(66 μM dissolved in CH_3CO_2Na, 0.3 M), which completely inhibits platelet aggregation. The inhibiting action was expressed as percent inhibition by comparing the aggregation curve of the test compound with that of the control.

Antithrombotic Activity in Vivo. The determination was carried out by a modification of the method of Minno and Silver.[12] Male Swisse mice weighing 20–30 g were divided into three groups of 10. Groups 1 and 2 were treated with test compounds and the reference drug (ASA), respectively, both dissolved in 1% hydroxymethylcellulose and orally administered in a volume of 50 mL/kg. The dose of the test compound was equimolar to that of ASA (20 mg/kg). One hour after medication, groups 1 and 2, along with group 3 (controls), received a thrombotic mixture (fetal bovine collagen, 200 μg/mL, and adrenaline, 200 μM) in a volume of 10 mL/kg administered iv in the tail (before injection animals were warmed at 27 °C for 30 min). Death of the animals or paralysis of the hind limbs for more than 15 min was considered as a thrombotic effect. The antithrombotic activity was characterized as percent protection (%P) by relating the number of the thrombotic effects in group 1 (treated) to those of group 3 (controls), according to the formula $\%P = [(N_c - N_t)/N_c] \times 100$. The protection of the test compound was then compared to that of the reference drug (group 2).

Antiinflammatory Activity. The activity was determined in the carrageenin-induced edema of the rat paw by a modification of the method of Winter et al.[13] Male Sprague-Dawley rats weighing 130–150 g, 18 h fasted, were randomly divided into groups of eight. Each test compound was suspended in 1% carboxymethylcellulose and administered by gavage, with controls receiving the vehicle only. One hour after medication 0.1 mL of 1% carrageenin in normal sterile saline was injected into the plantar tissue of the right hind paw. Paw volume was measured by a plethysmometer at time intervals of 0, 1, and 3 h after induction of inflammation. Mean percentages of edema inhibition were calculated at the third hour, according to the formula % inhibition = $[(\Delta V_c - \Delta V_t)/\Delta V_c] \times 100$ where ΔV_c and ΔV_t were the increase in paw volume for control and treated animals, respectively.

Antiulcer Activity. Indomethacin Ulcer. The technique described by Lee et al.[14] was employed. Male Albino rats of Sprague-Dawley strain, weighing 180–250 g, in groups of eight, were housed in individual cages and fasted for 24 h having free access to water. The test compounds were administered by gavage immediately after indomethacin (15 mg/kg, ip), while the control group received distilled water only. Five hours later animals were sacrificed and their stomachs excised and opened along the greater curvature. The number of severity of lesions were observed with a 10 × wide-field binocular microscope and evaluted with the method proposed by Moron et al.[15]

Registry No. **2b**, 103422-53-3; **2c**, 103422-54-4; **2d**, 103602-83-1; **2e**, 103794-16-7; **2f**, 103794-15-6; **3**, 58161-35-6; **4**, 103602-84-2; **5**, 103422-85-1; **6**, 103422-62-4; **8**, 95355-15-0; 3-[*p*-(acetylamino)benzoyl]propanoic acid, 5473-15-4.

(12) Minno, G.; Sliver, N. J. *J. Pharm. Exp. Ther.* **1983**, *225*, 57.
(13) Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.
(14) Lee, Y. H.; Mollison, K. W.; Cheng, W. D. *Arch. Int. Pharmacodyn.* **1971**, *191*, 370.
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(16) Winegar, R.; Schreiber, W.; Hugo, R. *J. Pharm. Exp. Ther.* **1969**, *166*, 1.

Synthesis and Antihypertensive Activity of 4-(Cyclic amido)-2*H*-1-benzopyrans

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Received January 23, 1986

The synthesis and antihypertensive activity of a series of novel 4-(cyclic amido)-2*H*-1-benzopyran-3-ols, administered orally to conscious spontaneously hypertensive rats, are described. The effects of lactam ring size, the presence of heteroatoms in the lactam ring, substitution at C(2) and C(3), relative stereochemistry at C(3) and C(4), and aromatic substitution pattern on the blood pressure lowering activity of this series have been determined. The key compound **2** from this work [BRL 34915; (±)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol] has been resolved, and antihypertensive activity was found to reside primarily in the (-) enantiomer. The key step in the preparation of this class of compounds is the action of a cyclo amidic anion on an appropriate epoxide. Another approach, involving a cyclization step to the lactam was found to be more convenient in certain cases, particularly in forming the cis analogue of compound **2**. Compound **2** has been shown to possess a novel mechanism of action, and it has been selected for progression to the clinic.

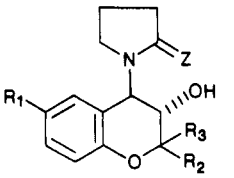
During the preparation of a series of substituted *trans*-4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ols and their evaluation as antihypertensive agents[1,2] it was discovered that introduction of a carbonyl group α to the C(4) nitrogen atom enhanced antihypertensive potency. This paper therefore describes the synthesis of a novel series of (cyclic amido)-2*H*-1-benzopyrans[3] and their effects on blood pressure in the spontaneously hypertensive rat (SHR). Included for comparison are *trans*-6-cyano-3,4-dihydro-2,2-dimethyl-4-pyrrolidin-1-yl-2*H*-1-benzopyran-3-ol (**1**; see Table I), the lead compound from the earlier work[1], and the calcium slow-channel blocker nifedipine. These (cyclic amido)benzopyranols have been shown to exert their antihypertensive action by a novel mechanism[4] in vascular smooth muscle involving the opening of potassium channels.

Chemistry. Convenient starting materials for the synthesis of the *trans*-4-(cyclic amido)-2*H*-1-benzopyran-3-ols shown in Tables I–III are the (±)-*trans*-3-bromo-3,4-dihydrobenzopyran-4-ols **52** or the corresponding (±)-epoxides **53** (Scheme I; only relative stereochemistry is shown). These compounds, with the exception of **8**, **9**,

(1) Evans, J. M.; Fake, C. S.; Hamilton, T. C.; Poyser, R. H.; Watts, E. A. *J. Med. Chem.* **1983**, *26*, 1582.
(2) Evans, J. M.; Fake, C. S.; Hamilton, T. C.; Poyser, R. H.; Showell, G. A. *J. Med. Chem.* **1984**, *27*, 1127.
(3) Ashwood, V. A.; Cassidy, F.; Evans, J. M.; Faruk, E. A.; Hamilton, T. C. Partly Presented at the VIIIth International Symposium on Medicinal Chemistry, Uppsala, Sweden, 1984; Dahlbom, R., Nilsson, J. L. G., Eds.; Swedish Pharmaceutical: Stockholm, 1984; Vol. 1, p 316.
(4) Hamilton, T. C.; Weir, S. W.; Weston, A. H. *Br. J. Pharmacol.* **1986**, *88*, 103.

0022-2623/86/1829-2194$01.50/0

Table I. 6-Substituted 3,4-Dihydro-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ols and Analogues



compd	R_1	R_2	R_3	Z	yield, %	mp, °C	solvent[a] of recryst	formula	anal.[b]	dose, mg/kg po	max fall[c] in bp, % ± SEM
1[d]	CN	Me	Me	H_2						0.1	8 ± 2
										0.3	21 ± 2
										1.0	29 ± 2
2	CN	Me	Me	O	56[e]	230–231	E	$C_{16}H_{18}N_2O_3$	C, H, N	0.1	13 ± 5
										0.3	39 ± 4
										1.0	47 ± 1
3[f]	CN	Me	Me	O	42	216–217	E	$C_{16}H_{18}N_2O_3$	C, H, N	1.0	23 ± 7
										10.0	46 ± 3
4[g]	CN	Me	Me	O	63	242–244	E	$C_{16}H_{18}N_2O_3$	C, H, N	0.1	31 ± 4
										0.3	47 ± 5
5[h]	CN	Me	Me	O	66	243–245	E	$C_{16}H_{18}N_2O_3$	C, H, N	3.0	14 ± 4
6	CN	Me	Me	S	22	175–177	B	$C_{16}H_{18}N_2O_2S$	C, H, N	0.1	16 ± 4
										0.3	39 ± 6
7	NO_2	Me	Me	O	31[e]	227–228	E	$C_{15}H_{18}N_2O_5$	C, H, N	0.3	27 ± 5
										1.0	63 ± 2
8	NO_2	Me[i]	H	O	9[j]	238–242	E	$C_{14}H_{16}N_2O_5$	C, H, N	10.0	34 ± 3
9	NO_2	H	H	O	16[e]	203–206	E	$C_{13}H_{14}N_2O_5$	C, H, N	10.0	5 ± 1
nifedipine										1.0	13 ± 4
										3.0	27 ± 1
										10.0	36 ± 3

[a] E = EtOAc; B = PhH. [b] Analyses for the elements indicated were within ±0.4% of the theoretical values. [c] Systolic blood pressure was measured indirectely at intervals from 1 to 6 h in groups of six or more SH rats per dose level. [d] See ref 2. [e] Method A. [f] Cis 3,4 isomer. [g] (−) enantiomer of compound 2. [h] (+) enantiomer of compound 2. [i] Stereochemistry unassigned. [j] Method B.

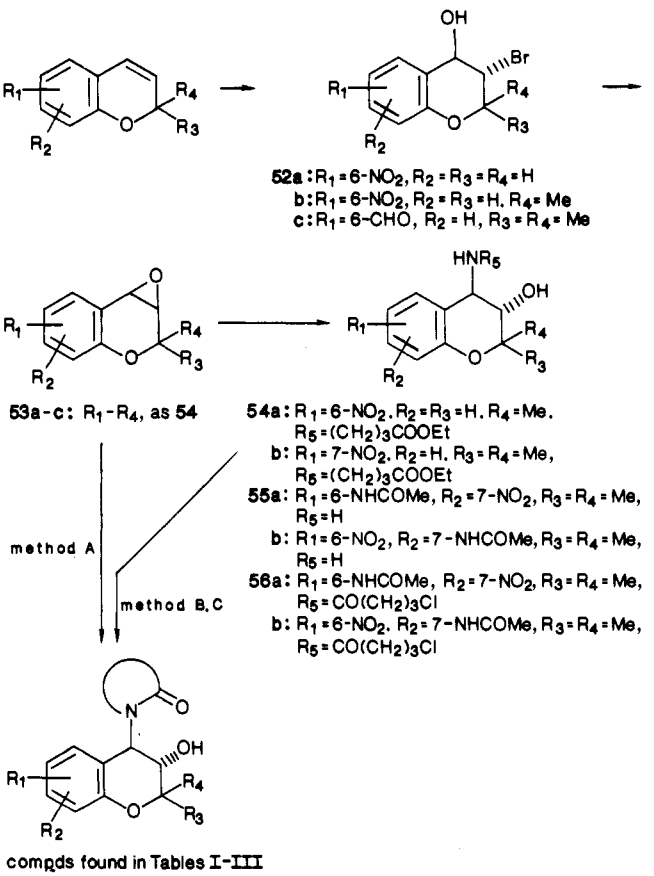
and **13**, have been described in the earlier work.[1,2] Compounds **8**, **9**, and **13** required 2-methyl-6-nitro-2*H*-1-benzopyran,[5] 6-nitro-2*H*-1-benzopyran,[6] and 6-formyl-2,2-dimethyl-2*H*-1-benzopyran,[7] respectively, as starting materials for the preparation of the appropriate bromohydrin or epoxide precursors.

Treatment of the bromohydrins **52** in a one-pot reaction, with 1 equiv of NaH in Me_2SO followed by 1 equiv of the appropriate cyclic amide and an additional 1 equiv of NaH, or treatment of the corresponding epoxides **53** with 1 equiv of the cyclic amide and 1 equiv of NaH (method A) furnished the majority of compounds in Tables I–III (Scheme I). Compounds **8** and **15** were prepared by thermal cyclization of the [(ethoxycarbonyl)propyl]amino derivatives, **54a** and **54b**, respectively (method B), obtained from the appropriate epoxides by treatment with ethyl 4-aminobutyrate. The 6,7-disubstituted compounds **17** and **19** were not conveniently prepared by method A; consequently, the corresponding epoxides were converted to the 4-amino compounds **55**, and these were treated with chlorobutyryl chloride to furnish compounds **56**, which were intramolecularly cyclized with NaH to give the desired compounds **17** and **19** (method C). Hydrolysis of the acetylamino compounds **17** and **19** provided the amino compounds **18** and **20**, respectively (Table II).

Thiolactam **6** (see Table I) was obtained by treating the 2-pyrrolidinone **2** with Lawesson's reagent,[8] while compounds **21** and **22** (Table II) were prepared from compound **2** by standard esterification procedures.

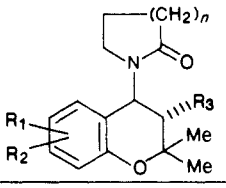
Compounds **29–43** (Table III) containing an additional heteroatom in the cyclic amide ring were prepared by method A using the appropriate epoxide and cyclic amide. Compound **33** required subsequent hydrolysis of compound **38**, and compound **37** was obtained by quaternization of compound **34** with MeI.

Scheme I



(5) Harfenist, M.; Thom, E. *J. Org. Chem.* **1972**, *37*, 841.
(6) Schweizer, E. E.; Liehr, J.; Monaco, D. J. *J. Org. Chem.* **1968**, *33*, 2416.
(7) Naidu, M. V.; Rao, G. S. K. *Synthesis* **1979**, 144.
(8) Pedersen, B. S.; Scheibye, S.; Clausen, K.; Lawesson, S.-O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 293.

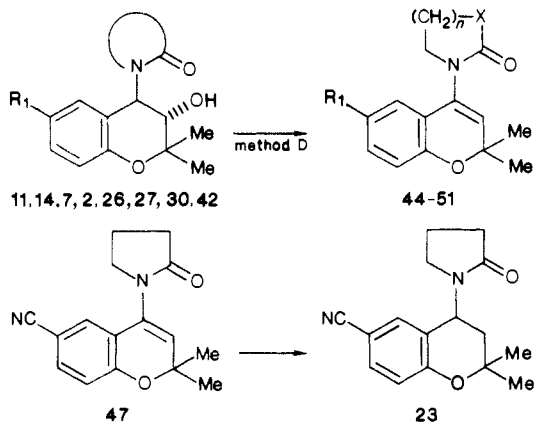
Table II. *trans*-4-(Cyclic amido)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans



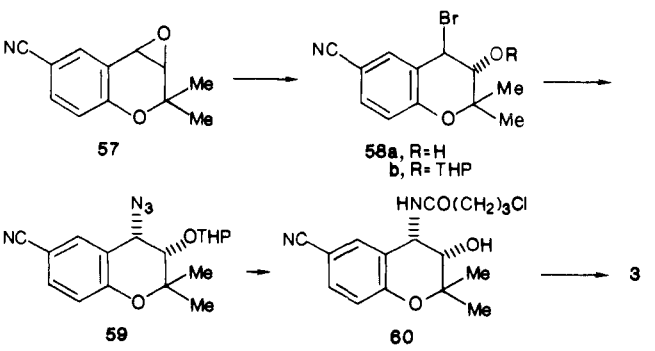
compd	R_1	R_2	n	R_3	yield, %	mp, °C	solvent[a] of recryst	formula	anal.[b]	dose, mg/kg po	max fall[c] in bp, % ± SEM
10	H	H	1	OH	30[d]	187–188	E-P	$C_{15}H_{19}NO_3$	C, H, N	10.0	9 ± 4
11	6-Cl	H	1	OH	15[d]	202–203	E	$C_{15}H_{18}NO_3Cl$	C, H, N, Cl	1.0	8 ± 3
										10.0	37 ± 7
12	6-COOMe	H	1	OH	5[d]	190–192	E-P	$C_{17}H_{21}NO_5$	C, H, N	0.3	11 ± 3
										1.0	20 ± 7
										10.0	44 ± 8
13	6-CHO	H	1	OH	12[d]	196–197.5	E	$C_{16}H_{19}NO_4$	C, H, N	10.0	25 ± 6
14	6-COMe	H	1	OH	46[d]	218–219	E	$C_{17}H_{21}NO_4$	C, H, N	0.3	33 ± 5
										1.0	56 ± 7
15	7-NO_2	H	1	OH	44[e]	211–212.5	E	$C_{15}H_{18}N_2O_5$	C, H, N	0.3	12 ± 3
										1.0	56 ± 7
										10.0	68 ± 2
16	8-NO_2	H	1	OH	6[d]	218–219	E	$C_{15}H_{18}N_2O_5$	C, H, N	1.0	7 ± 4
17	6-NO_2	7-NHCOMe	1	OH	87[f]	266–268	Et	$C_{17}H_{21}N_3O_6$	H, N; C[g]	0.1	37 ± 4
										0.3	62 ± 2
18	6-NO_2	7-NH_2	1	OH	20	310–313	Et	$C_{15}H_{19}N_3O_5$	*h*	0.1	51 ± 4
										0.3	68 ± 0
19	6-NHCOMe	7-NO_2	1	OH	53[f]	240–241	E	$C_{17}H_{21}N_3O_6$	C, H, N	1.0	3 ± 2
										3.0	14 ± 3
20	6-NH_2	7-NO_2	1	OH	39	244–245	E	$C_{15}H_{19}N_3O_5$	C, H; N[i]	1.0	25 ± 6
21	6-CN	H	1	OCOH	22	182–183	E-P	$C_{17}H_{18}N_2O_4$	C, H, N	0.3	31 ± 7
										1.0	36 ± 5
22	6-CN	H	1	OCOMe	19	152–153	E-P	$C_{18}H_{20}N_2O_4$	C, H, N	1.0	30 ± 9
										3.0	39 ± 5
23	6-CN	H	1	H	81	133–135	E-P	$C_{16}H_{18}N_2O_2$	C, H, N	1.0	15 ± 3
										3.0	45 ± 4
24	6-COOMe	H	2	OH	41[d]	249–250	E	$C_{18}H_{23}NO_5$	C, H, N	0.3	13 ± 12
										1.0	42 ± 0
25	6-COMe	H	2	OH	46[d]	225–226	E	$C_{18}H_{23}NO_4$	C, H; N[j]	0.3	29 ± 5
										1.0	66 ± 2
26	6-CN	H	2	OH	21[d]	198–199	E	$C_{17}H_{20}N_2O_3$	C, H, N	0.03	27 ± 8
										0.1	46 ± 7
										0.3	49 ± 4
										1.0	59 ± 5
27	6-CN	H	3	OH	38[d]	222–224	E	$C_{18}H_{22}N_2O_3$	C, H, N	1.0	10 ± 1
28	6-CN	H	4	OH	18[d]	130–133	E-P	$C_{19}H_{24}N_2O_3 \cdot H_2O$	C, H, N	10.0	11 ± 4

[a] E = EtOAc; Et = EtOH; P = pentane. [b,c] See footnotes *b* and *c* in Table I. [d] Method A. [e] Method B. [f] Method C. [g] C: calcd, 56.20; found, 55.52. [h] Consistent analyses could not be obtained. Mass ($C_{15}H_{19}N_3O_5 \cdot H_2O$) found *m/z* 303.1220, calcd 303.1219. [i] N: calcd, 13.07; found, 12.14. [j] N: calcd, 4.41; found, 3.92.

Scheme II

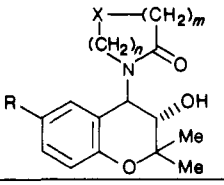


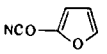
Scheme III



During preparation of certain of the *trans*-3,4-dihydro-4-(cyclic amido)benzopyran-3-ols described in Tables I–III, further reaction occurred, leading to formation of the corresponding 4-(cyclic amido)benzopyrans. For example, compounds **50** and **51** (see Table IV) were obtained as byproducts in the formation of compounds **30** and **42**, respectively. Alternatively, direct treatment of the *trans*-3,4-dihydro-4-(cyclic amido)benzopyran-3-ols with NaH in refluxing THF resulted in elimination of water (method D, Scheme II) to give the benzopyrans **44–49** (see Table IV). Hydrogenation of the 6-cyanobenzopyran **47**, followed by removal of unreacted material by reaction with NBS and chromatography, furnished the 4-(2-oxopyrrolidin-1-yl)-3,4-dihydrobenzopyran **23** (Table II) as depicted in Scheme II.

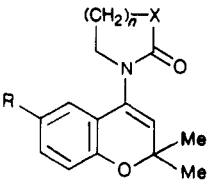
Table III. 6-Substituted *trans*-4-(Aza, Oxa, or Thia cyclic amido)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ols



compd	R	X	n	m	yield,[a] %	mp, °C	solvent[b] of recryst	formula	anal.[c]	dose, mg/kg, po	max fall[d] in bp, % ± SEM
29	CN	O	2	0	14	232–234	E	$C_{15}H_{16}N_2O_4$	C, H, N	0.3	20 ± 4
										1.0	29 ± 8
30	CN	S	2	0	5	239–240	E-P	$C_{25}H_{16}N_2O_3S$	C, H, N, S	0.3	27 ± 3
										1.0	30 ± 3
31	CN	O	2	1	16	249–250	E	$C_{16}H_{18}N_2O_4$	C, H, N	0.3	26 ± 3
										1.0	27 ± 6
32	CN	S	2	1	19	238–240	E	$C_{16}H_{18}N_2O_3S$	C, H, N, S	1.0	9 ± 3
										10.0	12 ± 4
33	CN	NH	2	1	35	207–210	E-P	$C_{16}H_{19}N_3O_3 \cdot 0.5H_2O$	C, H, N	0.1	28 ± 3
										0.3	53 ± 8
34	CN	NMe	2	1	35	196–197.5	E	$C_{17}H_{21}N_3O_3$	C, H, N	0.1	20 ± 2
										0.3	37 ± 3
35	CN	$N(CH_2)_3Me$	2	1	10	90–93	E-P	$C_{20}H_{27}N_3O_3 \cdot H_2O$	C, H, N	0.1	18 ± 3
										0.3	48 ± 4
										1.0	61 ± 3
36	CN	NCH_2Ph	2	1	23	178–179.5	E	$C_{23}H_{25}N_3O_3$	C, H, N	0.1	10 ± 2
										0.3	26 ± 5
										1.0	35 ± 3
37	CN	$N^+(Me)_2I^-$	2	1	55	252–254		$C_{18}H_{24}N_3O_3I$	C, H, N, I	1.0	9 ± 4
38	CN	NCOMe	2	1	19	230–231	E	$C_{18}H_{21}N_3O_4 \cdot 0.5H_2O$	C, H, N	1.0	17 ± 9
										3.0	23 ± 4
39	CN	NCOPh	2	1	13	250–251	E-P	$C_{23}H_{23}N_3O_4$	C, H, N	10.0	14 ± 7
40	COMe	NCOPh(*p*-F)	2	1	18	147.5–150	E	$C_{24}H_{25}N_2O_5F \cdot H_2O$	C, H, N	10.0	16 ± 2
41	CN		2	1	5	226–229	E-P	$C_{21}H_{21}N_3O_5 \cdot 0.5H_2O$	C, H, N	1.0	15 ± 3
										3.0	43 ± 4
42	CN	NMe	3	0	7	187–188.5	E-P	$C_{17}H_{21}N_3O_3$	C, H, N	1.0	23 ± 10
43	CN	O	0	2	5	128–129	E-P	$C_{15}H_{16}N_2O_4$	C, H, N	10.0	19 ± 5

[a] Method A. [b] E = EtOAc; P = pentane. [c,d] See footnotes *b* and *c* in Table I.

Table IV. 6-Substituted 4-(Cyclic amido)-2,2-dimethyl-2*H*-1-benzopyrans



compd	R	n	X	yield,[a] %	mp, °C	solvent[b] of recryst	formula	anal.[c]	dose, mg/kg po	max fall[d] in bp, % ± SEM
44	Cl	1	CH_2	20	123–125	Et-P	$C_{15}H_{16}NO_2Cl$	N, H; C[e]	10.0	36 ± 5
45	COMe	1	CH_2	<5	106–108	E-P	$C_{17}H_{19}NO_3 \cdot 0.3H_2O$	C, H, N	1.0	48 ± 6
46	NO_2	1	CH_2	<5	135–137	PLC	$C_{15}H_{16}N_2O_4$	C, H, N	1.0	68 ± 1
47	CN	1	CH_2	34	144–145	E	$C_{16}H_{16}N_2O_2$	C, H, N	0.1	12 ± 3
									0.3	47 ± 5
48	CN	2	CH_2	20	162–163	E	$C_{17}H_{18}N_2O_2$	C, H, N	0.3	25 ± 3
									1.0	55 ± 4
49	CN	3	CH_2	14	201–203	E	$C_{18}H_{20}N_2O_2$	C, H, N	1.0	15 ± 2
									10.0	36 ± 1
50	CN	1	S	<5	132.5–133.5	E-P	$C_{15}H_{14}N_2O_2S$	C, H, N, S	0.3	9 ± 1
									1.0	27 ± 6
51	CN	2	NMe	6	185–187	E-P	$C_{17}H_{19}N_3O_2$	C, H, N	1.0	29 ± 3

[a] Method D. [b] E = EtOAc; Et = Et_2O; P = pentane; PLC = preparative layer chromatography. [c,d] See footnotes *b* and *c*, respectively, in Table I. [e] C: calcd, 64.87; found, 64.42.

The preparation of *cis*-6-cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol (**3**) required an alternative synthesis (Scheme III) that employed 6-cyano-3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**57**) as the starting material. Treatment of the epoxide **57** with HBr gave the trans 4-bromo 3-ol **58a**, which, as the tetrahydropyranyl ether **58b**, was subjected to nucleophilic attack by azide ion to form a mixture of 4-azido compounds **59**. Hydrogenation, treatment with acid, and subsequent reaction with chlorobutyryl chloride yielded, after chromatography, the (4-chlorobutyryl)amino derivative **60**. Finally, intramolecular alkylation of compound **60** employing NaH as the base furnished the cis analogue **3**.

Compound 2 was resolved into its enantiomers 4 and 5 by reaction with (−)-α-methylbenzyl isocyanate. Fractional crystallization gave the two diastereomeric carbamates, which were hydrolyzed by treatment with trichlorosilane–triethylamine to yield the enantiomers 4 and 5.

Results and Discussion

Compounds were evaluated for oral antihypertensive activity in the SHR. Systolic blood pressure, recorded indirectly from the tail, was determined before dosing and at various time intervals during the ensuing 6 h. Maximum falls in blood pressure obtained for all the compounds (Tables I–IV) occurred at 1–4 h postdose, with some recovery to the predose level of blood pressure being observed at 6 h.

Initially the 2-pyrrolidinone 2 was compared with the pyrrolidine 1[1] and found to be more potent (Table I). Comparison of compound 2 with the cis analogue 3 showed that inversion of stereochemistry at C(4) resulted in a diminution of activity. The thioamide analogue 6 was equally as active as compound 2; however, in contrast, it was found[9] to have a shorter duration of action.

The importance of the *gem*-dimethyl group at C(2) was investigated in the nitro compounds 7–9 (Table I) in which the methyl groups present in compound 7 were successively removed. The reason for the attenuation of activity observed is not clear, as molecular modeling studies indicated very little difference in the spatial arrangement in the three compounds 7–9, so we speculate that the role of the methyl groups is to prevent metabolic degradation. In the monomethyl compound 8, the stereochemistry of the methyl group remains unassigned.

Increasing the cyclic amide ring size by one methylene unit to a 2-piperidinone moiety while maintaining the other molecular characteristics (compound 26, Table II) increased potency, particularly at the lower doses, compared with compound 2. This is in contrast to the earlier work[1] on cyclic amino compounds where pyrrolidine and piperidine substituents conferred approximately equal activity. The activity diminished if the cyclic amide ring was further increased in size (compounds 27 and 28).

In another group of compounds, the cyclic amido group was fixed as 2-pyrrolidinone or 2-piperidinone and the aromatic substitution pattern of the benzopyran structure was varied; the results (see Tables I and II) paralleled those observed[1,2] for the earlier series of 4-cyclic amino compounds. Thus, a powerful electron-withdrawing group such as cyano, nitro, or acetyl located at C(6), as in compounds 2, 26, 7, 14, and 25, or at C(7), as in compound 15, was a requisite for optimum activity. Compounds with other electron-withdrawing substituents such as methoxycarbonyl (12, 24) were less active, while those with chloro and formyl substituents (11 and 13, respectively) further diminished the activity. At the doses used, neither the unsubstituted compound 10 nor the C(8)-nitro compound 16 exhibited activity.

As in previous studies[2] on 4-(cyclic amino)benzopyran-3-ols, insertion of an acetylamino or amino group at C(7) adjacent to a 6-nitro group of the 4-(2-oxopyrrolidin-1-yl)benzopyran-3-ols (compounds 17 and 18, respectively) augmented the activity of the 6-nitro compound 7. Also, as before,[2] a decrease in the activity of the 7-nitro compound 15 was noted when an acetylamino group was located at C(6) (compound 19). However, in contrast to the previous work,[2] the amino group at C(6) in compound 20 decreased activity when compared with the 7-nitro compound 15 (Table II). The reason for the superior activity of the compounds containing the 7-(acetylamino)-6-nitro or 7-amino-6-nitro substituents over that possessed by the 6-nitro compound is not clear. Hydrogen bonding has been discounted[2] as a possible factor. Since it has been shown[10] that *o*-aminonitrobenzenes exhibit an enhanced electron density at the nitro group, we considered that this was a possible explanation for the enhanced activity seen with our compounds (17, 18). However, in this series, calculation of partial charges[11] for compounds 18 and 7 did not indicate any difference in electronic distribution at the 6-nitro group between these compounds.

It was previously shown[1] that introduction of an oxygen atom into compounds containing a C(4) cyclic amino moiety caused a decline in activity. This was also observed with the cyclic amido compounds when the activity of analogues 2 and 26 was compared with their counterparts 29 and 31, respectively (Table III). Moreover, incorporation of an oxygen atom α to the amide nitrogen atom as in the isoxazolidinone 43 reduced the activity even more markedly than compounds 29 and 31. Replacement of the ring oxygen atom in the oxazolidinone 29 by a sulfur atom as in the thiazolidinone 30 had little effect on activity, but a similar substitution in the 2-morpholinone 31 to give the thiamorpholinone 32 considerably reduced activity. The reason for this differential effect may lie in the size of the sulfur atom. Molecular modeling studies confirmed that when sulfur replaces methylene or oxygen in a five-membered ring, the volume occupied by such a ring approaches that of the 2-piperidinone ring. Thus, thiazolidinone 30 retains an effective ring size between 5 and 6, which is still optimum, whereas the thiamorpholinone 32 has an effective ring size above 6, which is beyond the optimum ring size for potent activity.

Heteroatom substitution in the cyclic amido moiety with a nitrogen atom incorporated β to the carbonyl group provided the most active compounds of this type. For example, the *N*-methyl-2-piperazinone 34 was more active than the *N*-methyltetrahydro-2-pyrimidinone 42. Other 2-piperazinones such as 33, 35, and 36 were also very active compounds. The high activity of the 2-piperazinones 33–36 may be due in part to the basic nature of the molecule as modification of this property, by quaternization (37) or amide formation (38–41), caused a reduction in potency.

Where comparisons can be made, the activities of the 4-(cyclic amido)-2*H*-1-benzopyrans presented in Table IV parallel those of the dihydrobenzopyranols (Tables I–III). Thus, the same conclusions can be drawn with regard to the nature of the aromatic substituent at C(6), as the activities of compounds 44–47 (Table IV) parallel compounds 11, 14, 7, and 2 (Tables I and II), respectively. A similar trend was also observed with the cyclic amide ring size when compounds 47–49 (Table IV) were compared with compounds 2, 26, and 27 (Tables I and II), respectively.

When the 3-hydroxyl group in the 2-pyrrolidinone 2 was esterified, as in compounds 21 and 22, activity declined slightly while the time course of action remained unaltered.[9] The effect on activity of removing the hydroxyl group at C(3) apparently depends on the structure of the product. Thus, the dihydrobenzopyran 23 was considerably less potent than compound 2, whereas the benzopyran 47 was similar in potency to compound 2.

Compound 2 has been resolved into its enantiomers 4 and 5, and the antihypertensive activity was found to reside mainly in the (−) enantiomer 4. Compound 2 is about 10 times more active than nifedipine (Table I) in the SHR

(9) Buckingham, R. E.; Hamilton, T. C., unpublished results.

(10) Politzer, P.; Abrahamsen, L.; Sjoberg, P. *J. Am. Chem. Soc.* 1984, *106*, 855.

and, like that drug, inhibits spontaneous contractions in rat isolated portal vein.[4] However, in direct contrast to nifedipine, compound **2** does not inhibit the contractile response due to a fully depolarizing (60 mM) concentration of K^+ ions.

Thus, compound **2** acts in a different manner to calcium slow-channel blockers such as nifedipine. Further investigations in rat isolated portal vein have shown that the compound **2** hyperpolarizes the cell membrane, with this action being achieved by the ability of this agent to open potassium channels and enhance the outward conductance of K^+ ions.[4] In conclusion, compound **2**, BRL 34915, possessing a novel chemical structure and mechanism of action for an antihypertensive agent, is currently undergoing clinical evaluation.

Experimental Section

Melting points were determined with a Büchi capillary melting point apparatus and are uncorrected. IR, NMR, and mass spectra, which were in agreement with the structures cited, were recorded on a Perkin-Elmer 197 or 599, a Varian EM 360A at 60 MHz, a Varian CFT-20 at 80 MHz, or a JEOL GX 270, and a VG 70-70 or 70 ZAB at 70 eV, respectively. Optical rotations were determined on a Perkin-Elmer 241. HF_{254} silica gel plates (2 mm) were used for chromatotron chromatography (radial chromatography).

Partial charges were calculated by the semiempirical CNDO method.[11] Molecular overlaps were carried out with a least-squares fitting program within the CHEMGRAF suite.[12]

***trans*-3-Bromo-3,4-dihydro-2*H*-1-benzopyran-4-ols (52).** Freshly recrystallized NBS (25 mmol) was added in one portion to a vigorously stirred solution of the appropriate 2*H*-1-benzopyran (12.5 mmol) in Me_2SO (35 mL) and H_2O (25 mmol). After the exothermic reaction, the solution was stirred for 40 min, poured into H_2O, and extracted with EtOAc. The organic phase was washed with H_2O, dried over anhydrous $MgSO_4$, filtered, and evaporated, leaving the crude bromohydrins. Recrystallization gave the following.

Compound 52a: 89%; mp 92–96 °C (60–80 °C petroleum ether); NMR ($CDCl_3$) δ 3.35–3.80 (m, OH), 4.15–4.80 (m, H-2 and H-3), 4.90 (d, 6, H-4), 6.95 (d, 9, H-8), 8.23 (m, 2H, aromatic). Anal. ($C_9H_8NO_4Br$) C, H, N, Br.

Compound 52b: 83%; mp 149 °C (60–80 °C petroleum ether). Anal. ($C_{10}H_{10}NO_4Br$) H, N, Br; C: calcd, 41.68; found, 40.97.

Compound 52c: 64%; mp 126–127 °C (EtOAc–pentane). Anal. ($C_{12}H_{13}O_3Br$) C, H; Br: calcd, 28.02; found, 29.08.

3,4-Epoxy-3,4-dihydro-2*H*-1-benzopyrans (53). *trans*-3-Bromo-3,4-dihydro-2*H*-1-benzopyran-4-ol (**52a,b**; 4 mmol) was stirred with NaOH (4.4 mmol) in dioxane (10 mL) and H_2O (8 mL) at room temperature for 3 h. Dilution of the reaction mixture with H_2O and extraction with Et_2O gave the crude epoxide. Compound **52c** (1.5 g, 5.3 mmol) and KOH (1.6 g, 30 mmol) were stirred in Et_2O (150 mL) at room temperature for 1 day. Filtration and evaporation of the solvent gave the crude epoxide. Recrystallization gave the following.

Compound 53a: 65%; mp 128 °C (60–80 °C petroleum ether); NMR ($CDCl_3$) δ 3.90 (d, 5, H-3), 4.05 (d, 5, H-4), 4.32 (d, 13, H-2), 4.73 (d, 13, H-2), 6.95 (d, 9, H-8), 8.14 (q, 9, 2, H-7), 8.31 (d, 2, H-5). Anal. ($C_9H_7NO_4$) C, H, N.

Compound 53b: 90%; mp 108–109 °C (60–80 °C petroleum ether). Anal. ($C_{10}H_9NO_4$) C, H, N.

Compound 54c: 49%; mp 108–109 °C (Et_2O–pentane). Anal. ($C_{12}H_{12}O_3$) C, H.

Method A. *trans*-4-(Cyclic amido)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ols. The requisite cyclic amide (0.1 mol) was treated with 80% NaH (0.1 mol) and the appropriate epoxide (0.1 mol) in Me_2SO (30 mL) with stirring under N_2 for 2–6 h. Alternatively, the corresponding 3-bromobenzopyran-4-ol (0.1 mol) in Me_2SO (30 mL) was treated with 80% NaH (0.1 mol) with stirring under N_2 for 0.5 h, and then the appropriate cyclic amide (0.1 mol) and 80% NaH (0.1 mol) were added and the solution was stirred for 2–6 h. The resulting solutions from both methods were cautiously diluted with H_2O and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over anhydrous $MgSO_4$. Filtration and evaporation of the solvent gave the crude 4-(cyclic amido)-2*H*-1-benzopyran-3-ols, which were purified after chromatography by recrystallization (see Tables I–III).

Compound 2: NMR ($CDCl_3$) δ 1.28 [s, 3 H, $C(Me)_2$], 1.55 [s, 3 H, $C(Me)_2$], 2.11 (m, NCH_2CH_2), 2.57 (m, $NCOCH_2$), 3.22 (br m, NCH_2 and OH), 3.64 (d, 10, H-3), 5.26 (d, 10, H-4), 6.87 (d, 9, H-8), 7.24 (narrow m, H-5), 7.45 (q, 9, 2, H-7); IR (KBr) 3260, 2220, 1652 cm^{-1}.

In certain instances, investigation of other chromatographic fractions or mother liquors from recrystallization revealed the presence of the corresponding 4-substituted 2,2-dimethyl-2*H*-1-benzopyrans as byproducts; for example, compounds **50** and **51** (see Table IV) were obtained in this manner.

Method B. *trans*-3,4-Dihydro-2,2-dimethyl-7-nitro-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol (15). 3,4-Epoxy-3,4-dihydro-2,2-dimethyl-7-nitro-2*H*-1-benzopyran (0.48 g, 2.2 mmol), ethyl 4-aminobutyrate hydrochloride (0.34 g, 2.6 mmol), and NaOH pellets (0.08 g, 2 mmol) were refluxed in EtOH (50 mL) for 12 h. The solution was cooled, filtered, and evaporated, and the residue was chromatographed (chromatotron, gradient elution pentane → EtOAc) to give recovered epoxide (0.2 g) and a crude amino ester fraction (0.21 g). This amino ester **54b** was boiled in xylene (30 mL) under N_2 for 3 days. Evaporation of solvent and chromatography, as above, furnished compound **15**, 40 mg (see Table II).

Compound **8** was prepared in a similar manner, from amino ester **54a**.

Method C. *trans*-6-(Acetylamino)-3,4-dihydro-2,2-dimethyl-7-nitro-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol (19). 6-(Acetylamino)-3,4-epoxy-3,4-dihydro-2,2-dimethyl-7-nitro-2*H*-1-benzopyran (1.0 g, 3.6 mmol) was dissolved in dry EtOH (150 mL) and saturated with dry NH_3 during 3 h with cooling. The reaction mixture was stirred for an additional 5 days at room temperature. The crude product obtained on evaporation of the solvent was purified on a chromatotron (80% EtOAc–pentane → 20% MeOH–EtOAc as a gradient elution) to give the 4-amino compound **55a** (0.41 g, 39%). A portion was converted to the HCl salt and recrystallized from EtOH–Et_2O: mp 258–261 °C. Anal. ($C_{13}H_{18}N_3O_5Cl$) C, H, Cl; N: calcd, 12.66; found, 12.19.

The corresponding 6-nitro-7-acetylamino compound **55b** was prepared in a similar manner from the appropriate epoxide **53** and used directly in the next reaction.

Compound **55a** (0.3 g, 1.02 mmol) and NaOH pellets (40 mg, 1 mmol) were stirred in $CHCl_3$ (5 mL) and H_2O (5 mL), and chlorobutyryl chloride (0.12 mL, 1.1 mmol) was added to the solution at room temperature. The layers were separated after 0.5 h of stirring, and the aqueous phase was extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with H_2O and brine, dried, and evaporated to give compound **56a** (0.31 g, 76%) as yellow crystals from EtOAc–pentane; mp 178–180 °C. Anal. ($C_{17}H_{22}N_3O_6Cl$) H, Cl; C, N: calcd, C, 51.07; N, 10.51; found, C, 50.51; N, 9.12.

The corresponding 6-nitro-7-acetylamino compound **56b** was prepared in a similar manner from compound **55b** and used directly in the next step.

Compound **56a** (250 mg, 0.63 mmol), anhydrous K_2CO_3 (2.0 g, 14.5 mmol), and KI (0.2 g, 1.2 mmol) in Me_2CO (60 mL) were stirred and heated under reflux in an atmosphere of N_2 for 18 h. The reaction mixture was cooled, filtered, and the crude residue purified on a chromatotron (EtOAc → 20% MeOH–EtOAc as a gradient elution) to give compound **19** (120 mg) as yellow crystals (see Table II). Compound **17** was prepared in an analogous manner (see Table II) from compound **56b**.

***trans*-6-Amino-3,4-dihydro-2,2-dimethyl-7-nitro-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol (20).** Compound **19** (65 mg, 0.2 mmol), 5 N HCl (2.5 mL), and EtOH (4.5 mL) were heated under reflux for 3 h. The reaction mixture was diluted with H_2O, basified with NaOH (10% aqueous), and extracted with EtOAc. Solvent removal and recrystallization of the crude product afforded compound **20** (20 mg) as brick red crystals (see Table II). Compound **18** was prepared in an analogous manner from

(11) Dobosh, P. A. *QCPE* **1968**, *No. 141*.
(12) Davies, E. K. *CHEMGRAF*; Chemical Crystallography Laboratory, Oxford University, developed and distributed by Chemical Design Ltd., Oxford.

compound 17: NMR ($CDCl_3$) δ 1.28 [s, 3 H, $C(Me)_2$], 1.47 [s, 3 H, $C(Me)_2$], 2.09 (m, NCH_2CH_2), 2.57 (q, 8, 7.5, $NCOCH_2$), 3.17 (irreg m, NCH_2), 3.73 (d, 10, H-3), 5.08 (d, 10, H-4), 6.27 (s, H-8), 7.67 (s, H-5).

Method D. 4-(Cyclic amido)-2,2-dimethyl-2*H*-1-benzopyrans. NaH (1.4 mmol, 80% dispersion in oil) was added to a solution of the corresponding dihydrobenzopyranol (1.4 mmol) in dry THF (12 mL) and the mixture stirred and refluxed for 16 h. The mixture was cooled, concentrated, treated cautiously with H_2O, and extracted with EtOAc to give the crude title compounds that were purified by recrystallization (see Table IV).

Compound 47: NMR ($CDCl_3$) δ 1.50 [s, 6 H, $C(Me)_2$], 2.22–2.58 (m, $NCOCH_2CH_2$), 3.61 (t, 7, NCH_2), 5.67 (s, H-3), 6.85 (d, 8, H-8), 7.19 (d, 2, H-5), 7.42 (q, 8, 2, H-7); IR (KBr) 2220, 1690 cm^{-1}.

***cis*-6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol (3).** To a stirred solution of 6-cyano-3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**57**; 20.1 g, 0.1 mol) in CCl_4 (500 mL) was added dropwise 45% HBr in HOAc (40 mL) at room temperature, and the solution was stirred for an additional 2 h. The CCl_4 solution was washed with H_2O and saturated $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. Filtration and evaporation of the solution gave a pale yellow solid (24.6 g, 88%), a small portion of which was recrystallized from EtOAc–pentane to give *trans*-4-bromo-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol (**58a**): mp 111–112 °C; NMR ($CDCl_3$) δ 1.23 [s, 3 H, $C(Me)_2$], 1.38 [s, 3 H, $C(Me)_2$], 2.83 (d, 4, exchangeable OH), 3.90 (q, 9, 4, collapsing to d, 9, with D_2O, H-3), 5.07 (d, 9, H-4), 6.78 (d, 9, H-8), 7.40 (q, 9, 2, H-7), 7.80 (d, 2, H-5). Anal. ($C_{12}H_{12}NO_2Br$) C, H, N.

The bromohydrin **58a** (12 g, 43 mmol), dihydropyran (25 mL, 0.27 mol), and concentrated HCl (0.2 mL) were stirred at room temperature for 2 days. The mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with H_2O and saturated $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. Filtration and evaporation of the solvent gave a gum that was triturated with pentane to give the crude tetrahydropyranyl ether **58b**, 14.25 g (95%).

The crude tetrahydropyranyl ether **58b** (14 g, 0.04 mol) was treated directly with NaN_3 (4 g, 0.06 mol) in DMF (50 mL) for 24 h at room temperature. The solution was diluted with H_2O and extracted with EtOAc. Evaporation of the solvent gave a gum that was triturated with pentane to give the crude 4-azide **59**: 12.2 g (98%); IR (film) 2100, 2225 cm^{-1}.

The azide **59** (7.5 g, 23 mmol) and 5% Pd/C (0.5 g) in EtOH (150 mL) were shaken in an atmosphere of H_2 for 25 h. Filtration and evaporation of the solvent gave a yellow foam (6.29 g), which was used directly in the next stage. This crude aminotetrahydropyranyl ether (5.91 g, 19.5 mmol) and Et_3N (2.8 mL, 19.5 mmol) were stirred in CH_2Cl_2 (60 mL) at 0 °C. Chlorobutyryl chloride (2.25 mL, 19.5 mmol) was added dropwise to the stirred solution at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. The organic phase was washed with H_2O and evaporated, and the resulting oil was treated with concentrated HCl (2 mL) and H_2O (2 mL) in EtOH (25 mL) during 2 h. The solution was diluted with H_2O and extracted with EtOAc to give a gum (4.50 g) that was chromatographed on silica gel. Chromatographically homogeneous fractions (1.08 g, 17%) were combined, and a portion was recrystallized from EtOAc to give *cis*-4-[(4-chlorobutyryl)amino]-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol (**60**): mp 172–173 °C. Anal. ($C_{16}H_{19}N_2O_3Cl$) C, H, N, Cl.

The (chlorobutyryl)amino compound **60** (484 mg, 1.5 mmol) was dissolved in dry THF (25 mL) and the resultant mixture treated with 80% NaH (48 mg, 1.6 mmol) under N_2. The solution was stirred for 4 h at room temperature and then treated with the cautious addition of H_2O (200 mL). Extraction with EtOAc gave a cream-colored solid (340 mg), which was recrystallized from EtOAc to give compound **3**: 173 mg (see Table I); NMR ($CDCl_3$) δ 1.36 [s, 3 H, $C(Me)_2$], 1.49 [s, 3 H, $C(Me)_2$], 2.12 (m, NCH_2CH_2), 2.54 (m, NCH_2), 2.79 (m, exchangeable OH), 3.34 (m, 1 H, $NCOCH_2$), 3.74 (m, 1 H, $NCOCH_2$), 3.88 (q, collapsing to d, 3.5, on addition of D_2O, H-3), 5.41 (d, 3.5, H-4), 6.94 (d, 9, H-8), 7.24 (narrow m, H-5), 7.48 (m, one coupling 9 discernible, H-7); IR (KBr) 2235, 1680 cm^{-1}.

***trans*-6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-thioxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol (6).** A solution of compound **2** (0.5 g, 1.7 mmol) and Lawesson's reagent[8] (354 mg, 0.9 mmol) in dry PhMe (100 mL) was refluxed under N_2 for 1 h. Removal of solvent gave a yellow foam that was chromatographed on silica gel (40 g). Elution with $CHCl_3$ and recrystallization gave the title compound (see Table I): NMR ($CDCl_3$) δ 1.36 [s, 3 H, $C(Me)_2$], 1.56 [s, 3 H, $C(Me)_2$], 2.17 (m, NCH_2CH_2), 2.68 (br m, OH), 3.07–3.73 (m, CH_2NCSCH_2), 3.90 (d, 10, H-3), 6.47 (d, 10, H-4), 6.95 (d, 8, H-8), 7.24 (narrow m, H-5), 7.53 (irreg q, 8, 2, H-7); IR (KBr) 3300, 2220, 1160cm^{-1}.

Resolution of *trans*-6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol (2). A solution of compound **2** (4.75 g, 16.6 mmol) and (−)-α-methylbenzyl isocyanate (2.84 g, 19.3 mmol) in dry PhMe (160 mL) was refluxed for 43 h and evaporated, leaving a gum. Fractional crystallization from EtOAc–pentane afforded the carbamate of the (−) enantiomer of the title compound: 1.50 g (21%); $[\alpha]^{26}_D$ (Me_2CO, *c* 1) −14.2°. Similarly, crystallization of the oil derived from the mother liquor gave the carbamate of the (+) enantiomer of the title compound: 1.00 g (14%); $[\alpha]^{26}_D$ (Me_2CO, *c* 1) −77.1°.

The foregoing carbamates (2 mmol) were hydrolyzed to the parent alcohols by treatment with Et_3N (4 mmol) and $SiCl_3H$ (4 mmol) in PhMe at 35–40 °C for 16 h. The crude products were purified by chromatography on silica gel. Elution with EtOAc gave the pure (−) enantiomer **4** [$[\alpha]^{26}_D$ ($CHCl_3$, *c* 1) −52.2°] and similarly the pure (+) enantiomer **5** [$[\alpha]^{26}_D$ ($CHCl_3$, *c* 1) +53.5°] (see Table I).

***trans*-6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-yl Acetate (22).** Compound **2** (0.5 g, 1.7 mmol), Ac_2O (10 mL, 0.11 mol), and pyridine (0.2 mL, 2.4 mmol) were refluxed for 24 h. The mixture was cooled and poured onto ice, and the resulting solution was extracted with EtOAc. The organic extract was washed with H_2O and aqueous $NaHCO_3$ and dried. Filtration and evaporation of the solvent and chromatography (chromatotron; elution with EtOAc) gave a solid (0.35 g) that was recrystallized to give compound **22** (see Table II).

Compound **21** was prepared in an analogous manner, from compound **2**, by refluxing in formic acid (see Table II).

6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran (23). Compound **47** (0.83 g, 3.1 mmol) and 10% Pd/C (80 mg) in EtOAc (30 mL) were shaken in an atmosphere of H_2 for 32 h. Filtration and evaporation gave a mixture (0.80 g) that was treated with NBS (0.7 g, 3.9 mmol) and H_2O (0.5 mL, 0.03 mol) in Me_2SO (30 mL) during 0.5 h at room temperature. Addition of H_2O and extraction with EtOAc gave a gum that was chromatographed on silica gel. The most polar fractions were combined and recrystallized to give compound **23** (see Table II): NMR ($CDCl_3$) δ 1.36 [s, 3 H, $C(Me)_2$], 1.49 [s, 3 H, $C(Me)_2$], 1.93 (d, 9, 2, H-3), 2.12 (m, NCH_2CH_2), 2.49 (m, NCH_2), 3.15 (m, $COCH_2$), 5.53 (t, 9, H-4), 6.87 (d, 8, H-8), 7.28 (irreg narrow m, H-5), 7.45 (m, H-7); IR (KBr) 2220, 1678 cm^{-1}.

***trans*-6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopiperazin-1-yl)-2*H*-1-benzopyran-3-ol (33).** Compound **38** (1.50 g, 44 mmol) was heated under reflux in EtOH (90 mL) containing 5 N HCl (50 mL) for 2 h. The solution was cooled, basified with 10% aqueous NaOH, diluted with H_2O (50 mL), and extracted with EtOAc to give the title compound, which was purified by recrystallization (see Table III).

***trans*-6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopiperazin-1-yl)-2*H*-1-benzopyran-3-ol Methiodide (37).** A solution of compound **34** (0.10 g, 0.32 mmol) and MeI (0.5 mL, 1.5 mmol) in EtOH (10 mL) was stirred at room temperature in the dark for 16 h. The precipitate of compound **37** (0.08 g) that formed was separated from the solution, by decanting the solvent, and was washed with Et_2O (see Table III).

Pharmacological Testing. Hypertensive Rats. All of the test compounds, and the standard drug, were evaluated for antihypertensive activity in conscious spontaneously hypertensive rats (14–24 weeks old), derived from the Japanese (Okamoto) strain. Animals with systolic blood pressure >180 mmHg (1 mmHg ≈ 133 Pa) were considered to be hypertensive.

Systolic blood pressure was recorded by the tail cuff method using a W+W bp recorder, Model No. 8005; each determination was the mean of at least six recordings. Blood pressure measurements were made prior to the oral administration of test compound and at intervals for up to 6 h postdose.

All compounds were administered (via an oral dosing needle placed in the esophagus) as a solution or suspension in 1% w/v methylcellulose solution.

With use of the above procedure, vehicle alone typically has little or no effect on blood pressure apart from a slight reduction (by 5–10%) at 6 h postdose.

Acknowledgment. We thank David Bragg, Helen Costema, Katharine Fish, and Graham Moore for skilled technical assistance.

Registry No. (±)-**1**, 75611-78-8; (±)-**2**, 94470-67-4; (±)-**3**, 103774-92-1; (±)-4, 94535-50-9; (−)-4 (carbamate), 94470-68-5; (±)-**5**, 94535-51-0; (+)-**5** (carbamate), 94470-69-6; (±)-**6**, 103732-19-0; (±)-**7**, 103732-20-3; (±)-**8** (isomer 1), 103751-07-1; (±)-**8** (isomer 2), 103732-70-3; (±)-**9**, 103732-21-4; (±)-**10**, 103732-22-5; (±)-**11**, 103732-23-6; (±)-**12**, 103732-24-7; (±)-**13**, 103732-25-8; (±)-**14**, 103732-26-9; (±)-**15**, 103732-27-0; (±)-**16**, 103732-28-1; (±)-**17**, 103732-29-2; (±)-**18**, 103732-30-5; (±)-**19**, 103732-31-6; (±)-**20**, 103732-32-7; (±)-**21**, 103751-08-2; (±)-**22**, 103732-33-8; (±)-**23**, 103732-34-9; (±)-**24**, 103732-35-0; (±)-**25**, 103732-36-1; (±)-**26**, 103732-37-2; (±)-**27**, 103732-38-3; (±)-**28**, 103732-39-4; (±)-**29**, 103732-40-7; (±)-**30**, 103732-41-8; (±)-**31**, 103732-42-9; (±)-**32**, 103732-43-0; (±)-**33**, 103732-44-1; (±)-**34**, 103732-45-2; (±)-**35**, 103732-46-3; (±)-**36**, 103732-47-4; (±)-**37**, 103732-48-5; (±)-**38**, 103732-49-6; (±)-**39**, 103732-50-9; (±)-**40**, 103732-51-0; (±)-41, 103732-52-1; (±)-**42**, 103732-53-2; (±)-43, 103732-54-3; **44**, 89080-74-0; **45**, 89080-75-1; **46**, 89080-73-9; **47**, 89080-71-7; **48**, 89080-72-8; **49**, 103732-55-4; **50**, 91236-94-1; **51**, 91224-99-6; (±)-**52a**, 103732-57-6; (±)-**52b** (isomer 1), 103732-56-5; (±)-**52b** (isomer 2), 103732-72-5; (±)-**52c**, 103732-59-8; (±)-**53a** (isomer 1), 103774-93-2; (±)-**53a** (isomer 2), 103774-95-4; (±)-**53b**, 103732-60-1; (±)-**53c**, 103732-65-6, (±)-**54b**, 103732-62-3; **54c**, 103732-61-2; (±)-**55a**, 103732-64-5; (±)-**55a** (epoxide), 103732-63-4; (±)-**55b**, 103732-66-7; (±)-**56a**, 103751-09-3; (±)-**56b**, 103751-10-6; (±)-**57**, 75611-72-2; (±)-**58a**, 103732-67-8; (±)-**58b**, 103774-94-3; (±)-**59**, 103732-68-9; (±)-**59** (amine), 103732-70-3; (±)-**60**, 103732-69-0; $H_2N(CH_2)_3C-O_2CH_2CH_3$, 5959-36-4; $Cl(CH_2)_3COCl$, 4635-59-0; 6-nitro-2*H*-1-benzopyran, 16336-26-8; 2-methyl-6-nitro-2*H*-1-benzopyran, 103732-58-7; 6-formyl-2,2-dimethyl-2*H*-1-benzopyran, 69964-40-5.

Phenethyl Ester Derivative Analogues of the C-Terminal Tetrapeptide of Gastrin as Potent Gastrin Antagonists

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A series of phenethyl ester derivative analogues of the C-terminal tetrapeptide of gastrin, in which the phenylalanyl residue has been replaced by a phenethyl group and the peptide bond between aspartic acid and phenylalanine by an ester bond, were synthesized. None of these derivatives were able to stimulate gastric acid secretion in the anesthetized rat, whereas they inhibited gastrin-induced acid secretion with ED_{50} values between 0.02 and 1.5 mg/kg. Among these derivatives, Boc-βAla-Trp-Leu-Asp phenethyl ester (**9**) and Boc-βAla-Trp-Leu-Asp *p*-fluorophenethyl ester (**16**) were very potent in inhibiting gastrin-induced acid secretion. From these studies, the significant role of the C-terminal dipeptide of gastrin was pointed out. More particularly, the functional role of the phenylalanine through the C-terminal carboxamide and its binding role through its aromatic ring were demonstrated.

The search for active in vivo gastrin antagonists is still an interesting challenge that greatly depends on a good knowledge of the mechanism of action of gastrin. Structure–activity relationships were studied by Morley[1] on an unprecedented scale and showed that all the diverse biological activities of gastrin were found to be closely related to the C-terminal tetrapeptide amide portion of the molecule of sequence Trp-Met-Asp-Phe-NH_2.[2] Owing to the significance of tryptophan and phenylalanine residues, many analogues of the type Trp-Met-X-Phe-NH_2 were prepared, but they were devoid of antisecretory activity. Attempts to apply the "Robard's multi-subsite receptor model"[3] to the concept of gastrin antagonists was also unsuccessful.[4] Previous studies dealing with the significance of the phenylalanyl residue in C-terminal gastrin and cholecystokinin related peptides[5a] and our recent results[5b] allowed us to propose a functional rather than a binding role for this residue. C-Terminal gastrin related peptides lacking the phenylalanyl residue bind to the gastrin receptor while they are devoid of biological activity and are able to inhibit gastrin-induced acid secretion.[6] However, the loss of the phenylalanyl residue resulted in a significant decrease in the affinity of the peptide for the gastrin receptor. Recently we showed the importance of the peptide bonds of the C-terminal tetrapeptide of gastrin for eliciting biological activity, particularly of the bond between methionine and aspartic acid.[7] The pseudopeptide Boc-Trp-Leu-ψ(CH_2NH)-Asp-Phe-NH_2 analogue of the C-terminal tetrapeptide of gastrin, in which the peptide bond between leucine and aspartic acid had been replaced by a CH_2NH bond, was able to bind to the gastrin receptor with the same affinity as the tetrapeptide, but was devoid of biological activity. In fact, this pseudopeptide inhibited gastrin-induced acid secretion with ED_{50} value of 0.3 mg/kg,[7] whereas the pseudopeptide Boc-Trp-ψ-(CH_2NH)-Leu-Asp-Phe-NH_2, in which the peptide bond between tryptophan and leucine has been replaced by a CH_2NH bond, behaved as a complete agonist as potent as the tetrapeptide of natural sequence. From these results, we postulated that, for exhibiting agonist activity on acid secretion, the bond between methionine (or leucine) and

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