Synthesis and Biological Activity of 4-(Diphenylmethyl)-α-[(4-quinolinyloxy)methyl]-1-piperazineethanol and Related Compounds

Ila Sircar,*,† Steve J. Haleen,‡ and Sandra E. Burke‡

Departments of Chemistry and Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

Hubert Barth

Department of Chemistry, Goedecke A. G. Postfach 569, Mooswaldalle 1-9, 7800 Freiburg i/Br, Germany

Received June 22, 1992

A series of 4-(diphenylmethyl)-α-[(4-quinolinyloxy)methyl]-1-piperazineethanol and closely related compounds was synthesized and evaluated for cardiac and vascular activity in isolated perfused rat and guinea pig hearts. Compound 1 produced greater inotropic effects in rat hearts than in guinea pig hearts, a phenomenon which was also observed with the prototype agent DPI 201-106. Compound 15 produced an inotropic effect with one-tenth the potency of compound 1. Both compounds 1 and 15 demonstrated direct inotropic and vasodilatory effects when administered iv in anesthetized dogs, although the vasodilatory activity was more pronounced with compound 15 than 1 and DPI compound. Compound 1 lacks the CN moiety which is a key structural requirement in DPI for positive inotropic activity. The synthesis, in vitro, and in vivo evaluations of these agents, and comparative data with DPI-201-106 (compound 17) are reported.

DPI 201-106, a piperazinylindole derivative, is a unique nonsympathomimetic and nonglycoside positive inotropic agent developed for the treatment of congestive heart failure (CHF).[1-8] This agent appears to possess a complicated mechanism of action such as (a) an increase in the intracellular Na^+ load and in the sensitivity of the contractile proteins of cardiac muscle for Ca^{2+} for the positive inotropy[9,10] and (b) calcium channel blockade in vascular smooth muscle for vasodilation.[11] Several in vitro and in vivo experiments have characterized DPI 201-106 as a substance with a positive inotropic action combined with a negative chronotropic, action-potential prolonging and coronary dilatory activities sharing common properties with cardiotonics, antiarrhythmics of class III, and antianginals.[12-14] These combinations of activity in a single entity resulted in a favorable therapeutic profile. For example, administration of DPI 201-106 produced dose-dependent improvements in left ventricular function in patients with severe congestive heart failure.[1,7,10] However, these negative chronotropic, dromotropic, and distinct vasodilator effects were manifested at relatively high doses compared to the positive inotropic effect, indicating that DPI is a highly selective positive inotropic agent. Very little is known to date on the structure–activity relationships (SAR) of DPI 201-106[15-17] (Chart I). Our work was directed toward the identification of agents with greater vascular selectivity than DPI compound. Herein, we report the synthesis and structure–activity relationships of several aryl ring modified compounds related to DPI (compound 17) and in depth in vitro and in vivo evaluation of two compounds 1 and 15 (Chart I).

† Department of Chemistry.
‡ Department of Pharmacology.

(1) Kostis, J. B.; Lacy, C. F.; Raia, J. J.; Dworkin, J. H.; Warner, R. G.; Casazza, L. A. DPI 201-106 for Severe Congestive Heart Failure. *Am. J. Cardiol.* **1987**, *60*, 1334-1339.

(2) Kostis, J. B.; Lacy, C. R.; Warner, R. G.; Casazza, L. A.; Raia, J. J. DPI 201-106, a novel inotropic agent: Hemodynamic Improvement in Patients with Congestive Heart Failure. *Acta Pharmacol. Toxicol.* **1986**, *59* (suppl. 5, Pt. 2), 183.

(3) Scholtysik, G.; Salzmann, R.; Berthold, R.; Herzig, J. W.; Quast, U.; Markstein, R. DPI 201-106, A Novel Cardioactive Agent. Combination of cAMP-Independent Positive Inotropic, Negative Chronotropic, Action Potential Prolonging and Coronary Dilatory Properties. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1985**, *329*, 316-325.

(4) Butrous, G. S.; Debbas, N. M. G.; Erwin, J.; Davies, D. W.; Keller, H. P.; Lunnon, M. W.; Nathan, A. W.; Camm, A. J. Clinical cardiac electrophysiologic evaluation of the positive inotropic agent, DPI 201-106. *Eur. Heart. J.* **1988**, *9*, 489-497.

(5) Ruegg, P. C.; Nuesch, E. The effect of a new inotropic agent, DPI 201-106, on systolic time intervals and the electrocardiogram in healthy subjects. *Br. J. Clin. Pharmacol.* **1987**, *24* (4), 453-458.

(6) Uretsky, B. F.; Murali, S.; Reddy, P. S.; Valdes, A. M.; Kolesar, J. A. Hemodynamic and Electrocardiographic Effects of the Fast Channel Activator, DPI 201-106. *J. Am. Coll. Cardiol.* **1987**, *9* (No. 2, Suppl. A), 161A.

(7) Kostis, J. B.; Lacy, C. R.; Warner, R. G.; Dworkin, J. H.; Casazza, L. A.; Raia, J. J. DPI 201-106, A New Inotropic Agent: Hemodynamic Improvement in Patients with Congestive Heart Failure. *J. Am. Coll. Cardiol.* **1987**, *9* (No. 2, Suppl. A), 162A.

(8) Linderer, T.; Heineking, M.; Hadad, E.; Schroeder, R. Hemodynamic Effects of DPI, A Novel Cardiotonic Drug with Negative Chronotropic Action. *J. Am. Coll. Cardiol.* **1987**, *9* (No. 2, Suppl. A), 162A.

(9) Boehm, M.; Diet, F.; Kemkes, B.; Waankerl, M.; Erdmann, E. Positive Inotropic Effect of DPI 201-106 in the Failing Human Heart. *Circulation* **1988**, *78* (No. 4, Pt. 2), 348.

(10) Cai, Y. D.; Lee, N. K. M.; Blinks, J. R. Effects of DPI 201-106 on Action Potentials, Ca^{++} Transients and Contractions of Mammalian Cardiac Muscle. *Pharmacologists* **1988**, *30* (no. 3), A41.

(11) Takahashi, K.; Endoh, M.; Taira, N. Inotropic Versus Chronotropic, Dromotropic, and Coronary Vasodilator Actions of DPI 201-106, a Novel Positive Inotropic Agent, in the Dog Heart. *J. Cardiovasc. Pharamacol.* **1987**, *9*, 451-460.

(12) Mortensen, E.; Tande, P. M.; Klow, N. E.; Platou, E. S.; Refsum, H. Positive Inotropy Linked with Class III Antiarrhythmic Action; Electrophysiological Effects of the Cardiotonic Agent DPI 201-106 in the Dog Heart In Vivo. *Cardiovasc. Res.* **1990**, *24* (No. 11), 911-917.

(13) Hof, R. P.; Hof, A. Mechanism of the Vasodilator Effects of the Cardiotonic Agent DPI 201-106. *J. Cardiovasc. Pharmacol.* **1985**, *7*, 1188-1192.

(14) Salzmann, R.; Scholtysik, G.; Clark, B.; Berthold, R. Cardiovascular Actions of DPI 201-106, a Novel Cardiotonic agent. *J. Cardiovasc. Pharmacol.* **1986**, *8*, 1035-1043.

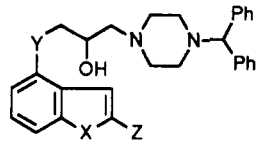
(15) Armah, B.; Pfeifer, T.; Ravens, U. Reversal of the Cardiotonic and Action-Potential Prolonging Effects of DPI 201-106 by BDF 8784, a methyl-indol derivative. *Br. J. Pharmacol.* **1989**, *96*, 807-816.

(16) Scholtysik, G. J. Cardiac Na^+ Channel Activation as a Positive Inotropic Principle. *J. Cardiovasc. Pharmacol.* **1989**, *14* (Suppl. 3), S24-29.

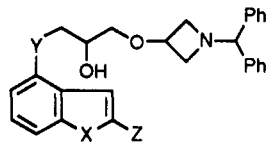
(17) Brasch, H.; Iven, H. Inotropic and Electrophysiological Effects of BDF 9148, a Congener of DPI 201-106, in Guinea-Pig Atria and Papillary Muscles. *Br. J. Pharmacol.* **1991**, *103*, 1939-1945.

0022-2623/92/1835-4442$03.00/0

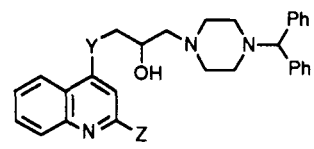
Chart I



17 (DPI 201-106): X = NH; Y = O; Z = CN
18 (BDF 8784): X = NH; Y = O; Z = CH_3
19 (SDZ 210921): X = NH; Y = NH; Z = CN
15: X = O; Y = O; Z = CN



20 (BDF 9148): X = NH; Y = O; Z = CN



1: Y = O; Z = H

Chemistry

The (aryloxy)propylamines (Tables I and II) were synthesized from the corresponding phenols[18-20] as shown in Scheme I. Method A involved the reaction of phenols with epichlorohydrin in the presence of NaH to provide the (aryloxy)propyl chloride compounds which were treated with the requisite amines to give the target compounds. Alternatively, the amines were reacted with epibromohydrin in the presence of K_2CO_3 to provide the epoxyalkylamine derivatives which were subsequently treated with the requisite phenols to provide the target compounds (method B). Method B is preferred for 4-hydroxyquinolines in which case method A gave a mixture of epoxy compounds. Scheme II outlines the synthetic routes for phenols in the isoquinoline series. (Benzyloxy)cinnamic acid was converted to the corresponding azide in two steps via treatment with $SOCl_2$ and NaN_3, respectively. The azide was rearranged and cyclized to give the 1-hydroxyisoquinoline derivative (22). This, in turn, was converted to the desired phenol (23) by catalytic reduction. Compound 22 was also converted to 5-hydroxy-1-(phenylthio)isoquinoline (24) in three steps.

Biological Results and Discussions

In Vitro Studies. Brief descriptions for all the protocols are provided in the Experimental Section.

Cardiovascular Isolated Heart Studies (CVIH). The compounds were evaluated in the isolated perfused rat and guinea pig hearts for inotropic, chronotropic, and vasodilator activities.[21] Compound 17 was also evaluated in these models for comparison. As seen in Table III, in the six-membered bicyclic series, the inotropic activity resides only in the quinoline compounds and very strict structure–activity relationships were observed even within that series. Compound 1 in which the (aminoalkyl)oxy chain resides at the 4-position was the most potent in this series. In the rat heart 1 increased LV + dP/dt_{max} by 25% (EC_{25}) at 100 nM with no change in heart rate (HR) and coronary flow (CF). Moving the side chain around the ring resulted in a (i) decrease in the inotropic activity and (ii) increase in the coronary flow activity. For example, compounds 2 and 5 were weakly active (LV + dP/dt_{max}; EC_{25} = 480 nM and 570 nM, respectively), whereas compound 6 was inactive. Weak CF activity was observed with 5 and 6. An alkyl or haloalkyl substitution in the ring abolished the positive inotropic activity (see compounds 4 and 7). Similar structure–activity relationships were observed with 17 and its 2-methyl analogue 18.[15] Replacement of the indole ring of 17 with furan produced compound 15, which retained modest activity (EC_{25} = 100 nM). The increase in LV + dP/dt_{max} with 15 was associated with a concomitant increase in CF with no change in HR. By contrast, compound 17 was very potent in increasing LV + dP/dt (EC_{25} = 20 nM) but relatively weak at increasing CF (EC_{25} = 300 nM). It is important to note that both in the indole as well as in the furan series the cyano group was critical for inotropic activity.[15,17] Compound 14, the descyano analogue of 17, was devoid of any inotropic activity, whereas in the quinoline series the inotropic activity resides in the parent ring system.

Compounds 15 and 1 were also evaluated in guinea pig hearts. In this species the inotropic potency of these agents was reduced by approximately 10-fold and only compound 1 was active in this model (Figure 1A,B). A similar species difference was also observed with 17 and the sodium channel stimulant veratridine[22] (Figure 2A,B). This phenomenon is consistent with the observation that the scorpion toxin, a naturally occurring toxin, increased force of contraction in rat heart yet had no similar effects on either guinea pig or rabbit hearts.[23] The basis for the species difference in response to these agents is not fully understood at the present time. Several investigators indicated either differences in the sodium channels[23] or variations in the activity of Na-K-ATPase[24] between the two species. Increased sensitivity of the contractile proteins and prolonged inactivation of the Na^+ channel have been reported[25-28] to contribute to the inotropic activity of 17. However, most of the inotropic activity of

(18) Agarval, S. K.; Kumar, Y.; Saxena, A. K.; Jain, P. C.; Anand, N. Synthesis and Biological Activities of 3-Substituted 1-Aryloxy-aminopropanes. *Indian J. Chem., Sect. B* **1982**, *21B* (5), 435–439.

(19) Mehrotra, S.; Barthwal, J. P.; Gupta, T. K.; Bhargava, K. P. Substituted Quinolines as Monoamine Oxidase Inhibitors and Analgesics. *Indian J. Physiol. Pharmacol.* **1982**, *26* (3), 253–257.

(20) Bennur, S. C.; Jagainni, V. B.; Badiger, V. V. The 1-Substituted Alkylamino-3-(4-quinazolyloxy)-2-propanol Trihydrochlorides and 1-Substituted Alkylamino-3-(8-quinolyloxy)-2-propanol Dihydrochlorides. *Rev. Roun. Chin.* **1975**, *20* (9–10), 1295.

(21) Haleen, S. J.; Steffen, R. P.; Sircar, I.; Major, T. C.; Taylor, M. D.; Pugsley, T. A.; Weishaar, R. E. PD 122860: A Novel Dihydropyridine With Sodium Channel Stimulating and Calcium Channel Blocking Properties. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 22–30.

(22) Haleen, S. J.; Steffen, R. P.; Weishaar, R. E. Species Differences in the Positive Inotropic Responses to DPI 201-106, a Novel Cardiotonic Agent. *Can. J. Physiol. Pharmacol.* **1989**, *67* (11), 1460–1463.

(23) Coraboeuf, E.; Derobaix, E.; Tazieff-Depierre, F. Effect of Toxin II Isolated from Scorpion Venom on Action Potential and Contraction of Mammalian Heart. *J. Mol. Cell. Cardiol.* **1975**, *7*, 643.

(24) Akera, T.; Yamamoto, S.; Chubb, J.; McNish, R.; Brody, T. M. Biochemical Basis for the Low Sensitivity of the Rat Heart of Digitalis. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1979**, *308*, 81–88.

(25) Romey, G.; Quast, U.; Peuron, D.; Frelin, C.; Renaud, J. F.; Lazdunski, M. Na^+ Channels as Sites of Action of the Cardioactive Agent DPI 201-106 With Agonist and Antagonist Enantiomers. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 896–900.

(26) Wang, G.; Dugas, M.; Armah, I. B.; Honerjager, P. Interaction between DPI 201-106 enantiomers at the cardiac sodium channel. *Mol. Pharmacol.* **1990**, *37* (1), 17–24.

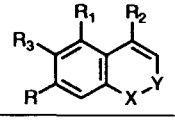
(27) Cingolani, H. E.; Wiedmann, R. T.; Lynch, J. J.; Wenger, H. C.; Scott, A. L.; Siegl, P. K.; Stein, R. B. Negative lusitropic effect of DPI 201-106 and E4031. Possible role of prolonging action potential duration. *J. Mol. Cell. Cardiol.* **1990**, *22* (9), 1025–1034.

(28) Hajjar, R. J.; Gwathmey, J. K. Modulation of calcium-activation in control and pressure overload hypertrophied ferret hearts: effect of DPI 201-106 on myofilament calcium responsiveness. *J. Mol. Cell. Cardiol.* **1991**, *23* (1), 65–75.

(29) Scholtysik, G.; Quast, U.; Schaad, A. Evidence for Different Receptor Sites for the Novel Cardiotonic s-DPI 201-106, ATX II and Veratridine on the Cardiac Sodium Channel. *Eur. J. Pharmacol.* **1986**, *125*, 111–118.

(30) Kohlhardt, M.; Frobe, U.; Herzig, J. W. Modification of Single Cardiac Na^+ Channels by DPI 201-106. *J. Membr. Biol.* **1986**, *89*, 163–172.

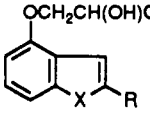
Table I. Physical Constants and Method of Preparation of Substituted (Aryloxy)alkylamine Derivatives

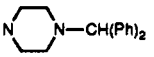
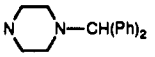
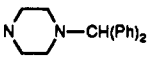


compd	R	R_1	R_2	R_3	X	Y	formula[a]	mp, °C (crystn solvent)	yield, %[b]	method[c]
1	H	H	Z—N⟨⟩N—CH(Ph)$_2$	H	N	CH	$C_{29}H_{31}N_3O_2$	180–181 (EtOH)	40	B
2	H	H	H	Z—N⟨⟩N—CH(Ph)$_2$	N	CH	$C_{29}H_{31}N_3O_2 \cdot 3HCl \cdot 0.5H_2O$	202–203 (EtOH)	23	A
3	H	H	Z-OH	H	N	CH	$C_{12}H_{13}NO_3 \cdot HCl$	168–169 (EtOH)	60	A
4	CF_3	H	Z—N⟨⟩N—CH(Ph)$_2$	H	N	CH	$C_{30}H_{30}F_3N_3O_2 \cdot 0.3H_2O$	foam	4	B
5	Z—N⟨⟩N—CH(Ph)$_2$	H	H	H	N	CH	$C_{29}H_{31}N_3O_2 \cdot 0.1DMF$	122–124	30	A
6	H	Z—N⟨⟩N—CH(Ph)$_2$	H	H	N	CH	$C_{29}H_{31}N_3O_2$	163–165	20	A
7	H	H	Z—N⟨⟩N—CH(Ph)$_2$	H	N	$CH(CH_3)$	$C_{30}H_{33}N_3O_2 \cdot 3HCl \cdot 2H_2O$	209–210 dec (EtOH)	42	A
8	H	H	Z—N⟨⟩	H	N	$CH(CH_3)$	$C_{22}H_{24}N_2O_2$	143–144	50	A
9	H	H	Z—N⟨⟩	H	N	$CH(CH_3)$	$C_{20}H_{19}N_3O_2 \cdot 0.1H_2O$	172–174	10	A
10	H	Z—N⟨⟩N—CH(Ph)$_2$	H	H	CH	N	$C_{29}H_{31}N_3O_2$	foam	23	A
10a							$C_{29}H_{31}N_3O_2 \cdot 3HCl$	220–223 dec (EtOH)		
11	H	Z—N⟨⟩N—⟨⟩—$PhCH_2S$	H	H	CH	N	$C_{29}H_{31}N_3O_2S$	foam	26	A
11a							$C_{29}H_{31}N_3O_2S \cdot 2HCl$	foam		
12	R	Z—N⟨⟩N—CH(Ph)$_2$	H	H	C(SPh)	N	$C_{35}H_{35}N_3O_2S$	146–148	54	B
13	H	Z—N⟨⟩N—CH(Ph)$_2$	H	H	CO	NH	$C_{29}H_{31}N_3O_3$	168–170 (EtOH)	55	B

[a] Elemental analysis were within ±0.4% of the calculated value. These compounds have a tendency to hold solvents. Amount of solvents were quantitated from the ^1H NMR spectra. [b] Yield was not optimized and represents final step. [c] Methods A and B were indicated in Scheme I. Z = $OCH_2CH(OH)CH_2$.

Table II. Physical Constants and Method of Preparation of Substituted (Aryloxy)alkylamine Derivatives

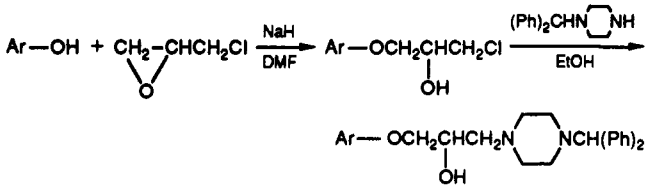


compd	X	R	R_1	formula[a]	mp, °C (crystn solvent)	yield,[b] %	method[c]
14	NH	H		$C_{28}H_{31}N_3O_2$	foam	36	A
15	O	CN		$C_{29}H_{29}N_3O_3$	143–144 (ether/hexane)	30	A
16	O	C(=NH)OEt		$C_{31}H_{35}N_3O_4$	123–124 (isopropyl ether)	33	B

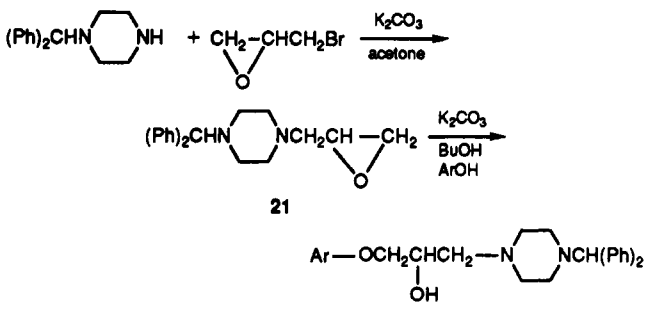
[a] Elemental analysis were within ±0.4% of the calculated value. [b] Yield was not optimized and represent final step. [c] Methods A and B were indicated in Scheme I.

Scheme I

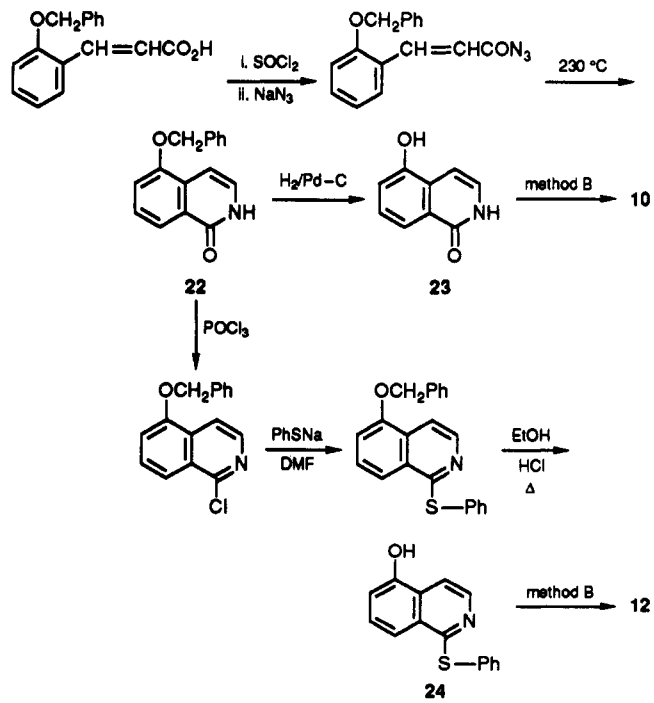
Method A



Method B



Scheme II



17 can be blocked with the Na channel blocker tetrodotoxin (TTX).[29,30] In this study, the inotropic response to 1 was reversed by TTX in a manner similar to that for 17 and veratridine. For these experiments compounds 1, 17, and veratridine were infused at a concentration which produced an increase in LV + dP/dt_{max} of about 50%–80% in the isolated rat heart. After a stable increase in contractility was achieved, 1.0 μM TTX was infused. As shown in Figure 3, TTX reversed the inotropic response to all three agents by about 80%. These results strongly suggest that compound 1, like 17 and veratridine, increases cardiac contractility by stimulating the inward movement of Na^+ ions through the Na^+ fast channel.

Compound 17 has been shown to allosterically bind to the slow Ca^{2+} channel[31] and to functionally block the slow Ca^{2+} channel[32] at relatively high concentration. These effects have been used to explain the compound's vasodilator activity. However, in our experiments in the isolated guinea pig heart, compounds 1, 15, and 17 produced significant coronary vasodilation at 1.0 μM but had no significant effect on Ca^{2+} channel binding in rat brain cortex (RBC) or functionally inhibiting the Ca^{2+} channel

Table III. Cardiovascular Activity in Isolated Rat Heart Model (CVIH)

	EC_{25}, μM[a,b]			
	LV + dP/dt_{max}, μM		CF, μM	
compd	EC_{25}	EC_{50}	EC_{25}	EC_{50}
1	0.14 ± 0.03	0.32 ± 0.10	N	
2	0.48 ● 0.11	0.80 ± 0.23	N	
4	N		0.2	0.40
5	0.70 ± 0.21	NA	0.16 ± 0.04	1.01 ± 0.28
6	N		0.8	2.0
7	-2.0		0.8	
8	N		N	
9	N		N	
10	N		0.20	1.50
11	N		1.0	
12	-1.3		-1.8	
13	-1.5		0.15	
14	N		0.12	2.50
15	0.10 ± 0.0	0.42 ± 0.28	0.10 ± 0.0	3.0
16	N		1.0	
17	0.02 ± 0.01	0.035 ● 0.01	0.30 ± 0.01	NA

[a] A minimum of two hearts were used for each compound tested expect for compounds 1 and 17 ($N = 4$–6). Effects on heart rate were negligible in all cases. [b] Values were concentrations producing a 25% increase (EC_{25}) and 50% increase (EC_{50}) in LV dP/dt_{max} (measure of contractility) and CF (coronary flow) which were obtained from a dose–response curve generated from 0.1–10 μM (for weakly active compounds) and 0.01–1 μM (for moderately active compounds). Values were average ± SEM (where indicated) or mean of two separate experiments. NA = not achieved; N = not active.

(31) Holck, M.; Osterrieder, W. J. Interaction of the cardiotonic agent DPI 201-106 with cardiac calcium channels. *Cardiovasc. Pharmacol.* **1988**, *11*, 478.

(32) Siegl, P. K.; Garcia, M. L.; King, V. F.; Scott, A. L.; Morgan, G.; Kaczorowski, G. J. Interactions of DPI 201-106, a novel cardiotonic agent, with cardiac calcium channel. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1988**, *338/6*, 684–691.

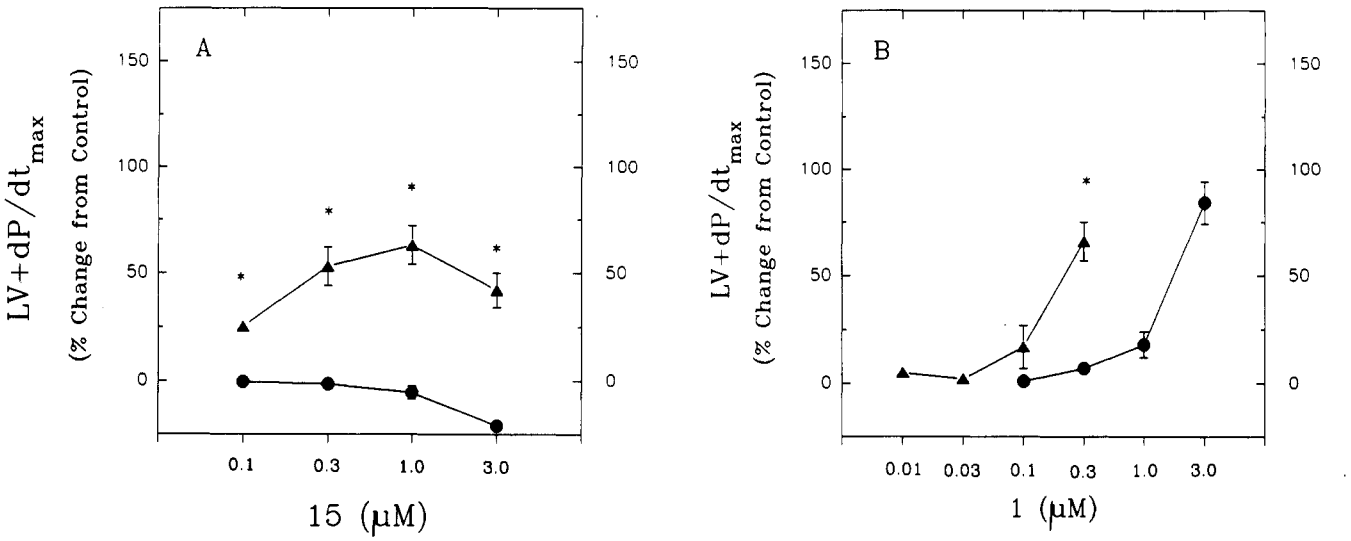


Figure 1. Effect of increasing concentrations of 15 and 1 on left ventricular contractility in isolated rat (▲) and guinea pig (●) hearts. Data represent the mean ± SEM for four to six hearts. Basal LV + dP/dt_{max} values for rat hearts were 1587 ± 165 mmHg (15) and 1561 ± 2 mmHg (1). The values for guinea pig hearts were 1062 ± 54 mmHg (15) and 779 ± 64 mmHg (1). (*) Significant difference between rat and guinea pig responses at the indicated concentrations ($p < 0.05$).

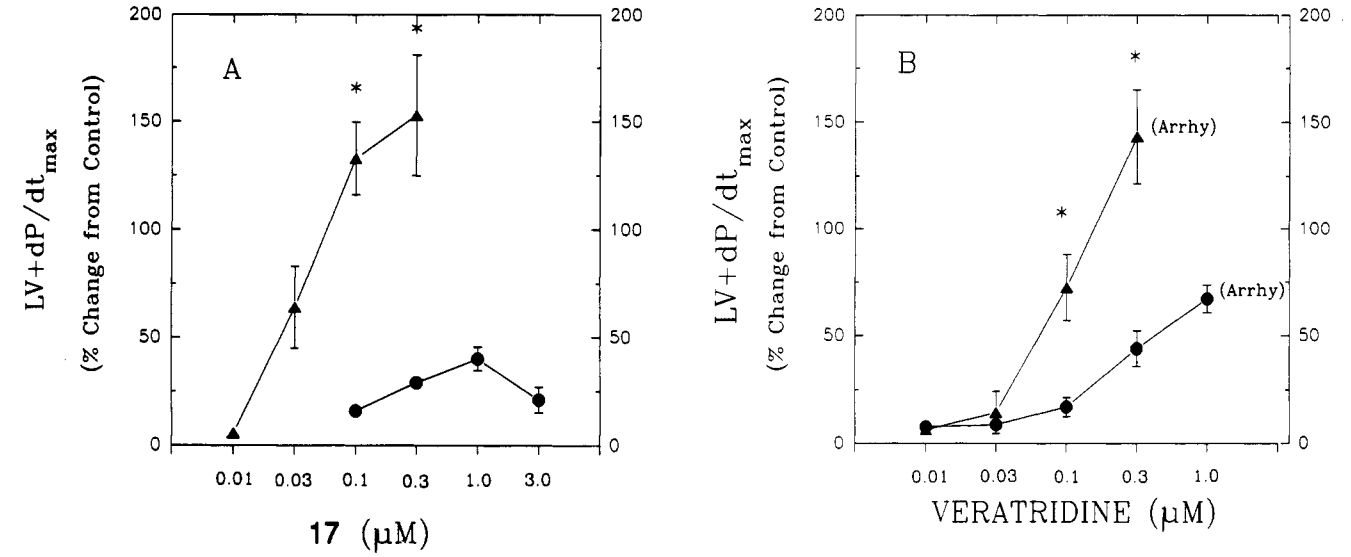


Figure 2. Effect of increasing concentrations of 17 and veratridine on left ventricular contractility in isolated rat (▲) and guinea pig (●) hearts. In B "Arrhy" indicates the occurrence of ventricular arrhythmias at 1.0 μM for rat hearts and at 3.0 μM for guinea pig hearts. Data represent the mean ± SEM for four to six hearts. Basal LV + dP/dt_{max} values for rat hearts were 1714 ± 159 mmHg. The values for guinea pig hearts were 1236 ± 86 mmHg. (*) Significant difference between rat and guinea pig responses at the indicated concentration ($p < 0.05$).

in isolated rabbit aortic rings (CACB, Table IV; see Experimental Section for methods). Although it is difficult to interpret the results due to tissue and species differences between these test systems, our results cannot confirm a correlation between vasodilation in the isolated rat heart and our assays of slow Ca^{2+} channel blockade.

In Vivo Studies. The hemodynamic responses to compounds 1 and 15 were evaluated in acutely instrumented anesthetized dogs as described previously (see Experimental Section for details).[33] Myocardial contractility (LV + dP/dt_{max}), heart rate (HR), mean blood pressure (MBP), and total peripheral resistance (TPR) were measured. Intravenous dose–response curves were determined with three doses of each compound.

Compound 1 infused over 1 min in doses ranging from 0.1 to 1.0 mg/kg produced a dose-related increase in contractility of 12 ± 7% to 115 ± 32% with no change in HR and MBP (Table V). By contrast, with compound 17[34] an increase in contractility of 9 ± 4% to 83 ± 31% in doses from 0.01 to 1.0 mg/kg was observed which were associated with a decrease in MBP of 21 ± 4% at the highest dose. The profile of the furan compound 15 was similar to compound 17 although the inotropic potency was significantly reduced. Compound 15 in doses from 0.1 to 1.0 mg/kg produced a dose-related increase in contractility of 2 ± 0% to 30 ± 9%. The decrease in MBP of 14 ± 6% was observed only at the highest dose of 1 mg/kg. No changes in heart rate were observed with any of these compounds. These increases in contractility for 1 and 15 (1 mg/kg) were associated with decreases in total peripheral resistance (TPR) of 17 ± 1% and 21 ± 3% in

(33) Sircar, I.; Steffen, R. P.; Bobowski, G.; Burke, S. E.; Newton, R. S.; Weishaar, R. E.; Bristol, J. A.; Evans, D. B. Cardiotonic Agents. 9. Synthesis and Biological Evaluation of a Series of (E)-4,5-Dihydro-6-[2-[4-(1H-imidazol-1-yl)phenyl]ethyl]-3(2H)-pyridazinones: A Novel Class of Compounds With Positive Inotropic, Antithrombotic, and Vasodilatory Activities for the Treatment of Congestive Heart Failure. *J. Med. Chem.* **1989**, *32*, 342–350.

(34) Gerard, J. L.; Berdeaux, A.; Guidicelli, J. F. Cardiac and Hemodynamic Profile of a New Cardiotonic Agent, DPI 201-106, in the Conscious Dog. *Eur. J. Pharmacol.* **1989**, *165* (1), 39–49.

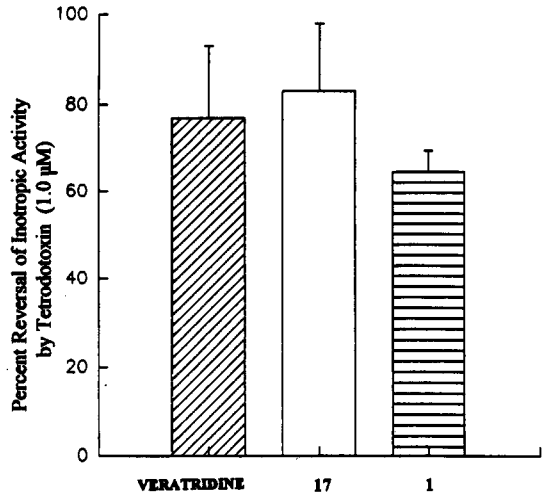


Figure 3. Effects of tetrodotoxin on inotropic responses to 1 (■), veratridine (▨), and 17 (□) in rat isolated hearts ($N = 2–4$). Height of the bars indicates the percent reversal of the inotropic response after addition of 1.0 μM TTX. To determine the effects of TTX on comparable inotropic responses, a concentration of the test agent that produced approximately a 50% increase in contractility was chosen. Data are the mean ± SEM of two to four hearts. (*) Significant from control ($p < 0.05$).

Table IV. Correlation of Calcium Channel Binding Activity (RBC), Functional Calcium Channel Blocking Activity (CCB), and Vasodilator Activity (CF)

compd	RBC (IC_{50}, μM)[a]	CF (EC_{25}, μM)[b]	CCB (EC_{25}, μM)[c]
1	>5000	0.9 ± 0.24 (4)	>100
15	>5000	2.25 ± 1.11 (2)	>100
17	5000	0.280 ± 0.11 (4)	>100

[a] Index of dihydropyridine receptor affinity; inhibition of [^{3}H]nitrendipine binding determined in rat brain cortical membranes. IC_{50} values represent the mean of three separate experiments. [b] Coronary flow activity determined in Langendorff perfused guinea pig heart. EC_{25} (concentration producing a 25% increase) values were average of two to four experiments ± SEM. [c] Calcium channel blocking activity determined in rabbit aortic rings. EC_{25} values represent the mean of three separate experiments.

comparison to 24 ± 10% with compound 17. The hemodynamic responses to compounds 1 and 15 were also evaluated after β-blockade with nadolol. The effects on contractility, HR, and MBP were not significantly reduced after administration of nadolol. These results indicate nonadrenergic inotropic mechanism of action of these agents. Figure 4 shows the comparative cardiovascular profile of 17, 1, and 15 after β-blockade in anesthetized dogs.

Although compound 1 was 10 times less potent than 17 in increasing LV dP/dt_{max} in vitro (rat and guinea pig hearts), the potency differences were less apparent and statistically not significant in vivo. This difference in potency may be attributed to differences in species and/or physicochemical properties of 1 and 17.

Conclusion

The synthesis and biological activity of (aryloxy)-propoxyamine derivatives from two different series are described. In general, a very strict correlation between structure and biological activity within these series was noted. Compound 1 demonstrated potent inotropic activity both in in vitro (rat) and in vivo (dog) model. This quinoline derivative lacks the CN group which seems to be the key structural feature responsible for the inotropic activity in the indole series. The benzofuran compound 15 which contains the CN group demonstrated weak inotropic activity but greater vasodilator activity compared to compound 17. Thus in the five-membered series the relative importance of a nitrogen heterocycle (NHET) vs the CN group (CN > NHET) seems to be different from that of six-membered series. These results indicate that the relative inotropic and vasodilator activities can be modulated by modifying the aromatic ring of compound 17.

Experimental Selection

Melting points were uncorrected and were taken on a Thomas-Hoover capillary melting point apparatus. Each analytical sample was homogeneous by TLC performed on silica gel plates. IR and ^{1}H NMR spectra of all new compounds were consistent with the proposed structures (data for selected compounds are presented). The requisite phenols either were commercially available or were synthesized by following literature methods. All target compounds are racemic and no attempt was made to obtain individual isomers. Veratridine and tetradotoxin (TTX) were purchased from Sigma Chemical Co.

4-(Diphenylmethyl)-α-[(4-quinolinyloxy)methyl]-1-piperazineethanol (1). 1-(Diphenylmethyl)-4-(2-oxiranylmethyl)piperazine (21). A mixture of 12.6 g (50 mmol) of diphenylmethylpiperazine, 13.7 g (100 mmol) of epibromohydrin, and 6.9 g (50 mmol) of K_2CO_3 was refluxed in 250 mL of dry acetone for 6 h. After cooling, the inorganics were filtered off, the filtrate was evaporated to dryness and the oily residue was crystallized twice from ethanol to yield 10 g (71.3%) of the title compound as colorless crystals: mp 110 °C (lit.[35] mp 99–100 °C). Anal. ($C_{20}H_{24}N_2O$) C, H, N.

A mixture of 3.4 g (11 mmol) of **21**, 1.4 g (10 mmol) of 4-hydroxyquinoline, and 1.4 g (10 mmol) of K_2CO_3 in 20 mL of 1-butanol was heated under reflux for 5 h. After the mixture cooled to room temperature, the alcohol was distilled off under vacuum (12 mm) and the residue was partitioned between CH_2Cl_2 and water. The organic phase was separated, the solvent evaporated to dryness, and the residue crystallized twice from ethanol to yield 2.0 g of colorless crystals: mp 180–181 °C; MS (454, M). Anal. ($C_{29}H_{31}N_3O_2$) C, H, N.

4-(Diphenylmethyl)-α-[(6-quinolinyloxy)methyl]-1-piperazineethanol, Trihydrochloride (2). A solution of 2.9 g (20 mmol) of 6-hydroxyquinoline in DMF (40 mL) was added to a slurry of NaH (60% oil suspension, 0.88 g, 22 mmol) in 10 mL of DMF with stirring. After hydrogen evolution ceased, epichlorohydrin was added and the reaction mixture was stirred at 60 °C for 5 h. DMF was removed by distillation and the residue was treated with water. The organic matter was extracted with $CHCl_3$, the chloroform layer was dried, and the solution was stripped to yield 3 g of the epoxy ether. This was dissolved in 30 mL of ethanol and 4 g of benzhydrylpiperazine and heated at reflux for 16 h. Ethanol was stripped and the residue chromatographed (SiO_2, CH_2Cl_2/CH_3OH, 9:1) to give the title compound in the free base form.

This was dissolved in EtOH (20 mL) and an ethanolic HCl solution was added. The precipitate was filtered, washed with small volume of ethanol, and dried at 80 °C for 4 h to give 0.8 g of the title compound: mp 202–203 °C dec; MS (454, M). Anal. ($C_{29}H_{31}N_3O_2 \cdot 3HCl \cdot 0.5H_2O$) C, H, N.

4-[3-[4-(Diphenylmethyl)-1-piperazinyl]-2-hydroxypropoxy]-2-benzofurancarbonitrile (15). A mixture of 2.5 g (15.7 mmol) of 2-cyano-4-hydroxybenzofuran,[36,37] 10 mL of epichlorohydrin, and 0.08 g of piperidine hydrochloride was refluxed for 2 h. Epichlorohydrin was distilled, and the residue was partitioned between water and CH_2Cl_2. The organic layer was separated, dried over anhydrous $MgSO_4$, and stripped. The

(35) Ott, H.; Pfeffingen, C. H. 4-Amino-indole-2-Carbonitrile Derivatives for use as Cardiotonics and Antiarrhythmics. DE 3,723,548, Jan 26, 1989.

(36) Rene, L.; Buisson, J. P.; Royer, R. Reactions induced by pyridine hydrochloride, XVI. The dealkylation of derivatives of methoxybenzene coumarilic nitriles and amides. *Bull. Chim. Soc.* **1974**, *No. 3–4*, 475–476.

(37) Moriyama, J. N.; Shiga, M. N.; Kosatsu, M. H.; Otsu, Y. S.; Shiga, Y. M. Benzofuran Derivatives, Process for Preparing the same and Antihypertensive Agents containing the same. US 4,652,566, March 24, 1987.

Table V. Cardiovascular Activity of 1, 15, and 17 in Anesthetized Dogs ($N = 3$)[a]

compd	dose, mg/kg	contractility (LV dP/dt_{max}), mmHg/s	heart rate (HR), beats/min	mean blood pressure (MBP), mmHg	total peripheral resistance (TPR), mmHg/mL per min
1	0.1	12 ± 7.0	−2 ± 0	3.0 ± 1.0	3.0 ± 1
	0.3	40 ± 20.0[b]	−3.5 ± 0.5	4.5 ± 2.1	6.5 ± 3.5
	1.0	115.5 ± 32[b]	−2.6 ± 1.2	3.0 ± 3.0	−17.0 ± 1.0
15	0.1	2 ± 0.0	0 ± 0	−0.66 ± 0.3	−0.66 ± 0.3
	0.3	9.3 ± 3.0	−1.66 ± 1.0	−7.3 ± 2.9	−8.66 ± 2.3
	1.0	30.3 ± 9.2[b]	−3.33 ± 1.3	−14.5 ± 6.3	−21.66 ± 3.5
17	0.01	9 ± 4	0.66 ± 0.3	4.3 ± 2.0	−9.6 ± 2.0
	0.1	21.3 ± 8.3[b]	−0.66 ± 0.3	2.3 ± 2.0	−17.3 ± 8.0
	1.0	83.3 ± 31.4[b]	−1.0 ± 0.3	−21.0 ± 4.0	−24.5 ± 10.0

[a] Drugs were infused in 0.5 mL of PEG 400 (0.5 mL of DMA for 17) iv over 1 min. Values are maximum response from control average ± SEM. [b] Significantly different from base line, $p < 0.05$.

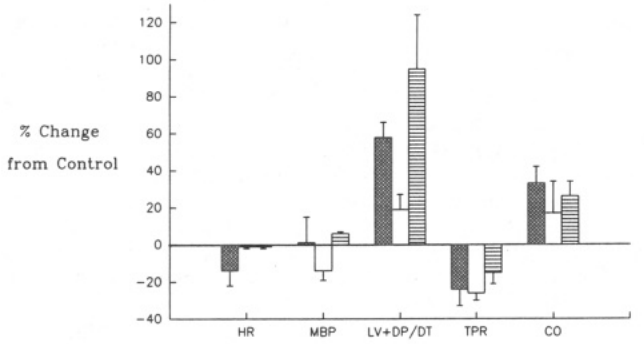


Figure 4. Cardiovascular activity of 17 (■), 15 (□), and 1 (▣) (1.0 mg/kg, iv) after β-adrenergic blockade in anesthetized dogs ($N = 3$). Drugs were infused in 0.5 mL of PEG 400 (0.5 mL of DMA for 17) iv over 1 min, following 15 min nadolol (1.0 mg/kg over 1 min) administration. HR = heart rate; MBP = mean arterial blood pressure; LV dP/dt = cardiac contractility; TPR = total peripheral resistance; CO = cardiac output.

residue was chromatographed over silica gel using toluene/EtOAc (10:1) to give the epoxypropoxy derivative.

A mixture of 0.71 g (3.3 mmol) of the above epoxide and 0.82 g (3.3 mmol) of (diphenylmethyl)piperazine in 5 mL of dioxane was heated at reflux for 2.5 h. The solution was evaporated and the residue chromatographed (silica gel, toluene/ethanol (10:1; $R_f = 0.33$) to yield 0.1 g of the desired compound. It was crystallized from ether/hexane to give a white solid: mp 143–144 °C; MS (468, M); IR (KBr) 2220 cm^{-1} (CN); ^1H NMR ($CDCl_3$) δ 2.25–2.95 (m, 10H, NCH_2), 3.95–4.3 (3H, OCH_2CHOH), 4.6 (s, 1H, $NCHPh_2$), 6.9–7.6 (m, 14H, aromatics).

Ethyl 4-[3-[4-(Diphenylmethyl)-1-piperazinyl]-2-hydroxypropoxy]-2-benzofurancarboximidate (16). A mixture of 1.4 g (9 mmol) of 2-cyano-4-hydroxybenzofuran, 1.24 g (9 mmol) of K_2CO_3, and 3.08 g (10 mmol) of the epoxide **21** in 30 mL of EtOH was heated at reflux for 4 h. Ethanol was distilled and the residue was partitioned between water and CH_2Cl_2. The aqueous solution was neutralized with 18 mL of 1 N HCl and extracted with CH_2Cl_2. The combined extract was washed with water, dried, and evaporated to give an oil which was chromatographed (CH_2Cl_2/CH_3OH, 20:1) to give one major product and several minor products. The major product was rechromatographed (toluene/EtOAc, 1:1) to give the title compound (0.8 g) which was crystallized from diisopropyl ether: mp 123–124 °C. The structure was confirmed by spectral data and elemental analysis. MS indicate correct molecular ion for the imino ester (514, M). IR (KBr) 1640 cm^{-1} (C=NH); ^1H NMR ($CDCl_3$) δ 1.3–1.6 (t, 3H, CH_3, $J = 6.96$ Hz), 2.35–2.95 (m, 10H, NCH_2), 3.95–4.25 (m, 3H, OCH_2CHOH), 4.39 (q, 2H, OCH_2CH_3, $J = 6.96$ Hz), 6.96–7.6 (m, 14H, aromatics), 8.1 (s, 1H, C=NH).

4-(Diphenylmethyl)-α-[[[1-(phenylthio)-5-isoquinolinyl]oxy]methyl]-1-piperazineethanol (12). 1-(Phenylthio)-5-isoquinolinol (24). A mixture of 10 g (40 mmol) of 2-(benzyloxy)-cinnamic acid[38] in 10 mL of $SOCl_2$ containing 3 drops of pyridine was allowed to stand at room temperature for 36 h. Thionyl chloride was distilled, 20 mL of dioxane was added, and the mixture was evaporated to dryness. This process was repeated once more to yield 10.5 g of the acid chloride as an oil. This was dissolved in 20 mL of dioxane, and the solution was added with stirring to a solution of 3.9 g of NaN_3 in 20 mL of dioxane/water (1:1) at 5–7 °C. After the reaction was over, the solution was poured over ice–water and extracted with toluene. Toluene solution was dried over Na_2SO_4 and filtered, and the filtrate was concentrated to ca. 10 mL. This solution was heated at 100 °C until the rearrangement was complete. It was cooled, toluene was replaced with diphenyl ether, and the solution was heated at 230 °C for 20 min under N_2. Ether was distilled under higher vacuum and the residue was triturated with $CHCl_3$/EtOH to yield 3 g of the product, 5-(benzyloxy)-1-hydroxyisoquinoline (**22**): mp 223–224 °C. The structure was confirmed by the spectral data. MS (251, M); ^1H NMR ($CDCl_3$) δ 5.2 (s, 2H, OCH_2) and 6.8–7.8 (m, 10H, aromatics). This was debenzylated (H_2/Pd–C) to give the hydroxy compound **23**, which was converted to the target compound **10**.

A mixture of 5 g of the above hydroxy compound in 30 mL of $POCl_3$ was heated at reflux for 1.5 h. Excess $POCl_3$ was distilled and the residue was poured into ice–water and $CHCl_3$ mixture. The $CHCl_3$ layer was separated, washed with water, dried, and evaporated to yield 3.0 g of a solid: mp 103–104 °C; MS (270, M) indicate correct molecular ion for the chloride.

To a solution of sodium ethoxide (prepared from 0.69 g of Na in 100 mL of EtOH) was added a solution of 3.4 g (30.8 mmol) of thiophenol in 100 mL of DMF. The above chloride (8.3 g, 30.7 mmol) was then added and the solution was heated at 50 °C for 2 h. The reaction mixture was cooled and DMF was distilled under high vacuum. The residue was partitioned between $CHCl_3$ and water, and the $CHCl_3$ layer was separated, dried, and evaporated. The residue was treated with ether to give a solid which was filtered and air-dried (6.2 g, mp 104–105 °C). The filtrate was chromatographed (toluene/EtOH 10:1) to give 1.5 g of additional quantity of the product. MS (343, M); ^1H NMR ($CDCl_3$) δ 5.2 (s, 2H, OCH_2), 6.8–8.4 (m, 15H, aromatics).

A solution of the above benzyloxy compound in a mixture of EtOH/HCl (10:10) was heated at reflux for 2 h. The solution was cooled and filtered to give 0.7 g of the desired phenol (**24**): mp 231 °C. The structure was confirmed by spectral data. MS (253, M); ^1H NMR ($CDCl_3$) δ 6.8–8.4 (m, 10H, aromatics). This hydroxy derivative was converted to the title compound **12** by following the procedure described for **16**: mp 146–148 °C; MS (564, M); ^1H NMR ($CDCl_3$) δ 2.30–2.80 (m, 10H, NCH_2), 4.05–4.25 (m, 3H, OCH_2CHOH), 4.28 (s, 1H, NCH), 6.85–8.28 (m, 20H, aromatics).

1-[2-(Benzylthio)phenyl]piperazine (25). This compound was prepared from 2-(benzylthio)aniline by following methodology described for 1-[2-(alkylthio)phenyl]piperazine.[39]

A solution of NaOH [11.2 g (0.28 mol) in 25 mL of H_2O] was added to a solution of 2-aminobenzenethiol [34 g (0.27 mol) in 150 mL of EtOH]. The solution was cooled to 0 °C, and benzyl chloride [31.6 g (0.25 mol)] was added dropwise with stirring. The ice bath was removed, and the reaction mixture was warmed to 50 °C for 30 min to complete the reaction. It was partitioned between water and ether, and the ether layer was separated. The ether layer was washed with water, dried over anhydrous $MgSO_4$ and evaporated. The residue was distilled to give 2-(benzylthio)-aniline (49.8 g, bp 136–140 °C/0.5 mm) which was used as is for the next step.

(38) Welter, T. R. Certain 2,3-Dihydrobenzofuran-3-yl Acetic Acids and their Method of Preparation. US 4,720,559, Jan 19, 1988.

(39) Parcell, R. F. N-Phenylpiperazines. US 3,028,390, April 3, 1962.

A mixture of the above aniline (49.8 g, 0.23 mol) and bis(2-bromoethyl)amine hydrochloride (25 g, 0.08 mol) in *n*-BuOH (180 mL) was heated under reflux for 16 h. Butanol was distilled, Na_2CO_3 (8.5 g) was added, and BuOH was removed via steam distillation. The residue was made strongly basic with NaOH, and the solution was extracted with ether. The ether layer was washed with water and then extracted with dilute HCl. The aqueous layer was separated, made basic, and extracted with ether. It was dried and evaporated, and the residue was distilled to give the title compound (16.4 g, bp 190–195 °C/0.6 mm). A portion was converted to the monohydrochloride salt which was crystallized from MeOH: mp 190–192 °C. Anal. ($C_{17}H_{20}N_2S$), C, H.

Biological Methods. Cardiovascular Isolated Heart (CVIH) Studies. Male Sprague-Dawley rats (350–500 g) were anesthetized with sodium pentobarbital (50 mg/kg ip) and heparinized (2000 units, ip) to prevent blood clotting. Hearts were isolated rapidly and perfused retrograde by the Langendorff method with a modified Krebs–Henseleit buffer containing the following (millimoles per liter): NaCl, 127.0; $NaHCO_3$, 25.0; KCl, 4.7; $MgSO_4$, 1.1; KH_2PO_4, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; Ca–Na_2 EDTA, 0.05; sodium pyruvate, 2.0; and dextrose, 5.5. This buffer was equilibrated with a gas mixture of 95% O_2–5% CO_2 and maintained at 37 °C and a pH of 7.4. Hearts were perfused at a constant pressure of 70 mmHg. Perfusion pressure and drug concentration were controlled using a computer-regulated pump system. Coronary perfusion was measured using a calibrated output signal from the perfusion pump. LV pressure was measured by inserting a catheter (3F) pressure transducer (Millar Instruments, Houston, TX) into the left ventricle via the mitral valve. ECG was measured using two platinum electrodes positioned at the base and apex of the heart connected to a differential amplifier with the low frequency cutoff set at 0.05 Hz and high-frequency cutoff set at 100 Hz (Gould Electronics, Oxnard, CA). Coronary perfusion rate, LV pressure, and ECG were recorded on a strip chart and digitized with a data analyzer (Buxco Electronics, Sharon, CT). Heart rate, coronary resistance, and LV dP/dt_{max} were derived from the primary signals by the data analyzer. The cardiovascular effects of increasing concentrations (half-log increments) of compounds were evaluated.

TTX Studies. For this protocol the test agent was infused at a concentration that increased contractility by about 50%, after which TTX (1.0 μM) was added to the perfusate. At 1.0 μM, TTX partially blocks the Na^+ fast channel (Brown et al., 1981). Higher concentrations of TTX produce asystole (personal observation) and were therefore avoided.

Calcium Channel Blocking (CCB) Activity. Isolated Aortic Ring Studies. Adult male New Zealand White rabbits (2.5–3 kg) were sacrificed by cervical dislocation and exsanguination. The thoracic aorta was removed quickly and cleaned of connective tissue. The aorta was cut into 4–5-mm wide rings and placed in physiological salt solution of the following composition (millimoles per liter): NaCl, 118.2; KCl, 4.6; KH_2PO_4, 1.2; $NaHCO_3$, 24.8; $MgSO_4$, 1.2; $CaCl_2$, 2.5; Ca–Na_2 EDTA, 0.026; and dextrose, 10.0. This buffer was equilibrated with a gas mixture of 95% O_2–5% CO_2 maintained at 37 °C and a pH of 7.4. Rings were suspended between two stainless steel wire triangles (32 gauge), one of which was attached to a fixed rod and the other connected via 7–0 surgical silk to a Grass FT 0.03 force displacement transducer (Grass Instruments, Quincey, MA) for measurement of isometric force. A resting force of 4 g was placed on the rings, which were allowed to equilibrate for 90 min before testing. Aortic rings were contracted with either 50 mM KCl or 10 μM NE, after which they were exposed to increasing concentrations of the test agent. Selective relaxation of KCl-induced contractions was taken to indicate Ca^{2+} channel blockade because depolarization by potassium triggers the voltage-dependent extracellular influx of Ca^{2+} via the slow channel.

Dihydropyridine Binding Studies Using Rat Brain Cortical Membranes (RBC). The brain was removed quickly and the cerebral cortex dissected. This and all subsequent procedures were performed at 4 °C. The tissue was weighed and homogenized in 10 volumes of 50 mM Tris-HCl (pH 7.7) using a Brinkmann PT-10 polytron (setting of 5 for 10 s). The homogenate was then centrifuged at 48000*g* for 10 min, and the resulting pellet was resuspended in 50 mM Tris-HCl and centrifuged as before. This latter procedure was repeated twice, and the final pellet was resuspended in 50 mM Tris-HCl to a concentration of 50 mg of original wet weight per milliliter.

For the binding studies, aliquots (200 μL) of the membrane preparation, in triplicate, were incubated with 50 mM Tris-HCl (pH 7.7) and 0.2 nM [^{3}H]nitrendipine (final volume = 2.0 mL). The reaction mixture also contained increasing concentrations of the drug being studied or the drug vehicle. Incubations were carried out in the dark for 90 min at 25 °C. Membrane-bound [^{3}H]nitrendipine was separated from free ligand by rapid vacuum filtration over Whatman GF-B glass fiber filters, followed by two consecutive washes with 4 mL of ice-cold 50 mM Tris-HCl. The filters were then placed in glass scintillation vials containing 8.0 mL of Beckman Ready-Solv MP. Nonspecific binding was determined by including 1.0 μM unlabeled nifedipine in the reaction mixture. Specific activity was defined as total [^{3}H]-nitrendipine binding minus the binding obtained in the presence of 1.0 μM nifedipine.

Statistical Evaluation. The direct cardiovascular effects of the test agents on isolated hearts was statistically evaluated using *F*-tests to identify significant differences in group means between the test agents and their respective vehicle control. Any slight vehicle effects were accounted for by this statistical method. Student's *t*-test was used to determine differences between the test agents in response to TTX. Binding experiments were analyzed by the iterative nonlinear least squares curve fitting technique.

Anesthetized Dog Model. Adult mongrel dogs of either sex were anesthetized with pentobarbital, 35 mg/kg, iv, and were subsequently maintained under anesthesia with a continuous infusion of pentobarbital, 5 mg/kg per hour. The trachea was intubated, but the animals were permitted to breathe spontaneously. A cannula was inserted into the femoral vein for administering test agents. A Millar catheter tip pressure transducer (Model PC-350) was inserted into the ascending aorta via the femoral artery for measuring aortic blood pressure. Another similar transducer was passed into the left ventricle via the left carotid artery for measuring left ventricular blood pressure. Needle electrodes were placed subcutaneously for recording a lead II electrocardiogram (ECG).

Left ventricular and aortic blood pressures were recorded on a strip chart recorder. Heart rate, using a biotachometer triggered from the R wave of the ECG, and the first derivative of left ventricular blood pressure (dP/dt), obtained with a differentiator amplifier coupled to the corresponding pressure amplifier, were also recorded. Data analyses were performed with a digital computer. A period of 30 min was utilized to obtain control data prior to administration of test agent. Each dose of the test agent was administered in a volume of 0.1 mL/kg over a period of 1 min.

Data are expressed as means ± SEM. Statistical analysis of the data was performed by using a Student's *t*-test for paired or unpaired data. The probability value, $p < 0.05$, was accepted as level of significance.

Acknowledgment. We thank our analytical departments for spectral determinations and microanalysis. We are also grateful to Mrs. Rosemarie Kraut-Boksan for her excellent technical assistance. We thank the Sandoz Chem. for supplying DPI 201-106.