from β-receptor down-regulation.[7–11]

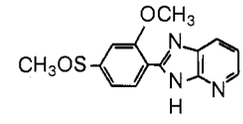
In recent years the growing number of patients suffering from congestive heart failure and the considerable therapeutic need for the management of this disease has prompted a worldwide search for safer orally active positive inotropic agents. Prototypical compounds such as sulmazole[12] and amrinone[13,14] were developed as nonsteroidal, noncatecholamine cardiotonic drugs. Both of them were tested clinically in humans, but severe side effects[15,16] stopped the development of these agents for chronic oral treatment. A second generation of more active compounds including enoximone (MDL-17043),[17,18] piroximone (MDL-19205),[19,20] milrinone[21,22] (**25**), CI-914, CI-930,[23–25] and UD-CG 115[26–28] have been reported to be effective in animals as well as in patients with congestive heart failure.

In addition to their positive inotropic properties, these compounds show vasodilatory effects on the peripheral vasculature. The exploration of the mechanism of action is still under development. However, it is clearly different from that of digitalis glycosides and sympathomimetic drugs. It is at least well accepted that part of the pharmacological action of these novel inotropic agents is mediated via inhibition of cardiac phosphodiesterases.[29,30]

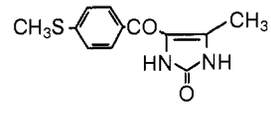
We report here on our efforts to develop a novel class of compounds with potent positive inotropic activity. Stimulated by the well-known cardiotonic property of imidazole[31] itself as well as some common structural requirements derived from the agents described above, we focused our interest on benzimidazole derivatives with an additional amide group in the molecule. Among the many compounds tested we finally found that 6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-ones bearing a pyridyl substituent in the 2-position are very potent positive inotropic agents. 7,7-Dimethyl-2-(4-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (1, BM 14.478) was the most active compound out of a series of analogues,



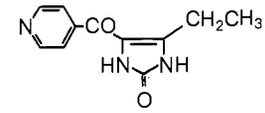
amrinone: R = H, $R_1 = NH_2$
milrinone: R = CH_3, R_1 = CN



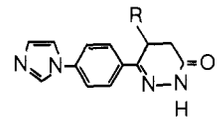
sulmazole



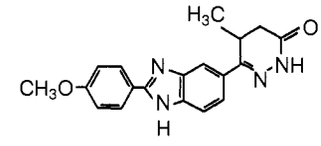
enoximone = MDL-17043



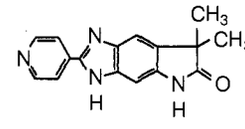
piroximone = MDL-19205



CI-914: R = H
CI-930: R = CH_3



pimobendan = UD-CG 115



1 = BM 14.478

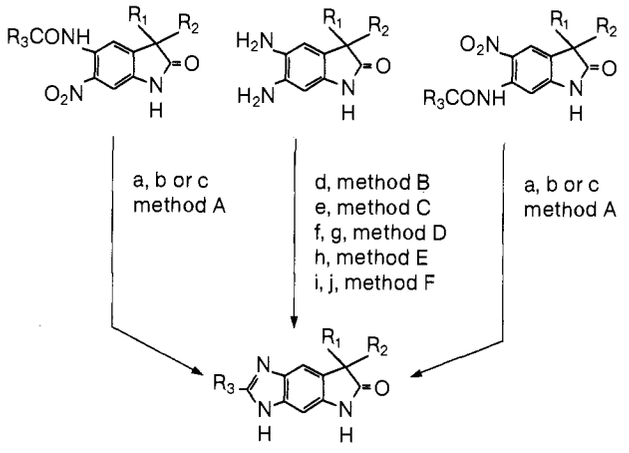
with even greater activity than milrinone (**25**) and enoximone when administered intravenously to rats, cats, and

† Department of Chemistry.
‡ Department of Pharmacology.

(1) Portions of this work were presented: Mertens, A.; Hölck, J.-P.; Kampe, W.; Müller-Beckmann, B.; von der Saal, W. IXth International Symposium on Medicinal Chemistry, Berlin (West), Sept 1986.
(2) Withering, W. *An Account of the Foxglove and Some of its Medical Uses with Practical Remarks on Dropsy and Other Diseases*; Robinson, G. G. J., Robinson, J., Eds.; Swinney: London, 1785.
(3) Mason, D. T. *Ann. Intern. Med.* **1974**, *80*, 520.
(4) Arnold, S. B.; Byrd, R. C.; Meister, W.; Melmon, K.; Cheitlin, M. D.; Bristow, J. D.; Parmley, W. W.; Chatterjee, K. N. *N. Engl. J. Med.* **1980**, *303*, 1443.
(5) Tuttle, R. R.; Mills, J. *Circulation* **1975**, *36*, 185.
(6) Stoner, J. D.; Bolen, J. L.; Harrison, D. C. *Br. Heart J.* **1977**, *39*, 536.
(7) Su, Y.-F.; Harden, T. K.; Perkins, J. P. *J. Biol. Chem.* **1980**, *255*, 7410.
(8) Mukherjee, C.; Caron, M. G.; Lefkowitz, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1945.
(9) Bristow, M. R.; Ginsburg, R.; Minobe, W.; Cubicciotti, R. S.; Sageman, W. S.; Lurie, K.; Billingham, M. E.; Harrison, D. C.; Stinson, E. B. *N. Eng. J. Med.* **1982**, *307*, 205.

0022-2623/87/1830-1279$01.50/0

Scheme I



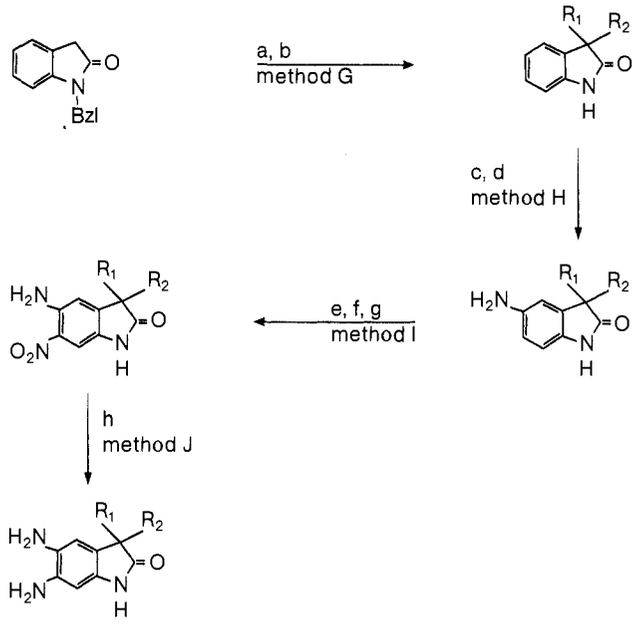
(a) H_2/Pd; (b) Acetic acid; (c) 2N HCl; (d) R_3CHO/EtOH; (e) R_3COOH/PPA; (f) R_3COCl/NEt_3; (g) conc. HCl; (h) R_3COOCH_3; (i) R_3COOH/DCC/N-Hydroxy-benzotriazole; (j) EtOH/conc. HCl.

dogs. After oral administration to conscious dogs, **1** (BM 14.478) produced a long-acting positive inotropic effect.

Chemistry

Depending on the substituents in position 2 of 6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-ones, three different synthetic routes were used to build up the heterocyclic ring system. Either a suitably substituted 5-[(pyridinylcarbonyl)amino]-6-nitrooxindole or the corresponding 6-[(pyridinylcarbonyl)amino]-5-nitro isomer were hydrogenated and subsequently cyclized with acetic acid or hydrochloric acid to the desired benzimidazole derivatives (method A). The third synthetic preparation started from an appropriate 5,6-diaminooxindole, which could be readily transformed with aldehydes (method B), carboxylic acids in polyphosphoric acid (method C), carboxylic chlorides (method D), methyl carboxylates (method E), or activated derivatives thereof such as hydroxybenzotriazole esters (method F) to the tricyclic heterocycle by using well-known literature procedures.[32] When labile or reactive substituents were present in the pyridine ring, the mild condensation of aldehydes or activated esters with the diamine were usually employed. When the pyridine derivative was unsubstituted or possessed unreactive substituents, either the carboxylic acid itself, the methyl carboxylate, or the corresponding carboxylic chloride was the reagent of choice. Best results were usually obtained in polyphosphoric acid (PPA).

Scheme II



(a) Alkylation; (b) Na/NH_3; (c) H_2SO_4/HNO_3; (d) H_2/Pd; (e) Ac_2O; (f) Ac_2O/HNO_3; (g) HCl; (h) H_2/PtO_2.

For preparation of the precursors shown in Scheme I, we have developed several different synthetic routes, depending on the desired substituents in position 3 of the oxindole nucleus. Since direct alkylation of oxindoles usually leads to a mixture of N-alkylated and 3,3-dialkylated products, either N-benzylated oxindole[33] was dialkylated and deprotected with sodium in liquid ammonia (method G) or the old Brunner reaction[34] for carboxylic hydrazides was applied to prepare 3,3-disubstituted indolin-2-ones. Following standard chemistry, these oxindoles can be readily converted to 5-amino-6-nitro or 5,6-diaminoindolin-2-ones, which serve as precursors for the final products (Scheme II).

4,4-Dialkylated-7-nitrohomophthalimides can be further used as starting material via Hofmann degradation[35,36]

(10) 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.
(11) Harden, T. K. *Pharmacol. Rev.* **1983**, *35*, 5.
(12) Koch, H. *Pharm. Int.* **1982**, *3*, 5.
(13) Ward, A.; Brodgen, R. N.; Heel, R. C.; Speicht, T. M.; Avery, G. S. *Drugs* **1983**, *26*, 468.
(14) Alousi, A. A.; Bobreck, H. P. *New Drugs Ann.: Cardiovasc. Drugs* **1983**, *1*, 259.
(15) Benotti, J. R.; Grossman, W.; Braunwald, E.; Davolos, D. D.; Alousi, A. A. *N. Engl. J. Med.* **1978**, *299*, 1373.
(16) Lejemtel, T. H.; Keung, E.; Ribner, H. S.; Davis, R.; Wexler, J.; Blaufox, M. D.; Sonnenblick, E. H. *Am. J. Cardiol.* **1980**, *45*, 123.
(17) Schnettler, R. A.; Dage, R. C.; Grisar, M. J. Eur. Pat. Appl. 58 435, August, 25, 1982.
(18) Schnettler, R. A.; Dage, R. C.; Grisar, M. J. *J. Med. Chem.* **19**; 2, *25*, 1477.
(19) Dage, R. C.; Schnettler, R. A.; Palopoli, F. P.; Grisar, M. J. Eur. Pat. Appl. 59 948, Sept. 15, 1982.
(20) Dage, R. C.; Roebel, L. E.; Hsieh, C. P.; Woodward, J. K. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 35.
(21) Alousi, A. A.; Helstosky, M. J. M.; Cicero, F. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1981**, *410*, 663.
(22) Alousi, A. A.; Canter, J. M.; Montenaro, M. J.; Fort, D. J.; Ferrari, R. A. *J. Cardiovasc. Pharmacol.* **1983**, *5*, 792.
(23) Sircar, J.; Bristol, J. A. Eur. Pat. Appl. 75 436, March 30, 1983.
(24) Sircar, J.; Duell, B. L.; Bobowski, G.; Bristol, J. A.; Evans, D. B. *J. Med. Chem.* **1985**, *28*, 1405.
(25) Bristol, J. A.; Sircar, J.; Moos, W. H.; Evans, D. B.; Weishaar, R. E. *J. Med. Chem.* **1984**, *27*, 1099.
(26) Austel, V.; Heider, J.; Eberlein, W.; Diederen, W.; Haarmann, W. U.S. Patent 4 361 563, November 30, 1982.
(27) Van Meel, J. C. A. *Arzneim.-Forsch.* **1985**, *35*, 284.
(28) Honerjäger, P.; Heiss, A.; Schaefer-Korting, M.; Schoensteiger, G.; Reiter, M. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1984**, *325*, 259.
(29) Weishaar, R. E.; Michael, H. C.; Bristol, J. A. *J. Med. Chem.* **1985**, *28*, 537.
(30) Heiss, A.; Schaefer-Korting, M.; Honerjäger, P. *Arch. Pharmacol.* **1982**, *321* (Suppl.), R 36.
(31) Knope, R.; Moe, G. K.; Saunders, J.; Tuttle, R. *J. Pharmacol. Exp. Ther.* **1973**, *185*, 29.
(32) Preston, P. N. *The Chemistry of Heterocyclic Compounds*; Weissberger, A., Taylor, E. C., Eds.; Wiley: New York, 1981; Part 1, p 40.
(33) Powers, J. C. *J. Org. Chem.* **1966**, *31*, 2627.
(34) Brunner, K. *Monatsh. Chem.* **1897**, *18*, 95.

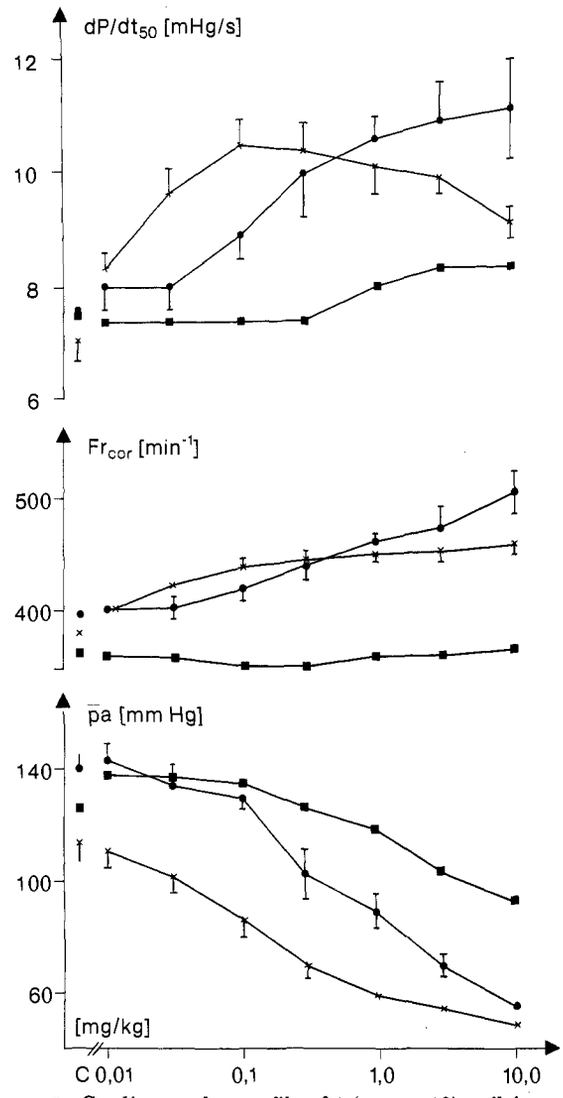


Figure 1. Cardiovascular profile of 1 (★, $n = 10$), milrinone (●, $n = 6$), and enoximone (■, $n = 2$) in anesthetized rats after iv doses. dP/dt was recorded in mmHg/s and converted to mHg/s in the figure. Fr_{cor} means heart rate and $\bar{p}a$ is the mean arterial blood pressure. C means control values. Each point is the mean ± range of experimental values.

(method L) for the synthesis of 3,3-dialkylated oxindoles. Monosubstituted oxindoles or compounds bearing an additional carboxylic group in position 3 of the oxindole ring are best prepared either by hydrogenation of 2-(2,4-dinitrophenyl)alkyl carboxylates (method K) or by condensation of known 6-(acetylamino)oxindole[37] with aldehydes and hydrogenation of the reaction product (method Q). These intermediates were converted to 6-pyridinoylamino-5-nitroindolin-2-ones or 5,6-diaminoindolin-2-ones (Scheme III) by applying straightforward chemistry. Again these precursors can easily react with different pyridine derivatives to 2-pyridyl-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-ones.

Some pyrrolo[2,3-*f*]benzimidazol-6-ones were readily converted to other derivatives of the tricyclic ring system. Actually the 3-chloro-4-pyridyl derivative **7** was transformed to the hydroxy compound **6** with diluted phosphoric acid (method R), 1 (BM 14.478) was oxidized with

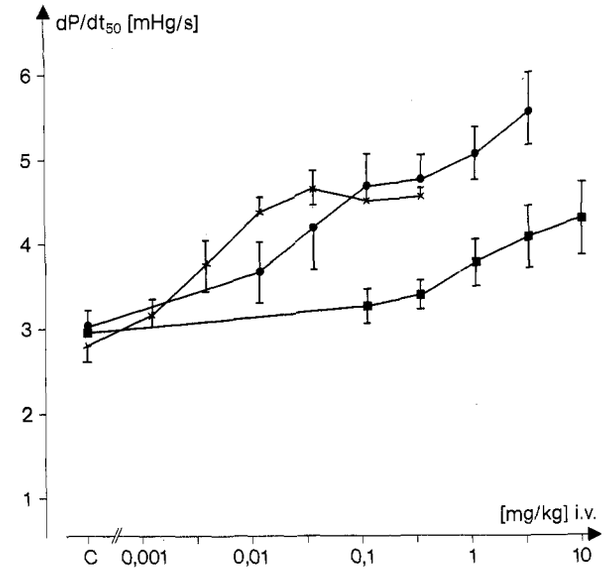


Figure 2. Cardiotonic activity of 1 (★, $n = 6$), milrinone (●, $n = 5$), and enoximone (■, $n = 2$) in anesthetized cats after a dose of 0.3 mg/kg iv desacetylmetipranolol. dP/dt was recorded in mmHg/s and converted to mHg/s in the figure. C means control values. Each point is the mean ± range of experimental values.

hydrogen peroxide in acetic acid (method S) to the *N*-oxide **4**, and the ethyl carboxylate derivative **16** was treated with hydrazine (method T) to produce the hydrazide **17**. All physical data and preparation methods are summarized in Table I.

Pharmacology

Anesthetized rats, open-chest cats, pretreated with 0.3 mg/kg desacetylmetipranolol (DAM) iv, and conscious dogs were prepared for recording dP/dt, blood pressure (BP), and heart rate (HR). Dose–response curves were performed for all compounds **1–24** by iv injection of incremental doses.

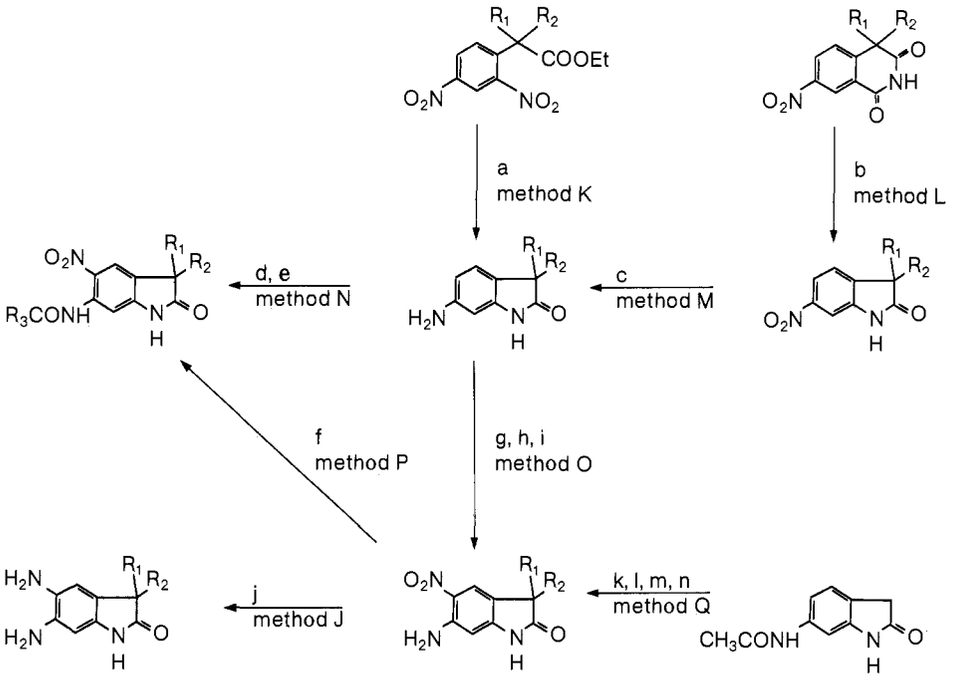
In rats, 0.01–10 mg/kg of tested compounds increased dP/dt and led to a dose-related decrease in blood pressure and a moderate increase in heart rate. The effective dose required to produce an increase in dP/dt_{50} by 1500 mmHg/s ($ED_{1.5}$) was 0.01 mg/kg for the most potent compound **1**. Up to 0.1 mg/kg iv, a dose-related increase of dP/dt was observed, whereas higher doses caused no further increase in contractility. Compound **1** ($ED_{1.5} = 0.01$ mg/kg) was 14 times more potent than milrinone (**25**) ($ED_{1.5} = 0.14$ mg/kg), and enoximone only showed a very weak increase in contractility in this animal model (Figure 1).

Similar dose–response curves were obtained for compounds **1–24** in open-chest cats and conscious dogs. Again, **1** was among the most active compounds. The inotropic effects of **1–24** were not blocked in cats by a prior dose of desacetylmetipranolol, which indicates that these agents are not acting via direct stimulation of β-adrenergic receptors or indirectly by release of catecholamines. Intravenous doses of 0.001–0.3 mg/kg of compound **1** produced a dose-related increase in cardiac contractile force and heart rate as well as a slight decrease in blood pressure. The effective dose that increased dP/dt by 1500 mmHg/s ($ED_{1.5}$) was 0.01 mg/kg for compound **1** in cats (Figure 2).

Figure 2 clearly demonstrates the strong positive inotropic properties of **1** ($ED_{1.5} = 0.01$ mg/kg), which was about 10 times more potent than milrinone ($ED_{1.5} = 0.13$ mg/kg). A significant increase in contractility with enoximone was first observed at doses above 1 mg/kg. Dose–response curves of compounds **1–24** in conscious dogs

(35) Joensson, N. A.; Moses, P. *Acta Chem. Scand., Ser. B* **1974**, *B28*, 441.
(36) Joensson, N. A.; Moses, P. *Acta Chem. Scand., Ser. B* **1974**, *B28*, 225.
(37) Ruggli, P.; Grand, R. *Helv. Chim. Acta* **1937**, *20*, 373.

Scheme III



(a) H_2/Pd; (b) NaOH, Br_2; (c) H_2/Pd; (d) R_3COCl/NEt_3; (e) H_2SO_4/HNO_3; (f) R_3COCl/Pyridine; (g) Ac_2O; (h) H_2SO_4/HNO_3; (i) NaOH; (j) H_2/PtO_2; (k).R_1CHO; (l) H_2/Pd; (m) Ac_2O/HNO_3; (n) HCl.

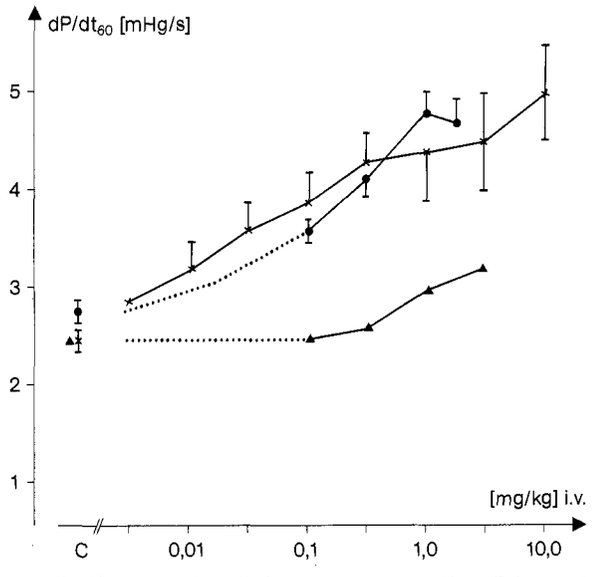


Figure 3. Cardiotonic activity of 1 (★, n = 10), milrinone (●, n = 6), and enoximone (▲, n = 2) in conscious dogs after iv injections of incremental doses. dP/dt was recorded in mmHg/s and converted to mHg/s in the figure. C means control values. Each point is the mean ± range of experimental values.

followed the same hemodynamic profile already described in rats and cats. However, the effective doses were usually slightly higher. Starting at 0.003 mg/kg, compound 1 produced a dose-related increase in contractility up to 10 mg/kg. Again, the cardiovascular effect was accompanied by a reduction in blood pressure and an increase in heart rate. Nevertheless, compound 1 ($ED_{1.5}$ = 0.20 mg/kg) was twice as potent as milrinone ($ED_{1.5}$ = 0.4 mg/kg) (Figure 3). A very weak increase of contractility with enoximone was first observed at doses of 0.3 mg/kg.

Encouraged by the positive inotropic effects of this novel heterocyclic ring system in different animal models after

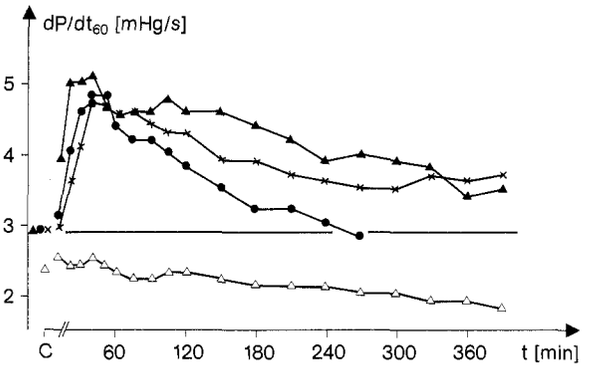


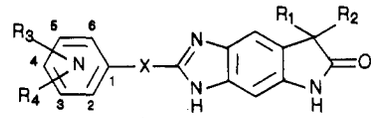
Figure 4. Duration of action of 1 (n = 6, ★), in comparison with pimobendan (n = 6, ▲), milrinone (n = 6, ●), and placebo (n = 6, △) in conscious dogs. Dose: 1 mg/kg po suspended in methylcellulose. dP/dt was recorded in mmHg/s and converted to mHg/s in the figure. C means the control values. Each point is the mean of experimental values.

iv administration, we further examined the oral effectiveness of the most potent compound 1 in conscious dogs. Since pimobendan[38] and milrinone[22] are reported to have a long duration of action, we included both compounds in our study. The dogs were given a single dose of 1 mg/kg po of each compound suspended in methylcellulose. At this dose compound 1, pimobendan, and milrinone were almost equipotent, the maximum increase in contractility being about 2000 mmHg/s. However, the cardiotonic activity of 1 and pimobendan was still evident 6 h after administration, while the effect of milrinone disappeared within 4 h (Figure 4).

In Table II we have summarized all the pharmacological data on 2-pyridyl-6,7-dihydro-3H,5H-pyrrolo[2,3-f]benz-

(38) Diederen, W.; Daemmgen, J.; Kadatz, R. *Arch. Pharmacol.* **1982**, *321* (*Suppl.*), R 36.

Table I. Structure and Properties of 6,7-Dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-ones



no.	R_1	R_2	position N	position R_3	position R_4	X	prepn methods	% yield	mp, °C	recrystn solvent
1	CH_3	CH_3	4	H	H		H, I, J, C	90.7	205–252	H_2O/ethanol
2	CH_3	CH_3	3	H	H		H, I, J, A	38	331–350	H_2O/dioxane
3	CH_3	CH_3	2	H	H		H, I, J, D	65	182–187	H_2O
4	CH_3	CH_3	4-*N*-oxy	H	H		S	22	260	H_2O/dioxane
5	CH_3	CH_3	4	3-CH_3	H		H, I, J, D	29	311–313	acetone
6	CH_3	CH_3	4	3-OH	H		R	23	>360	H_2O
7	CH_3	CH_3	4	3-Cl	H		H, I, J, D	42	341–344	ethyl acetate
8	CH_3	CH_3	3	4-CH_3	H		H, I, J, F	18	>360	*c*
9	CH_3	CH_3	3	2-OCH_3	4-CH_3		H, I, J, B	19	296–298	ethyl acetate
10	CH_3	CH_3	2	4-C_4H_9	H		H, I, J, D	40	176–178	ethyl acetate/CH_3OH
11	CH_3	CH_3	4	H	H	CH_2	H, I, J, E	16	333–337	H_2O/CH_3OH
12	CH_3	CH_3	4	H	H	CH_2CH_2	H, I, J, D	20	150–154	H_2O
13	CH_3	CH_3	3	H	H	CH=CH	H, I, J, D	16	203–207	H_2O/CH_3OH
14	C_2H_5	C_2H_5	4	H	H		G, H, I, J, D	37	216–219	H_2O/ethanol
15	$(CH_2)_4$		4	H	H		L, M, O, P, A	47	258–260	H_2O/dioxane
16	CH_3	$COOC_2H_5$	4	H	H		K, N, A	79	288–290[d]	ethanol
17	CH_3	$CONHNH_2$	4	H	H		T	70	>300	CH_2Cl_2/CH_3OH
18	CH_3	H	4	H	H		K, N, A	58	315–318	H_2O/ethanol
19	CH_3	H	3	H	H		K, N, A	32	>300	dioxane/CH_3OH
20	C_2H_5	H	4	H	H		K, N, A	36	270–272	ethyl acetate/CH_3OH
21	C_2H_5	H	3	H	H		K, N, A	63	>300	H_2O/ethanol
22	$(CH_3)_2CH$	H	4	H	H		K, N, A	66	215–220	H_2O/ethanol
23	$(CH_3)_2CHCH_2$	H	4	H	H		Q, J, D	25	200–202	CH_3OH
24	c-C_5H_9	H	4	H	H		K, N, A	75	200–204	dioxane/CH_3OH

[a] Yields are not optimized and corresponded to the final step. [b] Melting points are uncorrected. [c] Purification by column chromatography. [d] As HCl salt.

Table II. Pharmacological Data of Compounds 1–24 and Milrinone

no.	rat, iv $ED_{1.5}$[a]	rat, iv max[b] (dose)[c]	cat, iv $ED_{1.5}$[a]	cat, iv max[b] (dose)[c]	dog, iv $ED_{1.5}$[a]	dog, iv max[b] (dose)[c]
1	0.01	3.7 (0.1)	0.01	2.0 (0.3)	0.20	2.0 (1.0)
2	1.0	3.5 (3.0)	0.02	3.5 (0.3)	0.51	2.5 (3.0)
3	0.96	2.4 (3.0)	0.23	2.7 (3.0)	1.11	1.7 (3.0)
4	0.03	4.9 (1.0)	NT[e]	NT	0.44	1.8 (1.0)
5	0.29	2.5 (1.0)	0.03	2.7 (0.1)	0.51	1.8 (1.0)
6	0.87	3.4 (10.0)	0.21	2.4 (1.0)	NT	NT
7	0.07	3.9 (1.0)	0.07	3.7 (3.0)	NT	NT
8	>3	*d*	NT	NT	NT	NT
9	>10	*d*	NT	NT	NT	NT
10	>3	*d*	NT	NT	NT	NT
11	1.14	4.1 (10.0)	0.6	3.8 (10.0)	NT	NT
12	>10	*d*	NT	NT	NT	NT
13	1.21	4.3 (10.0)	NT	NT	NT	NT
14	>3	1.9 (10.0)	NT	NT	NT	NT
15	0.20	3.3 (3.0)	0.08	2.8 (1.0)	0.77	2.2 (3.0)
16	0.09	4.2 (3.0)	0.25	3.7 (3.0)	0.64	>3.6 (3.0)
17	>10	*d*	NT	NT	NT	NT
18	0.02	4.4 (1.0)	0.01	2.5 (0.1)	0.58	1.7 (3.0)
19	0.06	4.6 (3.0)	0.03	2.3 (0.3)	0.08	2.4 (1.0)
20	0.06	4.5 (3.0)	0.04	2.5 (0.3)	0.09	2.3 (1.0)
21	0.39	4 (10.0)	0.15	2.3 (1.0)	NT	NT
22	>3	2.2 (10.0)	NT	NT	NT	NT
23	>3	*d*	NT	NT	NT	NT
24	>10	*d*	NT	NT	NT	NT
25	0.14	3.4 (10.0)	0.13	3.0 (10.0)	0.4	2.0 (1.0)

[a] $ED_{1.5}$ is the effective dose (mg/kg) required to produce an increase in dP/dt_{50} by 1500 mmHg/s. dP/dt was recorded in mmHg/s and was converted to mHg/s in the table. Unless otherwise noted, each value is the mean of four experiments. [b] Max means the maximum increase in dP/dt recorded in mmHg/s from control and converted to mHg/s in the table. [c] Dose (mg/kg) at which the maximum increase in dP/dt was achieved. [d] Values not obtained. [e] NT = not tested.

imidazol-6-ones 1–24 and milrinone.

Structure–Activity Relationships

The data summarized in Table II provided us with more information about structural requirements necessary for optimal inotropic activity. If one considers $ED_{1.5}$ values in rats of three series of compounds 1–3, 17 and 18, and 19 and 20, then the 4-pyridyl derivatives always show a greater positive inotropic effect than the 3- and 2-pyridyl derivatives. Usually, nitrogen oxidation does not alter contractility since the parent compound 1 has a similar effect in rats as the *N*-oxy compound 4.

Further substituents in the pyridine ring (e.g., 5–10) again demonstrate the higher activity of 4-pyridyl deriv-

atives over their 3- and 2-isomers. However, any substituent always leads to a reduction in the force of contractility in comparison with the unsubstituted compounds.

Insertion of an alkyl chain between the pyridine ring and benzimidazole moiety (e.g., 11, 12) dramatically decreased contractility. In rats, the effective dose ($ED_{1.5}$) increased to 1.14 mg/kg in the case of a methylene group and increased further to more than 10 mg/kg for the ethylene group. However, a conjugated double bond between the two heterocyclic ring systems (13) again improved the positive inotropic activity ($ED_{1.5}$ = 1.2 mg/kg). We further prepared and tested several pyrrolo[2,3-*f*]benzimidazol-6-ones to determine the structural features necessary for inotropic activity at position 7 of the tricyclic ring system.

In the case of mono- and disubstituted derivatives we observed a rise of $ED_{1.5}$ values in rats with an increase in volume of the substituents in position 7. For example, compound 1 ($ED_{1.5}$ = 0.01 mg/kg) is more active than the spiro derivative 15 ($ED_{1.5}$ = 0.2 mg/kg), the diethyl compound 14 ($ED_{1.5}$ = 3 mg/kg), and the hydrazide 17 ($ED_{1.5}$ = 10 mg/kg). Surprisingly the ethyl carboxylate 16 ($ED_{1.5}$ = 0.09 mg/kg) does not fit into this row of disubstituted derivatives. A logical explanation of this fact could be the metabolic decarboxylation of this compound to the monomethyl derivative 18 ($ED_{1.5}$ = 0.02 mg/kg). Like the disubstituted pyrrolo[2,3-*f*]benzimidazolones, monosubstituted derivatives showed the same tendency with respect to contractility. Starting with 22, the positive inotropic activity further decreased with larger steric hindrance, while smaller substituents (e.g., 18–21) were still well tolerated.

Conclusions

We have prepared several new 2-pyridyl-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-ones and demonstrated that this series of compounds possess useful inotropic and vasodilator activity. In our studies we found in general that a 4-pyridyl ring directly bonded to the tricyclic ring system and small alkyl groups at position 7 are structural requirements necessary for potent inotropic activity.

One of the most potent compounds, 1, was chosen for comparison with milrinone and enoximone. After iv administration to rates, cats, and dogs, 1 was about 14-, 10-, and 2-fold more potent than milrinone, whereas enoximone only possessed a weak inotropic activity in these animal models.

In addition, 1, milrinone, and pimobendan were administered orally to conscious dogs and produced an equipotent effect. However, the inotropic effect of 1 and pimobendan were still evident after 6 h, while the activity of milrinone disappeared after 4 h. On the basis of these results, 2-pyridyl-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-ones represent a new class of potent, orally active, positive inotropic agents and suggest that they might be useful in the management of congestive heart failure. Further pharmacological and biochemical studies are in progress and will be reported elsewhere.[39]

Experimental Section

Melting points were determined on a Büchi capillary melting point apparatus and are not corrected. Identity of all compounds was confirmed by ^1H NMR, mass spectra, and combustion analysis. All reactions were followed by TLC carried out on Merck F 254 silica gel plates.

Unless literature references are given, the starting materials were commercially available or were prepared according to the literature.[40] The following methods (A–T) are described for specific products. However, identical procedures may be applied to analogous compounds.

Synthesis of Pyrrolo[2,3-*f*]benzimidazol-6-ones. Method A is illustrated by the preparation of 7,7-dimethyl-2-(3-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (2).

A 32.6-g (0.1 mol) sample of 3,3-dimethyl-5-nitro-6-(nicotinoylamino)indolin-2-one was hydrogenated in 500 mL of ethanol and 5 mL of triethylamine with 2 g of 10% Pd/C until the theoretical amount had been consumed. The reaction mixture was filtered, the solvent removed under reduced pressure, and the residue refluxed in 500 mL of ethanol and 100 mL of concentrated HCl for 6 h. The solvent was removed, the residue was taken up in water and neutralized, and the precipitate isolated by suction. Recrystallization from dioxane/water yielded 12.6 g of pure product: mp 331–335 °C (base·$3H_2O$); NMR (Me_2SO-d_6) δ 1.33 (s, 6 H, 2 CH_3), 7.08 (br s, 1 H, aromatic proton), 7.56 (br s, 1 H, aromatic proton), 8.44 (br s, 1 H, pyridine proton), 8.64 (br s, 1 H, pyridine proton), 9.29 (br s, 1 H, pyridine proton), 10.26 (s, 1 H, NH); mass spectrum, *m/e* 278 (M^+).

Preparation of Pyrrolo[2,3-*f*]benzimidazol-6-ones. Method B is illustrated by the preparation of 7,7-dimethyl-2-(2-methoxy-6-methyl-3-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]-benzimidazol-6-one (9).

A 3.8-g (0.02 mol) sample of 5,6-diamino-3,3-dimethylindolin-2-one, 3 g (0.02 mol) of 2-methoxy-6-methylpyridine-3-carbaldehyde,[41] 0.4 g (0.002 mol) of *p*-toluenesulfonic acid, and 50 mL of ethanol were refluxed for 1 h while air was bubbled through the solution. The solvent was removed, the residue was extracted with CH_2Cl_2, and the organic phase was washed with water and dried. Removal of the solvent and addition of ether yielded the product after 24 h, which was treated with charcoal and recrystallized from ethyl acetate to yield 1.2 g of pure product: mp 296–298 °C; NMR (Me_2SO-d_6) δ 1.31 (s, 6 H, 2 CH_3), 2.47 (s, 3 H, CH_3), 4.09 (s, 3 H, OCH_3), 7.00–7.08 (2 H, aromatic and pyridine protons), 7.50 (br s, 1 H, aromatic proton), 8.45–8.48 (1 H, pyridine proton), 10.58 (br s, 1 H, NH); mass spectrum, *m/e* 322 (M^+).

Preparation of Pyrrolo[2,3-*f*]benzimidazol-6-ones. Method C is illustrated by the preparation of 7,7-dimethyl-2-(4-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (1).

To 30 g of polyphosphorous acid were added 1.1 g (8.94 mmol) of pyridine-4-carboxylic acid, 1.71 g (8.94 mmol) of 5,6-diamino-3,3-dimethylindolin-2-one, and 9 g of P_2O_5. The mixture was heated under nitrogen at 150–160 °C for 7 h. After being poured into ice/water, the precipitate was isolated by suction, again suspended in water, and adjusted to pH 8 to liberate the free base. In order to start crystallization, the water phase was warmed to 50 °C and the product was isolated after recooling the solution to yield 2.84 g of pure product, mp 205–225 °C (base·$4H_2O$). Further recrystallization from methanol yielded a water-free compound: mp 285–288 °C; NMR (Me_2SO-d_6) δ 1.34 (s, 6 H, 2 CH_3), 7.02 (s, 1 H, aromatic proton), 7.57 (s, 1 H, aromatic proton), 8.02–8.04 (1 H, pyridine proton), 8.72–8.73 (1 H, pyridine proton), 10.29 (s, 1 H, NH); mass spectrum, *m/e* 278 (M^+).

Preparation of Pyrrolo[2,3-*f*]benzimidazol-6-ones. Method D is illustrated by the preparation of 7,7-dimethyl-2-(2-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (3).

A 3.8-g (20 mmol) sample of 5,6-diamino-3,3-dimethylindolin-2-one was suspended in 100 mL of CH_2Cl_2, 6.9 mL (50 mmol) of NEt_3 was added, and 5.3 g (30 mmol) of pyridine-2-carbonyl chloride hydrochloride was introduced with cooling. After 1 h water was added, the organic phase separated, and the solvent removed. The crude product was refluxed with 100 mL of ethanol and 30 mL of concentrated hydrochloric acid for 3 days. Ethanol was removed and the remaining water phase adjusted

(39) Further pharmacological data have been previously presented: (a) Müller-Beckmann, B.; Sponer, G. 27. Frühjahrstagung der Deutschen Pharmakologischen Gesellschaft, Mainz (West Germany), March 1986. (b) Strein, K.; Honerjäger, P.; Jäger, H.; Freund, P. 27. Frühjahrstagung der Deutschen Pharmakologischen Gesellschaft, Mainz (West Germany), March 1986.

(40) Hölck, J. P.; Mertens, A.; Kampe, W.; Müller-Beckmann, B.; Sponer, G.; Strein, K. Eur. Pat. Appl. 161 632, November 21, 1985.

(41) Beak, P.; Covington, J. B.; Smith, S. G.; White, J. M.; Zeigler, J. M. *J. Org. Chem.* **1980**, *45*, 1354.

(42) Späth, E.; Spitzer, H. *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 1477.

to pH 8. The precipitate was isolated and recrystallized from water to yield 3.6 g of pure product: mp 182–187 °C (base·0.3H_2O); NMR (Me_2SO-d_6) δ 1.33 (s, 6 H, 2 CH_3), 7.02 (s, 1 H, aromatic proton), 7.45–7.50 (1 H, pyridine proton), 7.53 (s, 1 H, aromatic proton), 7.92–8.00 (1 H, pyridine proton), 8.25–8.28 (1 H, pyridine proton), 8.67–8.70 (1 H, pyridine proton), 10.28 (s, 1 H, NH); mass spectrum, *m/e* 278 (M^+).

Preparation of Pyrrolo[2,3-*f*]benzimidazol-6-ones. Method E is illustrated by the preparation of 7,7-dimethyl-2-(4-pyridylmethyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (11).

A 7.3-g (38 mmol) sample of 5,6-diamino-3,3-dimethylindolin-2-one and 11.6 g (77 mmol) of methyl 4-pyridylacetate was heated to 180 °C under nitrogen for 16 h. The solid residue was purified by column chromatography on silica gel (eluent: CH_2Cl_2/CH_3OH saturated with NH_3, 20:1). The crude product was recrystallized from CH_3OH/H_2O to yield 1.8 g of pure product: mp 333–337 °C (base·H_2O); NMR (Me_2SO-d_6) δ 1.28 (s, 6 H, 2 CH_3), 4.16 (s, 2 H, CH_2), 6.86 (s, 1 H, aromatic proton), 7.26–7.29 (1 H, pyridine proton), 7.36 (s, 1 H, aromatic proton), 8.44–8.47 (1 H, pyridine proton), 10.00 (s, 1 H, NH); mass spectrum, *m/e* 292 (M^+).

Preparation of Pyrrolo[2,3-*f*]benzimidazol-6-ones. Method F is illustrated by the preparation of 7,7-dimethyl-2-(2-methyl-5-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (**8**).

A solution of 8.3 g (40 mmol) of *N,N'*-dicyclohexylcarbodiimide in DMF was added dropwise to 4.8 g (35 mmol) of 2-methylpyridine-5-carboxylic acid,[43] 5.4 g (40 mmol) of 1-hydroxy-1*H*-benzotriazole, and 5 g of anhydrous $CaSO_4$ in 50 mL of DMF at 0 °C. After 2 h at room temperature, 5.7 g (30 mmol) of 5,6-diamino-3,3-dimethylindolin-2-one was added in portions over 15 min. After evaporation of the solvent and addition of water, the precipitate was isolated by suction and added to 200 mL of ethanol and 40 mL of concentrated hydrochloric acid. The mixture was heated to reflux for 2 h, cooled, and filtered. The filtrate was evaporated to dryness and made alkaline with ammonia. The crude product was isolated and purified by column chromatography on silica gel (eluent: CH_2Cl_2/CH_3OH saturated with NH_3, 15:1) to yield 1.8 g of pure product: mp >360 °C; NMR (Me_2SO-d_6) δ 1.33 (s, 6 H, 2 CH_3), 2.58 (s, 3 H, CH_3), 6.96 (s, 1 H, aromatic proton), 7.36–7.40 (1 H, pyridine proton), 7.49 (s, 1 H, aromatic proton), 8.26–8.30 (1 H, pyridine proton), 9.12 (s, 1 H, pyridine proton), 10.13 (s, 1 H, NH); mass spectrum, *m/e* 292 (M^+).

Preparation of 3,3-Dialkylated Indolin-2-ones. Method G is illustrated by the preparation of 3,3-diethylindolin-2-one.

(a) A 102-g (0.46 mol) sample of 1-benzyloxindole[33] and 5 g of *N*-benzyltributylammonium bromide were suspended in 500 mL of concentrated sodium hydroxide solution. Ethyl iodide (85 mL) was added dropwise to the mixture with occasional cooling to keep the temperature below 60 °C. After 3 h 300 mL of toluene was added, the organic phase separated off, and the solvent removed to yield 126 g of 1-benzyl-3,3-diethyloxindole as oil.

(b) A 125-g sample of the above product was added to 1.5 L of liquid ammonia in portions at −30 °C and 35 g of sodium was introduced in small pieces until the blue color of the solution persisted for 5 min. Water (40 mL) was slowly added and the ammonia was allowed to evaporate overnight. After addition of 500 mL of water, the product was isolated by suction and recrystallized from ethanol to yield 70 g of pure product: mp 158–159 °C; NMR (Me_2SO-d_6) δ 0.36–0.62 (t, 6 H, 2 CH_3), 1.50–1.90 (q, 4 H, 2 CH_2), 6.66–7.30 (m, 4 H, aromatic protons), 10.20 (s, 1 H, NH).

Preparation of 5-Aminooxindoles. Method H is illustrated by the preparation of 5-amino-3,3-diethylindolin-2-one.

A mixture of 200 mL of 80% H_2SO_4 and 16 mL of concentrated HNO_3 (*d* = 1.5) was added dropwise to a solution of 68 g (0.37 mol) of 3,3-diethyloxindole in 500 mL of 80% H_2SO_4 at 10–20 °C. After 30 min, the mixture was poured into ice/water, and 5-nitro-3,3-diethyloxindole was isolated by suction and washed with water to yield 86.5 g of crude product, mp 174–176 °C. Without further purification, the above product was hydrogenated in 700 mL of ethanol with 4 g of 10% Pd/C. The 5-amino-3,3-diethyloxindole was isolated after separation of the catalyst and crystallization from ethanol to yield 53.6 g of pure product; mp 188–190 °C; NMR (Me_2SO-d_6) δ 0.4–0.63 (t, 6 H, 2 CH_3), 1.43–1.83 (q, 4 H, 2 CH_2), 4.62 (br s, 2 H, NH_2), 6.23–6.60 (m, 3 H, aromatic protons), 9.76 (s, 1 H, NH).

Preparation of 5-Amino-6-nitroindolin-2-ones. Method I is illustrated by the preparation of 5-amino-6-nitro-3,3-diethylindolin-2-one.

(a) A 100-g (0.49 mol) sample of 5-amino-3,3-diethylindolin-2-one was slowly introduced into 1000 mL of acetic anhydride. After 1 h the precipitate was isolated and recrystallized from ethyl acetate to yield 113 g of 5-acetamido-3,3-diethyloxindole, mp 196–197 °C.

(b) Concentrated HNO_3 (*d* = 1.5, 24 mL) was added dropwise to a solution of 84 g (0.35 mol) of 5-acetamido-3,3-diethyloxindole in 800 mL of acetic anhydride with cooling. After 2 h at room temperature, the mixture was aded dropwise to ice/water and the product was isolated by suction and washed with water to yield 72 g of 5-acetamido-6-nitro-3,3-diethyloxindole, mp 182–184 °C.

(c) A 72-g (0.25 mol) sample of the above product was refluxed in 500 mL of ethanol and 100 mL of concentrated hydrochloric acid for 3 h. The mixture was diluted with 1000 mL of water, and the precipitate was isolated and washed with ethanol/water (3:1) to yield 54.7 g of pure product: mp 267–272 °C; NMR (Me_2SO-d_6) δ 0.46–0.70 (t, 6 H, 2 CH_3), 1.50–1.90 (q, 4 H, 2 CH_2), 6.86 (s, 1 H, aromatic proton), 7.22 (s, 1 H, aromatic proton), 7.33 (br s, 2 H, NH_2), 10.25 (br s, 1 H, NH).

Preparation of 5,6-Diaminooxindoles. Method J, starting either from 5-amino-6-nitrooxindole or 6-amino-5-nitrooxindole, is illustrated by the preparation of 5,6-diamino-3,3-diethylindolin-2-one.

A 10-g (41 mmol) sample of 5-amino-6-nitro-3,3-diethylindolin-2-one was hydrogenated with 0.6 g of PtO_2 in 150 mL of ethanol. Usual workup and crystallization from ethanol yielded 8.5 g of 5,6-diamino-3,3-diethylindolin-2-one, mp 167–173 °C.

Preparation of 3-Alkylated 6-Aminooxindoles. Method K is illustrated by the preparation of 6-amino-3-methyloxindole.

A 40-g (0.15 mol) sample of ethyl 2-(2,4-dinitrophenyl)-propionate (prepared by nitration of 2-phenylpropionic acid and esterification[44]) were hydrogenated with 2 g of Pd/C in 1000 mL of ethanol. After removal of the catalyst, the solution was acidified with ethanolic HCl, the solvent evaporated, and the product isolated by recrystallization from ethanol to yield 13.7 g of pure product: mp 192–194 °C (HCl salt); NMR (Me_2SO-d_6) δ 1.25–1.43 (d, 3 H, CH_3), 3.20–3.70 (q, 1 H, CH), 6.85–7.06 (m, 2 H, aromatic protons), 7.23–7.43 (br d, 1 H, aromatic proton), 8.00–9.50 (br, 2 H, NH_2), 10.56 (s, 1 H, NH amide); mass spectrum, *m/e* 162 (M^+).

Preparation of 6-Nitrooxindoles. Method L is illustrated by the preparation of 6′-nitrospiro[cyclopentane-1,3′-indolin]-2′-one.[36]

A 54-mL (1.05 mol) sample of bromine was added dropwise at 0 °C to a solution of 210 g (5.25 mol) of sodium hydroxide in 1700 mL of water and subsequently 91 g (0.35 mol) of 7′-nitrospiro[cyclopentane-1,4′-2′*H*,4′*H*-isoquinoline]-1′,3′-dione was introduced. After being stirred for 1 h at ambient temperature, the reaction mixture was heated to 80 °C for 1 h and, after cooling, acidified with acetic acid. The product obtained was filtered off with suction, washed with water, dried, and recrystallized from ethanol to yield 66.5 g of pure product: mp 226–228 °C; NMR (Me_2SO-d_6) δ 1.83–2.20 (m, 8 H, 4 CH_2), 7.36–7.90 (3 H, aromatic protons), 10.60 (br s, 1 H, NH).

Preparation of 6-Aminooxindoles. Method M is illustrated by the preparation of 6′-aminospiro[cyclopentane-1,3′-indolin-2′-one.

A suspension of 164.7 g (0.71 mol) of 6′-nitrospiro[cyclopentane-1,3′-indolin]-2′-one in 3.5 L of methanol and 300 mL of glacial acetic acid were hydrogenated in the presence of 16 g of

(43) Preparation analogous to Tracy, A. H.; Elderfield, R. C. *J. Org. Chem.* **1941**, *6*, 70.

(44) Preparation analogous to Ruggli, P.; Grand, R. *Helv. Chim. Acta* **1937**, *20*, 373.

(45) Preparation analogous to Horner A. *Ann. Chem.* **1941**, *548*, 117.

10% Pd/C at 40 °C, while being well stirred. The clear solution obtained was filtered off with suction from the catalyst, the filtrate evaporated, and the compounds recrystallized from ethyl acetate to yield 140.6 g of pure product: mp 165–170 °C; NMR (Me_2SO-d_6) δ 1.50–2.10 (m, 8 H, 4 CH_2), 4.90 (br s, 2 H, NH_2), 5.93–7.15 (3 H, aromatic protons), 9.85 (br s, 1 H, NH).

Preparation of 6-[(Pyridinylcarbonyl)amino]-5-nitrooxindoles. Method N is illustrated by the preparation of 6-(isonicotinoylamino)-5-nitro-3-methyloxindole.

(a) A 5.1-g (27.1 mmol) sample of 6-amino-3-methylindolin-2-one hydrochloride was suspended in 100 mL of methylene chloride, mixed with 3.7 mL (27.1 mmol) of triethylamine, and stirred for 10 min. While cooling with ice, 6.73 g (37.8 mmol) of isonicotinic acid chloride hydrochloride and 5.24 mL (37.8 mmol) of triethylamine were added. After 3 h, the methylene chloride was distilled off and the residue was worked up with water and filtered with suction. The residue was stirred with hot ethanol to yield 5.6 g of 6-(isonicotinoylamino)-3-methyloxindole, mp >300 °C.

(b) A 5.0-g (18.7 mmol) sample of the above product was dissolved in 20 mL of concentrated sulfuric acid, cooled with ice, and 1.84 g (18.7 mmol) of potassium nitrate dissolved in concentrated sulfuric acid was slowly added dropwise. After 2 h, the solution was poured onto ice and filtered with suction and the crude product again suspended in water and neutralized. The precipitate isolated was dissolved in ethyl acetate, treated with charcoal and Na_2SO_4, and crystallized from ethyl acetate to yield 4.85 g of pure product: mp >300 °C; NMR (Me_2SO-d_6) δ 1.26–1.46 (d, 3 H, CH_3), 3.35–3.80 (q, 1 H, CH), 7.55 (s, 1 H, aromatic proton), 7.90–8.60 (3 H, two pyridine protons and one aromatic proton), 8.80–8.96 (2 H, pyridine protons), 10.98 (s, 1 H, NH), 11.16 (s, 1 H, NH); mass spectrum, *m/e* 312 (M^+).

Preparation of 6-Amino-5-nitroindolin-2-ones. Method O is illustrated by the preparation of 6′-amino-5′-nitrospiro[cyclopentane-1,3′-indolin]-2′-one.

(a) A 20.4-g (0.2 mol) sample of acetic anhydride was added dropwise, with cooling, to a suspension of 36.4 g (0.18 mol) of 6′-aminospiro[cyclopentane-1,3′-indolin]-2′-one in 500 mL of ethyl acetate and then the mixture was stirred for about 1 h at ambient temperature. The resultant product was filtered off with suction, washed well with ethyl acetate, dried, and crystallized from ethanol to yield 32.9 g 6′-acetamidospiro[cyclopentane-1,3′-indolin]-2′-one, mp 263–265 °C.

(b) A solution of 7.6 mL (0.18 mol) of fuming nitric acid in 7.6 mL of concentrated sulfuric acid was added dropwise, with cooling, to a solution of 39 g (0.16 mol) of 6′-acetamidospiro[cyclopentane-1,3′-indolin]-2′-one in 200 mL of concentrated sulfuric acid. The reaction mixture was further stirred for 1 h and poured onto ice. The crystals obtained were filtered off with suction, washed well with water, dried, and crystallized from ethanol to yield 38.4 g of 6′-acetamido-5′-nitrospiro[cyclopentane-1,3′-indolin]-2′-one, mp 290–292 °C.

(c) A solution of 40.5 g (0.14 mol) of 6′-acetamido-5′-nitrospiro[cyclopentane-1,3′-indolin]-2′-one in 180 mL of ethanol was heated under reflux for about 2 h with 18 mL of concentrated aqueous sodium hydroxide solution, subsequently evaporated in vacuum, adjusted to pH 6, and cooled in an ice bath. The crystals obtained were filtered off with suction, washed with water, dried, and crystallized from ethanol to yield 30.1 g of pure product: mp 300–303 °C; NMR (Me_2SO-d_6) δ 1.70–2.06 (m, 8 H, 4 CH_2), 6.35 (s, 1 H, aromatic proton), 7.60 (br s, 2 H, NH_2), 7.72 (s, 1 H, aromatic proton).

Preparation of 6-(Pyridinoylamino)-5-nitrooxindoles. Method P is illustrated by the preparation of 6-(isonicotinoylamino)-5-nitro-3,3-dimethylindolin-2-one.

A solution of 48.3 g (0.22 mol) of 6-amino-3,3-dimethyl-5-nitroindolin-2-one in 400 mL of pyridine was mixed portionwise with 78.8 g (0.44 mol) of isotonic acid chloride hydrochloride and then the mixture was stirred for about 2 h. Subsequently, the reaction mixture was poured onto water, rendered neutral, and filtered with suction and the compound obtained was recrystallized from ethanol to yield 68.1 g of pure product: mp 225–230 °C; NMR (Me_2SO-d_6) δ 1.33 (s, 6 H, 2 CH_3), 7.62 (s, 1 H, aromatic proton), 7.76–7.90 (3 H, two pyridine protons and one aromatic proton), 8.73–8.86 (2 H, pyridine protons), 10.98 (s, 1 H, NH), 11.10 (s, 1 H, NH).

Preparation of 3-Alkylated 6-Amino-5-nitrooxindoles. Method Q is illustrated by the preparation of 6-amino-5-nitro-3-(2-methylpropyl)indolin-2-one.

(a) A solution of 1.9 g of sodium hydroxide in 2 mL of water was added dropwise to a suspension of 9 g (0.047 mol) of 6-acetamidoindolin-2-one[37] in 50 mL of ethanol containing 3.4 g (0.047 mol) of isobutylraldehyde.[44] After about 5 h, the reaction mixture was evaporated to dryness and the residue purified on silica gel (elution agent: methylene chloride/ammonia-saturated methanol) to yield 7.6 g of 6-acetamido-3-isobutylideneindolin-2-one as foam, mp 93 °C.

(b) A solution of 6.4 g (0.026 mol) of 6-acetamido-3-isopropylideneindolin-2-one in 100 mL of methanol was hydrogenated in the presence of 0.6 g of 10% Pd/C. Subsequently, the catalyst was filtered off with suction and the filtrate was evaporated to dryness to yield 5.5 g 6-acetamido-3-(2-methylpropyl)indolin-2-one, mp 214–216 °C.

(c) A solution of 4.2 g (0.017 mol) of 6-acetamido-3-(2-methylpropyl)indolin-2-one in 50 mL of acetic anhydride was mixed, with cooling, with 0.8 mL (0.019 mol) of fuming nitric acid and then the mixture was stirred for about 30 min. Subsequently, the reaction mixture was carefully poured onto ice, and the crystals obtained were filtered off with suction, washed with water, and dried to yield 3.2 g of 6-acetamido-3(2-methylpropyl)-5-nitroindolin-2-one.

(d) A solution of 2.9 g (0.01 mol) of 6-acetamido-3-(2-methylpropyl)-5-nitroindolin-2-one in 50 mL of ethanol was heated under reflux for about 30 min with 3 mL of concentrated hydrochloric acid and evaporated and the residue purified on silica gel (elution agent: methylene chloride/ammonia-saturated methanol, 20:1, v/v) to yield 1.5 g of pure product: mp 192–197 °C; NMR (Me_2SO-d_6) δ 0.80–1.00 (d, 6 H, 2 CH_3), 1.50–2.10 (m, 3 H, CH_2 and CH), 3.40–3.70 (t, 1 H, CH), 6.38 (br s, 2 H, NH_2), 7.63 (s, 1 H, aromatic proton), 7.75 (s, 1 H, aromatic proton), 10.76 (br s, 1 H, NH).

Preparation of 7,7-Dimethyl-2-(2-hydroxy-4-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (6). Method R. A 20-g (64 mmol) sample of 7,7-dimethyl-2-(2-chloro-4-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (7) was stirred in 250 mL of semiconcentrated phosphoric acid at 100 °C for 6 days. The mixture was poured onto 1500 mL of ice/water, and the precipitate was isolated and purified by column chromatography (eluent: CH_2Cl_2/ammonia-saturated CH_3OH, 15:1 to 10:1). The crude product was recrystallized from water to yield 4.8 g of pure product: mp >360 °C (base·2H_2O); NMR (Me_2SO-d_6) δ 1.32 (s, 6 H, 2 CH_3), 6.87–7.00 (3 H, aromatic proton and two pyridine protons), 7.46–7.56 (2 H, aromatic proton and pyridine proton), 10.27 (s, 1 H, NH), 11.56 (s, 1 H, OH); mass spectrum, *m/e* 294 (M^+).

Preparation of 7,7-Dimethyl-2-(*N*-oxy-4-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (4). Method S. A solution of 3.9 g (0.014 mol) of 7,7-dimethyl-2-(4-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one in 50 mL of glacial acetic acid was stirred for 2 days at 50 °C with 20 mL of 30% hydrogen peroxide and the reaction mixture then was diluted with water. The precipitated substance was filtered off with suction and recrystallized from dioxane/water (1:1, v/v) to yield 1.4 g of pure product: mp 260–262 °C (base·3H_2O); NMR (Me_2SO-d_6) δ 1.32 (s, 6 H, 2 CH_3), 6.98 (s, 1 H, aromatic proton), 7.53 (s, 1 H, aromatic proton), 8.02–8.05 (2 H, pyridine protons), 8.31–8.34 (2 H, pyridine protons), 10.27 (s, 1 H, NH); mass spectrum, *m/e* 294 (M^+).

Preparation of 7-(Hydrazinocarbonyl)-7-methyl-2-(4-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo[2,3-*f*]benzimidazol-6-one (17). Method T. A solution of 1 g (2.7 mmol) of 7-(ethoxycarbonyl)-7-methyl-2-(4-pyridyl)-6,7-dihydro-3*H*,5*H*-pyrrolo-[2,3-*f*]benzimidazol-6-one hydrochloride (16) in 30 mL of ethanol was stirred at 80 °C for about 8 h with 6 mL of hydrazine hydrate. Subsequently, the reaction mixture was filtered with suction, the product obtained was boiled up with a solution of 1000 mL of methanol and 500 mL of methylene chloride and concentrated somewhat, and the crystals obtained were filtered off with suction, washed with methanol, and dried to yield 0.6 g of pure product: mp >300 °C; NMR (Me_2SO-d_6) δ 1.58 (s, 3 H, CH_3), 4.25 (br s, 2 H, NH_2), 6.94 (br s, 1 H, aromatic proton), 7.54 (br s, 1 H, aromatic proton), 7.92–7.98 (2 H, pyridine protons), 8.64–8.68 (2

H, pyridine protons), 8.78 (s, 1 H, NH), 10.40 (s, 1 H, NH); mass spectrum, m/e 322 (M^+).

Pharmacological Methods. 1. **Anesthetized Rat Model.** Male SPF Sprague–Dawley rats (350–450 g, Charles River WIGA, 8741 Sulzfeld, FRG) were anesthetized with thiabutabarbital sodium (80–100 mg/kg ip). After tracheotomy and insertion of a cannula to maintain airway patency, a Millar Microtip manometer PR-249 was introduced into the left ventricle via the right carotid artery for recording left ventricular pressure (LVP). Arterial blood pressure (BP) was registered via a polypropylene catheter (PP 50) which had been inserted into the femoral artery and connected to a Statham Transducer P 23 Db.

Change in left ventricular pressure was determined by electronic differentiation of pressure signal with a physiodifferentiator and taken as an index of inotropic state. Heart rate (HR) was deduced from the LVP signal by a pulse counter. All parameters were continuously recorded by a universal amplifier 47/Varioscript V 8008. All test doses of compounds 1–**25** were injected in a volume of 0.5 mL/kg into the jugular vein as single bolus injection in increasing doses at an interval of 10 min. The hemodynamic parameters were always 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 injections.

2. **Anesthetized Cat Model.** Cats of both sexes were anesthetized with pentobarbitone sodium (45 mg/kg ip) and after tracheotomy respirated by a Starling pump with open air (10 mL/kg and 30 strokes/min). Anesthesia was continued by a maintenance infusion of pentobarbitone sodium at a dose of 0.1 mg/kg × min. After thoracotomy, LVP and HR were recorded by means of a Millar Tip manometer, PC-350, which had been introduced into the left ventricle via the auricle. BP and dP/dt_{50} were recorded as described in rats. The animals received dextran (MW 75000) at a dose of 10 mL/kg via a jugular vein to stabilize the hemodynamic situation. This was followed by an injection of desacetylmetipranolol (0.3 mg/kg iv) to induce cardiovascular failure by β-blockade.

The test compounds were intravenously injected at an interval of 10 min in increasing doses. Again the hemodynamic parameters obtained 10 min after the bolus injection were taken as results.

3. **Intravenous Administration to Conscious Dogs.** In mongrel dogs of both sexes, weighing 25–30 kg, a left-side thoracotomy was performed under aseptic conditions and general anesthesia (haloethane, N_2O, O_2). The dogs were implanted with polyethylene catheters to measure BP in the femoral artery, to guide a Millar Tip PC-350, and to inject the test compounds in the femoral vein. dP/dt_{60} was obtained as described before, but at a LVP of 60 mmHg. All parameters were continuously recorded on a universal amplifier 47/Varioscript 8008. The dogs were recruited for the experiments after a recovery period of 10 days at least. The animals received the test compounds at an interval of 10 min in increasing doses intravenous hemodynamic parameters were determined every 10 min after each drug administration.

4. **Oral Administration to Conscious Dogs.** The dogs were instrumented as described before and a Konigsberg manometer guided into the left ventricle. Each animal received 1 mg/kg 1, milrinone, and pimobendan by gavage in a suspension of 1% of methylcellulose or an equivalent volume of methylcellulose as placebo for control experiments. LV dP/dt_{60} and blood pressure were recorded for 6 h after drug administration. During the experiments, the dogs were kept in small cages permitting only limited movement. In addition, the dogs were largely shielded from environmental effects (noise, moving people, etc.).

Analysis of Structure–Activity Relationships in Renin Substrate Analogue Inhibitory Peptides†

Kwan Y. Hui,* William D. Carlson, Michael S. Bernatowicz, and Edgar Haber

Cardiac Unit, Massachusetts General Hospital, Boston, Massachusetts 02114. *Received October 31, 1986*

On the basis of the minimal octapeptide sequence of the renin substrate, a series of peptides was synthesized containing (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (statine) or (3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA) at the P1P1′ position. Some of these peptides also contained N^{in}-formyltryptophan at the P5, P3, or P3′ position. Renin-inhibitory potency varied over a wide range (from inactive to IC_{50} = 3 nM). Potency was reduced by at least 10-fold when the peptide was shortened by two residues at either the amino or carboxy terminus. The AHPPA-containing inhibitors were several-fold less potent than the statine-containing inhibitors. Analysis of models for the three-dimensional structure of inhibitors at the active site of human renin suggests that the diminished potency of the AHPPA peptides in comparison with the statine-containing peptides was caused by a shift in the peptide backbone due to a steric conflict between the phenyl ring of the AHPPA residue and the S1 subsite. The importance of the side chain and the 3(*S*)-hydroxyl group of the statine residue was demonstrated by substituting 5-aminovaleric acid for a dipeptide unit at the P1P1′ position, which resulted in a peptide devoid of renin-inhibitory activity. Substitutions of other basic amino acids for histidine at the P2 position caused a great loss in potency, possibly due to disruption of a hydrogen bond as suggested by molecular modeling. Studies on the plasma renins of four nonhuman species suggest that the isoleucine-histidine segment at the P2′P3′ position is important to defining the human specificity of the substrate. This work suggests a number of properties important to the design of potent renin inhibitors, and demonstrates the usefulness of three-dimensional models in the interpretation of structure–activity data.

RIP (Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys), a competitive renin inhibitory peptide based on the sequence of equine angiotensinogen, was developed in this laboratory several years ago.[1,2] This substrate analogue has been studied both in primates[1,2] and in humans.[3,4] Although it appears to be a specific renin inhibitor, it is of relatively low potency (K_i = 5 μM) and may exhibit a lack of selectivity for renin when studied in vivo at high doses.[3,4] Others subsequently reported the synthesis of more potent renin inhibitors containing the amino acid residue (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (sta-

† Address for reprints: Editor's Office, Cardiac Unit, Jackson 13, Massachusetts General Hospital, Boston, MA 02114.

(1) Burton, J.; Cody, R. J., Jr.; Herd, J. A.; Haber, E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5476.
(2) Cody, R. J.; Burton, J.; Evin, G.; Poulsen, K.; Herd, J. A.; Haber, E. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 230.
(3) Zusman, R. M.; Burton, J.; Christensen, D.; Nussberger, J.; Dodds, A.; Haber, E. *Trans. Am. Assoc. Physicians* **1983**, 365.
(4) Haber, E.; Zusman, R.; Burton, J.; Dzau, V. J.; Barger, A. C. *Hypertension* **1983**, *5* (*Suppl V*), V-8.

0022-2623/87/1830-1287$01.50/0