Novel Positive Inotropic Agents: Synthesis and Biological Activities of 6-(3-Amino-2-hydroxypropoxy)-2(1*H*)-quinolinone Derivatives

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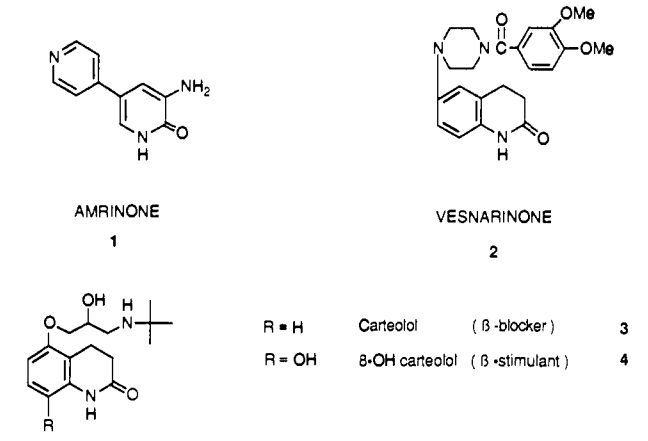
A series of 6-(3-amino-2-hydroxypropoxy)-2(1*H*)-quinolinones has been synthesized and evaluated for positive inotropic activity on the canine heart. Some of these derivatives have a potent activity with none or negative chronotropic effect in isolated, blood-perfused dog heart preparations. They also display a high selectivity for positive inotropic effect over chronotropic and vasodilatory effects in anesthetized dogs. (±)-6-[2-Hydroxy-3-[(3-methoxybenzyl)amino]propoxy]-2(1*H*)-quinolinone (**39**) and (±)-6-[3-(3,4-dimethoxybenzyl)amino]-2-hydroxypropoxy]-2(1*H*)-quinolinone (**40**) were further investigated in conscious dogs. After iv administration, they did not affect heart rate or mean blood pressure at the dose producing a 50% increase in the peak of the first derivative of the left ventricular pressure. The compounds (**39**, OPC-18750, and **40**, OPC-18790) are the most promising agents with desirable biological activities, and now are currently undergoing clinical evaluation.

The discovery of amrinone (1, Chart I) led to the synthesis of a number of agents with promise for congestive heart failure treatment in the fields of nonsympathomimetic and non-glycoside agents. However, almost all of these compounds are not strict digitalis replacements[1] because they have substantial vasorelaxant activity, as so-called inodilators,[2] and also increase heart rate. For many years, we have been searching for compounds in a series of 2(1*H*)-quinolinone derivatives having potent positive inotropic activities without chronotropic effect. At the outset of our studies, 3,4-dihydro-6-[4-(3,4-dimethoxybenzoyl)-1-piperazinyl]-2(1*H*)-quinolinone (Vesnarinone, **2**) was found to be the most promising agent with desirable biological activities,[3] and the compound has since been launched in Japan. In addition, we also found that 5-(3-*tert*-butylamino-2-hydroxypropoxy)-3,4-dihydro-8-hydroxy-2(1*H*)-quinolinone (8-hydroxycarteolol, **4**) increased cardiac contractile force, and therefore, many 8-hydroxycarteolol analogues were synthesized.[4] However, none of these demonstrated the desired characteristics with regard to the selectivity for positive inotropic effect over chronotropic effect in in vitro study. To find the agents having better selectivity for positive inotropic activity, we have studied the most suitable amine and the best position of the side chain on the nucleus in a series of 2(1*H*)-quinolinones substituted with an aminopropoxy system. As a result, we concluded that phenylalkylamines

Chart I



and 6-substitution might be the best. Further modification of 8-hydroxycarteolol was focused on the synthesis of 6-substituted isomers and culminated in the discovery of compound **26**. We describe herein the synthesis and biological properties of 6-(3-amino-2-hydroxypropoxy)-2(1*H*)-quinolinone derivatives, which display marked cardiac stimulant activity with little effect on heart rate and blood pressure. They may be the next digitalis replacements on the heels of vesnarinone.

Chemistry

The 2(1*H*)-quinolinone derivatives which were evaluated for cardiac stimulant activity are listed in Tables I and II. These compounds, apart from **27**, were prepared either by the treatment of the epoxides **5** with appropriate amines or by the reaction of epoxide **7** with methylamine and subsequent alkylation (**51–54**) as shown in Scheme I. The synthetic route of compound **27** is shown in Scheme II. Thus, compound **10**[5] was alkylated by epichlorohydrin in the presence of K_2CO_3 in MeOH to afford epoxide 11.

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(2) Coates, W. J.; Prain, H. D.; Reeves, M. L.; Warrington, B. H. 1,4-Bis(3-oxo-2,3-dihydropyridazin-6-yl)benzene Analogues: Potent Phosphodiesterase Inhibitor and Inodilators. *J. Med. Chem.* **1990**, *33*, 1735–1741 and references therein.
(3) Tominaga, M.; Yo, E.; Ogawa, H.; Yamashita, S.; Yabuuchi, Y.; Nakagawa, K. Synthesis of 3,4-Dihydro-6-[4-(3,4-Dimethoxybenzoyl)-1-piperazinyl]-2(1*H*)-quinolinone and Related Compounds. *Chem. Pharm. Bull.* **1984**, *32* (6), 2100–2110.
(4) Tominaga, M.; Ogawa, H.; Yo, E.; Yamashita, S.; Yabuuchi, Y.; Nakagawa, K. Synthesis of 5-(3-amino-2-hydroxypropoxy)-3,4-dihydro-8-hydroxy-2(1*H*)-quinolinone Derivatives. *Chem. Pharm. Bull.* **1988**, *35* (9), 3699–3704.
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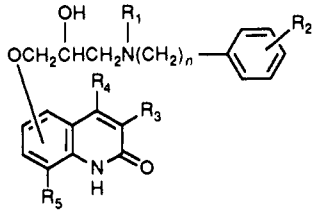
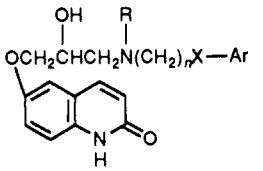
Table I. Physicochemical Data and Data of in Vitro Study for Compounds **25–49**



compd	R_1	R_2	R_3	R_4	R_5	position	n	mp, °C	anal.[a]	recrystn solvent	CF[b]	SR[b]
25	Me	H	dihydro		H	6	1	137–138	$C_{20}H_{24}N_2O_3 \cdot C_2H_2O_4$	MeOH–EtOH–Et_2O	0.1	−0.2
26	Me	H	H	H	H	6	1	129–132	$C_{20}H_{22}N_2O_3$	EtOH	5.1	0
27	Me	H	CN	H	H	6	1	122–126	$C_{21}H_{21}N_3O_3 \cdot {}^3/_4H_2O$	EtOH–H_2O	1.6	1.1
28	Et	H	H	H	H	6	1	135–137	$C_{21}H_{24}N_2O_3$	EtOH	4.0	0
29	Et	H	H	H	Me	6	1	108–110	$C_{22}H_{26}N_2O_3$	AcOEt–hexane	0.8	−0.4
30	Et	H	H	H	F	6	1	130–134	$C_{21}H_{23}FN_2O_3$	EtOH	4.1	0
31	Me	4-Cl	H	H	H	6	1	174–176	$C_{20}H_{21}ClN_2O_3$	EtOH–$CHCl_3$	4.0	0.1
32	Me	4-Me	H	H	H	6	1	181–183	$C_{21}H_{24}N_2O_3 \cdot {}^1/_4H_2O$	MeOH–$CHCl_3$	1.7	0
33	Me	4-OMe	H	H	H	6	1	134–136	$C_{21}H_{24}N_2O_4$	EtOH	2.8	0.2
34	Me	4-F	H	H	H	6	1	141–143	$C_{20}H_{21}FN_2O_3$	EtOH	2.8	0
35	H	H	H	H	H	6	1	155–157	$C_{19}H_{20}N_2O_3 \cdot {}^5/_4H_2O$	EtOH	2.2	0
36	H	4-Cl	H	H	H	6	1	178–179	$C_{19}H_{19}ClN_2O_3 \cdot {}^1/_4H_2O$	MeOH–$CHCl_3$	4.9	0
37	H	4-F	H	H	H	6	1	182–184	$C_{19}H_{19}FN_2O_3$	MeOH–$CHCl_3$	3.5	0
38	H	4-OMe	H	H	H	6	1	190–192	$C_{20}H_{22}N_2O_4$	MeOH	4.9	0
39	H	3-OMe	H	H	H	6	1	138–140	$C_{20}H_{22}N_2O_4$	EtOH	3.8	0
40	H	3,4-$(OMe)_2$	H	H	H	6	1	156–157	$C_{21}H_{24}N_2O_5$	MeOH	5.2	0
41	H	3,4-$(OMe)_2$	H	H	H	5	1	258–260	$C_{21}H_{24}N_2O_5 \cdot HCl$	EtOH–H_2O	0	0
42	H	3,4-$(OMe)_2$	H	H	H	7	1	158–160	$C_{21}H_{24}N_2O_5 \cdot {}^1/_4H_2O$	EtOH	0	−0.2
43	H	3,4-$(OMe)_2$	H	H	H	8	1	178–180	$C_{21}H_{24}N_2O_5$	EtOH	0	0
44	H	3,4-$(OMe)_2$	H	H	Me	6	1	158–160	$C_{22}H_{26}N_2O_5 \cdot {}^1/_4H_2O$	EtOH	0.5	0
45	H	3,4-$(OMe)_2$	H	H	H	6	2	190–191	$C_{22}H_{26}N_2O_5 \cdot HCl$	EtOH	1.7	−0.5
46	H	H	H	H	H	6	2	147–149	$C_{20}H_{22}N_2O_3$	EtOH	1.5	0
47	H	H	H	H	H	6	3	162–164	$C_{21}H_{24}N_2O_3$	MeOH–EtOH	3.6	0.4
48	Et	H	H	H	H	6	2	88–90	$C_{22}H_{26}N_2O_3$	AcOEt	6.9	0.8
49	Et	H	H	H	H	6	3	102–104	$C_{23}H_{28}N_2O_3 \cdot C_2H_2O_4 \cdot H_2O$	EtOH	3.2	0.2

[a] Analysis for C, H, N within ±0.4% of calculated values for the indicated empirical formula. [b] See the Experimental Section.

Table II. Physicochemical Data and Data of in Vitro Study for Compounds **50–62**



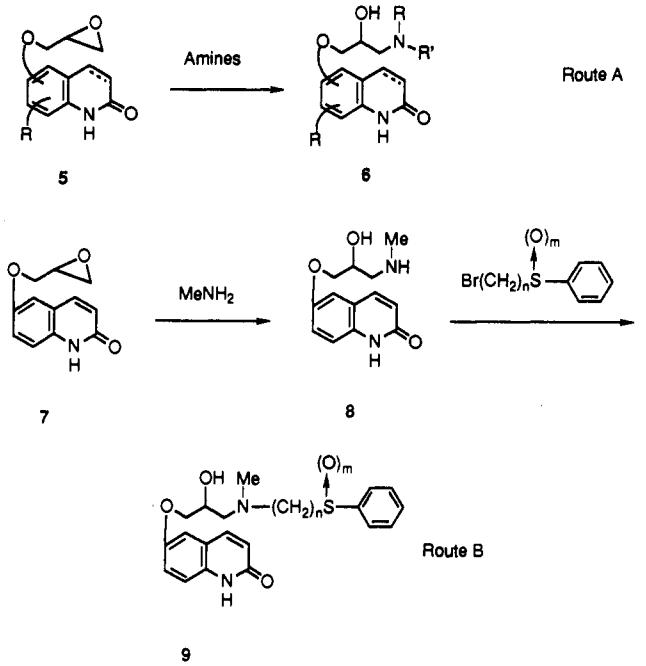
compd	R	n	X	Ar	mp, °C	anal.[a]	recrystn solvent	CF[b]	SR[b]
50	Me	2	NMe	Ph	129–132	$C_{22}H_{27}N_3O_3 \cdot C_2H_2O_4 \cdot H_2O$	EtOH–H_2O	5.0	0
51	Me	2	S	Ph	138–140	$C_{21}H_{24}N_2O_3S$	MeOH–$CHCl_3$	4.5	−1.1
52	Me	2	SO	Ph	84–86	$C_{21}H_{24}N_2O_4S \cdot {}^1/_2H_2O$	CH_2Cl_2–Et_2O	2.3	0
53	Me	2	SO_2	Ph	154–155	$C_{21}H_{24}N_2O_5S$	MeOH	2.1	0.1
54	Me	3	S	Ph	84–86	$C_{22}H_{26}N_2O_3S$	*i*-PrOH	1.3	4.3
55	Et	2	O	Ph	114–116	$C_{22}H_{26}N_2O_4$[c]	AcOEt	5.3	0
56	Me	3	NMe	Ph	103–105	$C_{23}H_{29}N_3O_3$	AcOEt–hexane	1.3	0
57	Me	1		2-Py	129–131	$C_{19}H_{21}N_3O_3$	EtOH–H_2O	2.2	0
58	Me	1		3-Py	160–162	$C_{19}H_{21}N_3O_3$	EtOH	1.7	0.3
59	Me	1		4-Py	135–138	$C_{19}H_{21}N_3O_3$	EtOH–AcOEt	2.3	0.1
60	Me	2		2-Py	95–97	$C_{20}H_{23}N_3O_3$	AcOEt	2.2	0
61	H	1		2-thienyl	*d*	$C_{17}H_{18}N_2O_3S \cdot H_2O$	EtOH	3.1	0.8
62	H	1		2-Py	142–144	$C_{19}H_{19}N_3O_3 \cdot {}^1/_4H_2O$	EtOH	0.6	0

[a] Analysis for C, H, N within ±0.4% of calculated values for the indicated empirical formula except where indicated. [b] See the Experimental Section (same as Table I). [c] Anal. C: calcd, 69.27; found, 68.84. [d] Definite melting point was not observed.

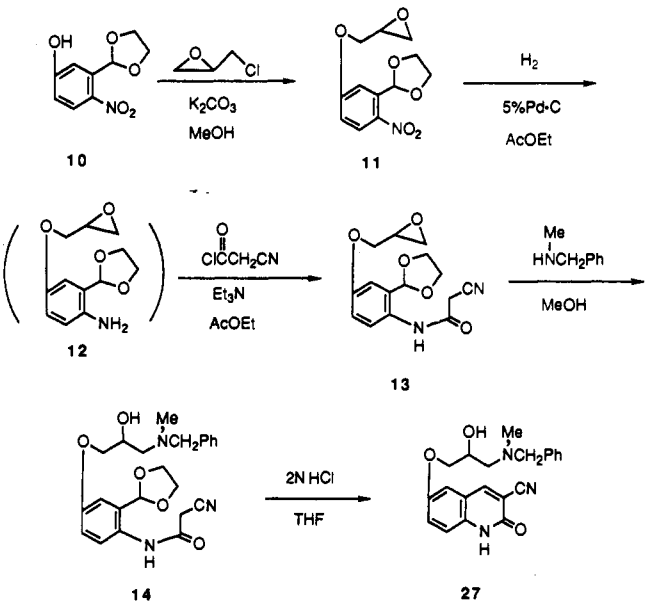
Reduction of the nitro function of **11** by catalytic hydrogenation gave unstable **12**, which was immediately acylated by cyanoacetyl chloride. Ring opening of the epoxide **13** with *N*-methylbenzylamine followed by treatment with 2 N hydrochloric acid gave **27**, proceeding hydrolysis and ring closure. 6-(2,3-Epoxypropoxy)-8-fluoro-2(1*H*)-quinolinone (**20**) was synthesized by the introduction of an acetyl group into the 6-position of 8-fluoro-3,4-dihydro-2(1*H*)-quinolinone (**15**)[6] followed by the further conversion to the acetoxy group (Scheme III). Thus, Friedel–Crafts reaction of **15** with acetyl chloride afforded 6-acetyl derivative (**16**) as the major product. The mixture was purified by single recrystallization to give a pure 6-acetyl derivative. Conversion of **16** into the phenol derivative (**18**) was accomplished by Baeyer–Villiger oxydation with *m*-chloroperbenzoic acid followed by hydrolysis with aqueous sodium hydroxide. Alkylation of **18** with epichlorohydrin gave **19**. Finally, DDQ oxidation of **19** in dioxane afforded **20**. The 6-(2,3-epoxypropoxy)-8-methyl-2(1*H*)-quinolinone (**24**) was synthesized by usual 2(1*H*)-

(6) Uematsu, T.; Inoue, S.; Yamashita, N. 3,4-Dihydrocarbostyril derivatives. *Jpn. Kokai Tokkyo Koho* **1979**, *90*, 183.

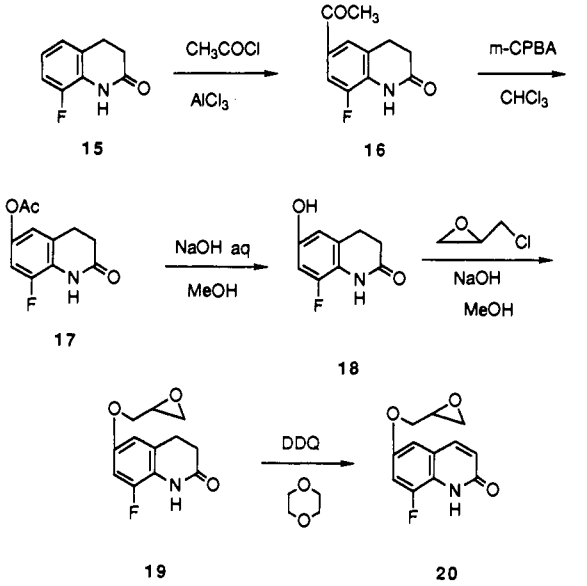
Scheme I



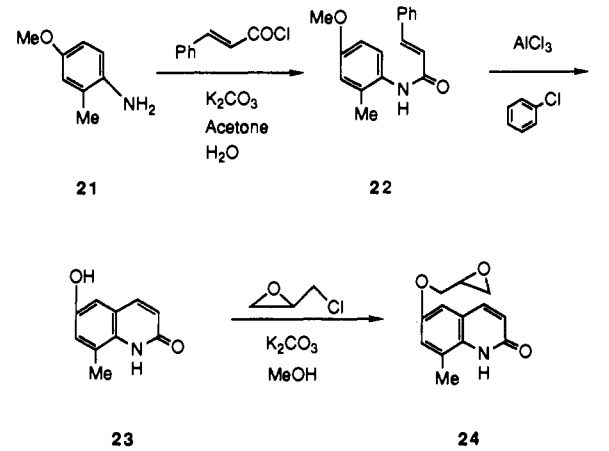
Scheme II



Scheme III



Scheme IV



quinolinone synthesis[7] (Scheme IV). Shotten–Baumann reaction of **21** with cinnamoyl chloride gave amide **22**. Friedel–Crafts cyclization of **22** in chlorobenzene afforded phenol derivative **23**. In this reaction condition, no methyl-migrating product by ipso attack was observed. Finally, alkylation with epichlorohydrin gave **24**.

Biological Results and Discussion

The inotropic (CF) and chronotropic (SR) effects of the 2(1*H*)-quinolinones were compared with those of amrinone (Tables I and II). As for the relationship between the activity and the position of the substituent, the 6-substituted isomers exhibited potent positive inotropic effect and did not increase sinus rate (**40–43**). On the other hand, the 3,4-dihydro derivative (**25**) did not show positive inotropic effect. Previous results[3] showed that the 6-positional isomer had the most potent inotropic effect and that 3,4-dihydro derivatives also displayed inotropic activity similar to that of 3,4-unsaturated derivatives. Our result was consistent with the previous one for the former case but not for the latter case. Therefore, our interest was focused on the synthesis of 6-substituted 3,4-unsaturated derivatives. In the case of tertiary amines (**26–34**), the modification of the parent structure (**26**) by the introduction of the substituents (R_2) in the phenyl ring decreased the potency, whereas, in the case of the secondary amines (**35–40**), the derivatization of **35** increased it. Some structural modifications of the quinolinone nucleus were examined, but none provided any improvement (**27**, **29**, **30**, **44**). Alabaster et al.[8] demonstrated that the introduction of an 8-methyl group afforded beneficial effects for the activity in a series of 6-heteroaryl

(7) Manimaran, T.; Thiruvengadam, T. K.; Ramakrishnan, V. T. Synthesis of Coumarins (2-oxo-2H-1-benzopyrans), Thiocoumarins (2-oxo-2H-1-benzothiopyrans), and Carbostyrils (2-oxo-1,2-dihydroquinolines). *Synthesis* **1975**, 739–741.

(8) Alabaster, C. T.; Bell, A. S.; Campbell, S. F.; Ellis, P.; Henderson, C. G.; Roberts, D. A.; Ruddock, K. S.; Samuels, G. M. R.; Stefaniak, M. H. 2-(1*H*)-Quinolinones with Cardiac Stimulant Activity. I: Synthesis and Biological Activities of (Six-Membered Heteroaryl)-Substituted Derivatives. *J. Med. Chem.* **1988**, *31*, 2048–2056.

(9) Mull, R. P.; Tannenbaum, C.; Dapero, M. R.; Bernier, M.; Yost, W.; Destevens, G. N,N'-Disubstituted Compounds with Diverse Biological Activities. *J. Med. Chem.* **1965**, *8*, 332–338.

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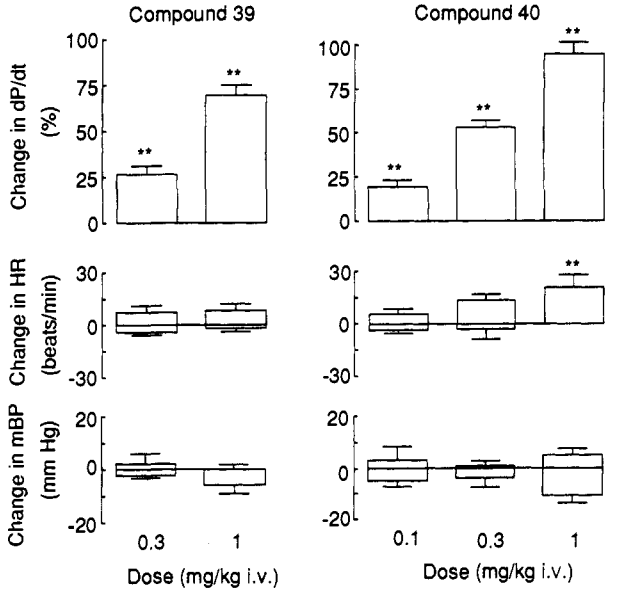

Figure 1. Effects of compounds **39** (left panel) and **40** (right panel) on peak LV dp/dt, heart rate, and mean blood pressure in conscious instrumented dogs. Control values of the peak LV dp/dt, which is an index of cardiac contractility, heart rate, and mean blood pressure of compounds **39** and **40** in the treated groups were 4054 ± 135 and 3959 ± 152 mmHg/s, 88 ± 3 and 89 ± 2 beats/min, and 116 ± 3 and 111 ± 3 mmHg, respectively. Data points represent mean ± SEM of six or seven dogs. $*P < 0.05$, $**P < 0.01$ vs the control values.

quinolinones. However, the reverse phenomenon was observed in our result. For example, modification of **28** and **40** by introduction of an 8-methyl group (**29**, **44**) caused a marked decrease in activity. The data we compiled while searching for a positive inotropic agent having a 2(1*H*)-quinolinone nucleus showed that, in general, incorporating a substituent into the nucleus did not result in any improvement. As for the length between the nitrogen and the phenyl ring, the best profile was methylene (Table I). Furthermore, when one of the methylenes was replaced by a heteroatom, the ethylene chain afforded the better profile (Table II, **50** and **51**). In order to see the necessity of the benzene ring at the amine part, it was replaced by a pyridyl ring (**57–60**, **62**) or a thienyl ring (**61**); neither significant increasing potency nor selectivity was observed. Some of the compounds in Tables I and II were administered intravenously to open-chest anesthetized dogs and the effect on cardiac contractility measured by strain gauge arch and heart rate was determined. As expected, all of these compounds showed a dose-related increase in the cardiac contractility with little change in heart rate (data not shown). More detailed studies in conscious dogs were undertaken with compounds **39** and **40** (Figure 1). Compounds **40** (at doses of 0.1–1.0 mg/kg) and **39** (at doses of 0.3 and 1.0 mg/kg) increased peak LV dp/dt, an index of cardiac contractility, by 20–95% and 25–75%, respectively. A slight increase in heart rate was seen at the highest dose (1 mg/kg) of compound **40**. The doses producing a 50% increase in the LV dp/dt were about 0.3 mg/kg for compound **40** and 0.6 mg/kg for compound **39**. Neither an increase in heart rate nor a decrease in mean blood pressure was observed at these doses.

The compounds discussed herein have a aminopropoxy side chain like compounds exerting cardiovascular action through β-adrenoreceptors. However, one of these compounds (**40**, OPC-18790) has no β-agonistic action.[13] The structure–activity relationship of β-blocking action in a field of 2(1*H*)-quinolinone derivatives[14] suggests that these compounds have weaker β-blocking action. At the present time, there is thought to be two possible mechanisms for the positive inotropic effects of **40**. One is cardiac peak III phosphodiesterase inhibition and the other is prolongation of action potential duration.[13]

Conclusion

Starting with 8-hydroxycarteolol (β-stimulant), we tried to find compounds increasing cardiac contractility without increasing heart rate. As a result, we got compounds having desirable biological properties and a mechanism of action which is not mediated through β-adrenoreceptors.

Selected compounds (**39**, OPC-18750, and **40**, OPC-18790) displayed positive inotropic and coronary vasodilating effects (data not shown), but did not affect heart rate or mean blood pressure. Their cardiovascular profiles are different from those of so-called inodilators.

Vesnarinone showed a profile similar to these compounds in in vitro and in vivo studies, and good results were obtained in a clinical trial. Thus, compounds **39** and **40**, which are now undergoing clinical trial, may join vesnarinone as digitalis replacements.

Experimental Section

All melting points were determined on a Yamato melting apparatus Model MP-21 and are uncorrected. IR spectra were recorded on a JASCO IRA-2 spectrometer and mass spectra were measured on a Shimadzu QP-1000 instrument.

^{1}H-NMR spectra were measured on a Brucker AC-200 or AC-250 spectrometer operating at 200 or 250 MHz, respectively. Chemical shifts are reported in ppm, referenced to tetramethylsilane or DMSO-d_6 (0.00 and 2.50, respectively). Elemental analyses were within 0.4% of the theoretical value unless otherwise stated. Some benzylamines (route A) were prepared by usual reductive amination with appropriate benzaldehydes and alkylamines. Ethylenediamines and propylenediamines (route A) were prepared according to the method of Mull et al.[9] Bromides (route B) were prepared by the method of Jushih et al.[10]

Chemistry. General Methods: Route A, 6-[2-Hydroxy-3-(*N*-methyl-*N*-benzylamino)propoxy]-2(1*H*)-quinolinone (26). A mixture of the 6-(2,3-epoxypropoxy)-2(1*H*)-quinolinone (0.6 g, 2.8 mmol) and *N*-methylbenzylamine (0.42 g, 3.5 mmol) in MeOH (30 mL) was stirred under reflux for 3 h and concentrated in vacuo. The residue was purified by chromatography and recrystallization from EtOH to afford **26** as white powder (0.65 g, 69.9%): ^{1}H NMR ($CDCl_3$) δ 2.31 (3 H, s), 2.30–2.75 (2 H, m), 3.55 (1 H, d, J = 13.0 Hz), 3.71 (1 H, d, J = 13.0 Hz), 4.00 (2 H, d, J = 5.0 Hz), 4.13 (1 H, m), 6.71 (1 H, d, J = 9.4 Hz), 7.00 (1 H, d, J = 2.4 Hz), 7.16 (1 H, dd, J = 9.4 and 2.4 Hz), 7.32 (6 H, m), 7.73 (1 H, d, J = 9.4 Hz). In the case of the reaction with primary amine, 10–20 equimolar amines were used.

Route B, 6-[2-Hydroxy-3-[*N*-methyl-*N*-[(2-phenylthio)-ethyl]amino]propoxy]-2(1*H*)-quinolinone (51). A mixture of 8 (1.5 g, 6.0 mmol), 2-bromoethyl phenyl sulfide (2.6 g, 12.1 mmol),

(11) Endoh, M.; Hashimoto, K. Pharmacological evidence of autonomic nerve activities in canine papillary muscle. *Am. J. Physiol.* **1970**, *218*, 1459–1463.

(12) Kubota, K.; Hashimoto, K. Selective stimulation of the parasympathetic preganglionic nerve fibers in the excised and blood-perfused SA-node preparation of the dog. *Naunyn-schmiederg's Arch. Pharmacol.* **1973**, *278*, 135–150.

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and K_2CO_3 (1.7 g, 12.1 mmol) in CH_3CN (30 mL) was stirred under reflux for 3 h. The mixture was then cooled, filtered to remove salts, and concentrated in vacuo. The residue was purified by chromatography and recrystallization from MeOH–$CHCl_3$ to afford **51** as white powder (1.8 g, 78.3%): ^{1}H NMR (DMSO-d_6) δ 2.29 (3 H, s), 2.35–2.55 (2 H, m), 2.63 (2 H, t, J = 7.4 Hz), 3.06 (2 H, t, J = 7.4 Hz), 3.80–4.15 (3 H, m), 4.84 (1 H, d, J = 3.8 Hz), 6.47 (1 H, d, J = 9.6 Hz), 7.05–7.40 (8 H, m), 7.80 (1 H, d, J = 9.6 Hz), 11.62 (1 H, s).

5-(2,3-Epoxypropoxy)-2-nitrobenzaldehyde Ethylene Acetal (11). A solution of **10** (33.0 g, 156.3 mmol), potassium carbonate (22.5 g, 162.5 mmol) and epichlorohydrin (120 mL, 1554.5 mmol) in MeOH (200 mL) was stirred under reflux for 20 h and concentrated in vacuo. The residue was dissolved in water (50 mL) and CH_2Cl_2 (100 mL). The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel by eluting with CH_2Cl_2–hexane (1:1). The solid product was recrystallized from AcOEt to give **11** (25.0 g, 59.9%) as colorless prisms: mp 77–79 °C; ^{1}H NMR ($CDCl_3$) δ 2.86 (1 H, dd, J = 4.6 and 2.8 Hz), 3.06 (1 H, t, J = 4.6 Hz), 3.45 (1 H, m), 4.00–4.10 (5 H, m), 4.38 (1 H, dd, J = 11.0 and 2.8 Hz), 6.56 (1 H, s), 6.95 (1 H, dd, J = 9.0 and 2.8 Hz), 7.33 (1 H, d, J = 2.8 Hz), 8.00 (1 H, d, J = 9.0 Hz). Anal. ($C_{12}H_{13}NO_6$) C, H, N.

2-[(Cyanoacetyl)amino]-5-(2,3-epoxypropoxy)benzaldehyde Ethylene Acetal (13). A mixture of **11** (25.3 g, 94.7 mmol), 5% palladium on charcoal (2.5 g), and AcOEt (500 mL) was stirred at room temperature under atmospheric pressure of hydrogen until the absorption of hydrogen ceased. The catalyst was filtered off and Et_3N (20 mL, 143.5 mmol) was added to the filtrate. To this solution, cyanoacetyl chloride (15.0 g, 144.9 mmol) was added dropwise at 0 °C over 1 h, and then the reaction mixture was poured into water (2000 mL). The organic layer was separated, the aqueous layer was extracted with AcOEt (3 × 200 mL), and the extracts were combined with the organic layer. After drying ($MgSO_4$) and concentration, the residue was triturated with AcOEt (200 mL). The resulting precipitate was collected by filtration. Recrystallization from AcOEt gave **13** as colorless needles (10.0 g, 34.7%): mp 114–115 °C; ^{1}H NMR (DMSO-d_6) δ 2.72 (1 H, dd, J = 4.4 and 2.6 Hz), 2.83 (1 H, t, J = 4.4 Hz), 3.32 (1 H, m), 3.65–4.20 (7 H, m), 4.34 (1 H, dd, J = 11.3 and 2.6 Hz), 5.82 (1 H, s), 6.90–7.10 (2 H, m), 7.35 (1 H, d, J = 8.5 Hz), 9.60 (1 H, brs). Anal. ($C_{15}H_{16}N_2O_5$) C, H, N.

2-[(Cyanoacetyl)amino]-5-[3-(*N*-methyl-*N*-benzylamino)-2-hydroxypropoxy]benzaldehyde Ethylene Acetal (14). A mixture of **13** (1.0 g, 3.3 mmol) and *N*-methylbenzylamine (0.6 g, 4.9 mmol) in MeOH (30 mL) was stirred at reflux for 3 h and concentrated in vacuo. The residue was chromatographed on silica gel by eluting with CH_2Cl_2–MeOH (25:1) and gave **14** (1.2 g, 78.9%). This was employed in the next step without further purification.

3-Cyano-6-[3-(*N*-methyl-*N*-benzylamino)-2-hydroxypropoxy]-2(1*H*)-quinolinone (27). A mixture of **14** (1.2 g, 2.6 mmol) and 2 N HCl (10 mL) in THF (100 mL) was stirred for 2 h at room temperature and concentrated in vacuo. The residue was dissolved in aqueous 5% K_2CO_3 (100 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined, dried ($MgSO_4$) organic extracts were evaporated, and the residue was chromatographed on silica gel by eluting with CH_2Cl_2–MeOH (20:1). The resulting solid was recrystallized from EtOH–H_2O to give **27** as yellow powder (0.6 g, 58.8%): mp 122–126 °C; ^{1}H NMR (DMSO-d_6) δ 2.18 (3 H, s), 2.40–2.70 (2 H, s), 3.30–3.70 (5 H, m), 3.70–4.10 (3 H, m), 7.20–7.40 (8 H, m), 8.63 (1 H, s). Anal. ($C_{21}H_{21}N_3O_3$) C, H, N.

6-Acetyl-3,4-dihydro-8-fluoro-2(1*H*)-quinolinone (16). Aluminum chloride (100 g, 750 mmol) was added portionwise to a suspension of **15** (24.0 g, 145.0 mmol) and acetyl chloride (21 mL, 290.0 mmol) in CS_2 (200 mL) at 0 °C. The mixture was warmed gradually to evaporate the solvent, and then stirred at 100 °C. After 3 h, the hot mixture was poured into ice–water (1000 mL) and was extracted with $CHCl_3$ (3 × 300 mL). The combined, dried ($MgSO_4$) extracts were evaporated, and the residue was chromatographed on silica gel (CH_2Cl_2–MeOH 100:1). The solid product was recrystallized from EtOH–H_2O (1:1) to give **16** as colorless needles (17.0 g, 56.5%): mp 184–186 °C; ^{1}H NMR ($CDCl_3$) δ 2.56 (3 H, s), 2.71 (2 H, t, J = 7.0 Hz), 3.08 (2 H, t, J = 7.0 Hz), 7.59 (2 H, m), 7.89 (1 H, brs). Anal. ($C_{11}H_{10}FNO_2$) C, H, N.

6-Acetoxy-3,4-dihydro-8-fluoro-2(1*H*)-quinolinone (17). *m*-Chloroperbenzoic acid (80%, 5.0 g, 23.2 mmol) was added to a solution of **16** (4.0 g, 19.3 mmol) in $CHCl_3$ (80 mL) at room temperature. After heating under reflux overnight, the cooled mixture was washed with K_2CO_3 solution (5%, 2 × 40 mL) and brine, dried ($MgSO_4$), and evaporated. The resulting solid was recrystallized from AcOEt to give **17** as colorless needles (3.5 g, 81.2%): mp 170–172 °C; ^{1}H NMR ($CDCl_3$) δ 2.29 (3 H, s), 2.67 (2 H, t, J = 6.8 Hz), 3.00 (2 H, t, J = 6.8 Hz), 6.70–6.90 (2 H, m), 7.74 (1 H, brs). Anal. ($C_{11}H_{10}FNO_3$) C, H, N.

8-Fluoro-6-hydroxy-3,4-dihydro-2(1*H*)-quinolinone (18). Sodium hydroxide solution (10%, 30 mL) was added to a solution of **17** (4.2 g, 18.8 mmol) in MeOH (100 mL) at room temperature. The mixture was stirred for 1 h and the organic solvent was evaporated. Water (50 mL) was added to the residue and acidified to pH 2 (concentrated HCl). The resulting precipitate was collected by filtration and washed with water. Recrystallization from MeOH gave **18** as colorless prisms (2.1 g, 61.6%): mp 237–238 °C; ^{1}H NMR ($CDCl_3$) δ 2.61 (2 H, t, J = 6.9 Hz), 2.91 (2 H, t, J = 6.9 Hz), 6.40–6.60 (2 H, m), 8.07 (1 H, brs), 9.93 (1 H, s). Anal. ($C_9H_8FNO_2$) C, H, N.

3,4-Dihydro-6-(2,3-epoxypropoxy)-8-fluoro-2(1*H*)-quinolinone (19). A solution of **18** (2.1 g, 11.6 mmol), sodium hydroxide (0.5 g, 12.5 mmol), and epichlorohydrin (10 mL, 127.9 mmol) in MeOH (8 mL) was stirred under reflux for 3 h and concentrated in vacuo. The residue was dissolved in water (20 mL) and CH_2Cl_2 (20 mL). The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel by eluting with CH_2Cl_2–MeOH (20:1). The solid product was recrystallized from MeOH to give **19** as colorless needles (1.4 g, 50.9%): mp 153–155 °C; ^{1}H NMR ($CDCl_3$) δ 2.55–2.75 (3 H, m), 2.90–3.05 (3 H, m), 3.32 (1 H, m), 3.85 (1 H, dd, J = 11.1 and 5.8 Hz), 4.20 (1 H, dd, J = 11.1 and 2.8 Hz), 6.55–6.65 (2 H, m), 7.50 (1 H, brs). Anal. ($C_{12}H_{12}FNO_3$) C, H, N.

6-(2,3-Epoxypropoxy)-8-fluoro-2(1*H*)-quinolinone (20). A mixture of **19** (5.1 g, 21.4 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (7.2 g, 31.7 mmol) in dioxane (200 mL) was stirred under reflux for 20 h and concentrated in vacuo. The residue was dissolved in potassium carbonate solution (2.5%, 200 mL) and extracted with $CHCl_3$–MeOH (20:1, 3 × 200 mL). The combined dried ($MgSO_4$) organic extracts were evaporated, and the residue was chromatographed on silica gel by eluting with CH_2Cl_2–MeOH (20:1). The solid product was recrystallized from MeOH to give **20** as colorless prisms (1.0 g, 20.2%): mp 225–228 °C; ^{1}H NMR (DMSO-d_6) δ 2.72 (1 H, dd, J = 5.0 and 2.6 Hz), 2.85 (1 H, t, J = 5.0 Hz), 3.30–3.40 (1 H, m), 3.86 (1 H, dd, J = 11.4 and 6.8 Hz), 4.37 (1 H, dd, J = 11.4 and 2.6 Hz), 6.57 (1 H, d, J = 9.6 Hz), 7.10–7.25 (2 H, m), 7.84 (1 H, d, J = 9.6 Hz), 11.66 (1 H, brs). Anal. ($C_{12}H_{10}FNO_3$) C, H, N.

4-Methoxy-2-methylcinnamanilide (22). To a stirred solution of 4-methoxy-2-methylaniline (10.0 g, 72.9 mmol), potassium carbonate (15.0 g, 108.5 mmol), water (100 mL), and acetone (50 mL) was added dropwise cinnamoyl chloride (15.0 g, 90.0 mmol) at 0 °C. The mixture was stirred at 0 °C for 0.5 h and then poured into ice–water (1000 mL). The resulting precipitate was collected by filtration and washed with water. Recrystallization from $CHCl_3$–MeOH gave **22** as colorless needles (15.0 g, 51.3%): mp 195–196 °C; ^{1}H NMR ($CDCl_3$) δ 2.28 (3 H, s), 3.80 (3 H, s), 6.60–7.10 (4 H, m), 7.30–7.80 (6 H m). Anal. ($C_{17}H_{17}NO_2$) C, H, N.

6-Hydroxy-8-methyl-2(1*H*)-quinolinone (23). Aluminum chloride (13.0 g, 97.6 mmol) was added portionwise to a suspension of **22** (5.0 g, 18.7 mmol) in chlorobenzene (100 mL) at 0 °C. The reaction mixture was gradually warmed to 120 °C and then stirred for 3 h. After the mixture was poured into ice–water, the resulting precipitate was collected by filtration and washed with water. The solid was chromatographed on silica gel (CH_2Cl_2–MeOH 20:1) and recrystallized from MeOH to give **23** as colorless prisms (2.4 g, 73.2%): mp 270–272 °C; ^{1}H NMR (DMSO-d_6) δ 2.36 (3 H, s), 6.44 (1 H, d, J = 9.4 Hz), 6.82 (1 H, s), 6.85 (1 H, s), 7.75 (1 H, d, J = 9.4 Hz), 9.30 (1 H, brs), 10.69 (1 H, brs). Anal. ($C_{19}H_6NO_2$) C, H, N.

6-(2,3-Epoxypropoxy)-8-methyl-2(1*H*)-quinolinone (24). A mixture of **23** (5.0 g, 28.5 mmol), epichlorohydrin (22 mL, 285.0 mmol), and potassium carbonate (4.7 g, 34.0 mmol) in MeOH (25 mL) was stirred at reflux for 2 h, and concentrated in vacuo. The residue was dissolved in water (150 mL) and CH_2Cl_2 (50 mL). The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was recrystallized from MeOH to give **24** as colorless prisms (3.6 g, 54.5%): mp 228–230 °C; ^1H NMR ($CDCl_3$) δ 2.44 (3 H, s), 2.78 (1 H, dd, J = 4.8 and 2.9 Hz), 2.93 (1 H, t, J = 4.8 Hz), 3.37 (1 H, m), 3.95 (1 H, dd, J = 11.1 and 5.8 Hz), 4.28 (1 H, dd, J = 11.1 and 2.9 Hz), 6.66 (1 H, d, J = 9.5 Hz), 6.88 (1 H, d, J = 2.7 Hz), 7.04 (1 H, d, J = 2.7 Hz), 7.68 (1 H, d, J = 9.5 Hz), 9.20 (1 H, brs). Anal. ($C_{13}H_{13}NO_3$) C, H, N.

Pharmacological Study (in Vitro). The inotropic and chronotropic effects of the test compounds were examined by the use of isolated, blood-perfused dog heart preparations. The hearts were excised from mongrel dogs of either sex weighing 8–14 kg. The isolated, blood-perfused papillary muscle and sinoatrial node preparations were prepared according to the methods of Endoh and Hashimoto[11] and Kubota and Hashimoto,[12] respectively. The preparations were cross-circulated through the cannulated arteries with blood from a donor dog anesthetized with sodium pentobarbital and receiving heparin. The perfusion pressure was kept constant at 100 mmHg. The papillary muscle was stimulated at a frequency of 2 Hz and the tension developed by the muscle was measured with a force displacement transducer (Shinkoh, UL-20-240). Sinus rate was measured by the use of a cardiotachometer (Data Graph, T-149) triggered by the developed tension of the right atrium. Blood flow through the cannulated arteries was measured with an electromagnetic flow meter (Nihon Kohden, MF-27). Signals of these parameters were recorded on a thermal pen recorder (NEC-Sanei, Recti-Horiz 8K). The compounds were injected intra-arterially with microsyringes. The potency of the inotropic and chronotropic effects of the test compounds was evaluated at the dose (ED_{50}) producing the half-maximal response of amrinone as follows: activity ratio of the test compound = (ED_{50} of amrinone)/(dose of the test compound producing the same response as ED_{50} of amrinone). A larger activity ratio indicates that the test drug is more potent. The highest dose (1 μmol) of amrinone used in these experiments increased developed tension by about 50% of the basal tension, and increased sinus rate by about 15 beats/min.

Pharmacological Study (in Vivo, in Normal Conscious Dogs). Experiments were performed on male beagle dogs, weighing 8.6–13.5 kg. The animals were anesthetized with pentobarbital sodium at 30 mg/kg iv and were respired with room air by use of a respirator. Aseptically, the chest was opened through the left fifth intercostal space and a solid-state pressure microtransducer (Konigsberg, p-5) and a polyethylene catheter for transducer calibration were implanted into the left ventricular chamber through a stab incision at the apex for the measurement of left ventricular pressure and its first derivative (LV dp/dt), which is the index of cardiac contractility. A polyethylene catheter (Intramedic, PE-60) for measurement of blood pressure was implanted into the lower part of the abdominal aorta through a stump of a branch of the femoral artery. A polyethylene catheter for injection of drugs was implanted into the femoral vein through a stump of its branch. The proximal ends of the catheters and that of the pressure microtransducer were passed under the skin and exteriorized at the back of the neck. The catheters were filled with sterile 0.9% saline containing heparin sodium. All dogs were allowed to recover at least 1 week after the operation before the experiments were initiated. The conscious dogs were placed in a sling in a silent room with sound insulation. The left ventricular pressure signal from the Konigsberg pressure transducer was adjusted to match that recorded from the polyethylene catheter. The first derivative of the left ventricular pressure was obtained by electronic differentiation of the left ventricular pressure pulse. The implanted arterial catheter was connected to a pressure transducer (NEC-San-ei, MPU-0.5) for the measurement of arterial blood pressure. The heart rate was counted from the arterial blood pressure pulse wave for 30 s. The lead II ECG was obtained by means of needle electrodes inserted subcutaneously. When these parameters became stable, the test drugs were administered intravenously through the implanted venous catheter.

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