Synthesis and Cardiotonic Activity of a Series of Substituted 4-Alkyl-2(1*H*)-quinazolinones

Victor T. Bandurco,* Charles F. Schwender,* Stanley C. Bell, Donald W. Combs, Ramesh M. Kanojia, Seymour D. Levine, Dennis M. Mulvey, Mary A. Appollina, Marianne S. Reed, Elizabeth A. Malloy, Robert Falotico, John B. Moore, and Alfonso J. Tobia

Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, New Jersey 08869-0602. *Received July 18, 1986*

The synthesis, cardiac fraction III cyclic nucleotide phosphodiesterase (PDE-III) inhibition, and positive inotropic activity of a series of 2(1*H*)-quinazolinones are reported. A general synthesis of the series involved the cyclization of 2-aminoacetophenones with potassium cyanate in acetic acid. Modifications at the 4-position of the quinazoline nucleus were best achieved by formation of the intermediate N^1-acyl-N^3-phenylurea from the substituted phenyl isocyanate and appropriate carboxamide. PPA was used to ring close to the quinazoline product. Generally the SAR for the series paralleled the five-point model previously published for PDE-III inhibition. The most active analogue of the series was 5,6-dimethoxy-4-methyl-2(1*H*)-quinazolinone (1) (ORF 16600), which had about twice the intravenous potency of amrinone. Compound 1 is currently under development as an orally active cardiotonic.

The management of chronic heart failure includes therapy utilizing diuretics, systemic vasodilators, and stimulation of myocardial contractility with cardiac glycosides such as digitalis. Newer inotropes currently available include dobutamine, a β-sympathomimetic, and amrinone, an agent reported to act through selective inhibition of cardiac fraction III cyclic nucleotide phosphodiesterase (PDE-III).[1,2] Since these newer agents are approved only for parenteral use, research has focused on the development of an orally potent agent. Several additional series of the amrinone type have been reported and are under clinical investigation.[1,3]

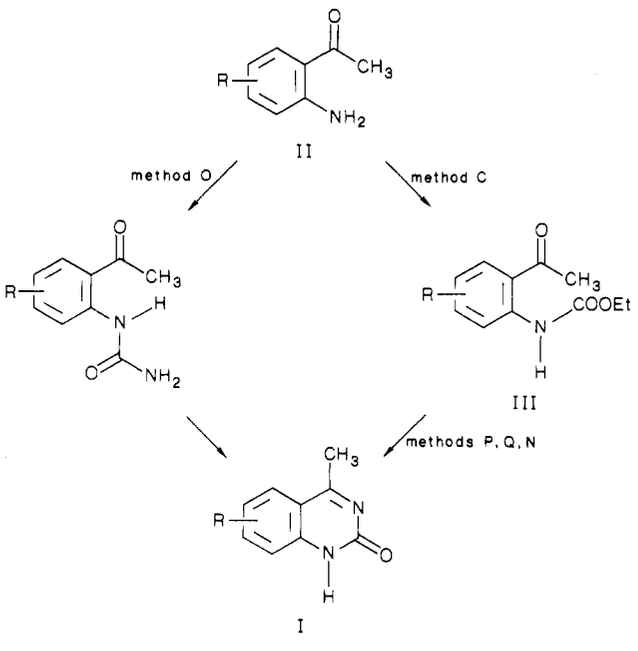
Recently, the cardiotonic activity of 5,6-dimethoxy-4-methyl-2(1*H*)-quinazolinone (1) (ORF 16600) was presented.[4] We now report the synthesis, PDE-III inhibition, and cardiotonic activity of ORF 16600 and a series of related 2(1*H*)-quinazolinones.

Chemistry

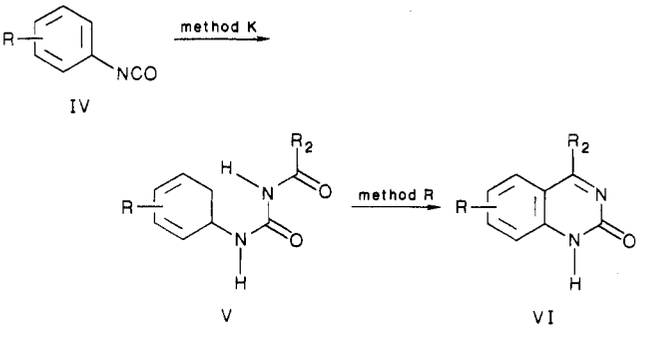
The synthesis of 4-methyl-2(1*H*)-quinazolinones (I) was accomplished generally (Scheme I) from the appropriately substituted 2-aminoacetophenone obtained by reduction of the corresponding nitroacetophenone (methods B, G, I, J). Ring closure to the desired quinazoline could be achieved by reacting the aminoacetophenone (II) directly with potassium cyanate in aqueous acetic acid (method O). Conversion of the amine to the carbamate (III) using ethyl chloroformate (method C) and subsequent ring closure using ammonia in ethanol under pressure (method N) gave the desired quinazolinone (I). Fusion with ammonium acetate (method P) or reaction with ammonia and ammonium acetate in hot DMF solution (method Q) also achieved ring closure to the quinazolinone product.[5,6]

Alternatively (Scheme II), reaction of certain substituted phenyl isocyanates (IV) with carboxamides using method K yielded intermediate acylureas (V), which were cyclized with polyphosphoric acid to give the quinazolinones (VI) (method R).[7] This approach facilitated variation at the 4-position, providing analogues **11**, **17**, and **21–26**.

Scheme I



Scheme II



The physical data for intermediates **28–65** are included in Table II.

Biological Results and Discussion

A description of the biological assays employed appears in the Experimental Section. The cardiotonic activity of a series of 2(1*H*)-quinazolinones was evaluated in an-

(1) Bristol, J. A.; Sircar, I.; Moos, W. H.; Evans, D. B.; Weishaar, R. E. *J. Med. Chem.* **1984**, *27*, 1099.
(2) Weishaar, R. E.; Quade, M. J.; Schenden, J. A.; Evans, D. B. *J. Cyclic Nucleotide Protein Phos. Res.* **1985**, *10*, 551.
(3) Wetzel, B.; Havel, N. *Annu. Rep. Med. Chem.* **1984**, *19*, 71.
(4) Falotico, R.; Moore, J. B.; Tolman, E. L.; Bandurco, V. T.; Bell, S. C.; Tobia, A. J. *Pharmacologist* **1985**, *27*, Abstr 679.
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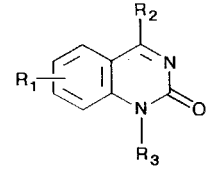
(6) Armarego, W.; Smith, J. *J. Chem. Soc. C* **1966**, 234.
(7) Budesinsky, Z.; Lederer, P. *Collect. Czech. Chem. Commun.* **1972**, *37*, 2779.

0022-2623/87/1830-1421$01.50/0

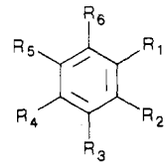
Table I. Cardiotonic and Phosphodiesterase III Inhibition of 4-Alkyl-2(1*H*)-quinazolinones

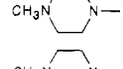
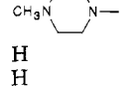


no.	R_1	R_2	R_3	formula	anal.	mp, °C	method	solvent[a]	dose,[b] mg/kg, iv	(*n*)	CF,[c] % Δ	HR, %Δ	MAP, %Δ	CF,[d] ED_{50}	PDE-III[e] IC_{50}, μM
1	5,6-$(OCH_3)_2$	CH_3	H	$C_{11}H_{12}N_2O_3$·HCl-H_2O	CHN	208–210	N, O, P	A	0.875	(5)	114	20	−16	0.23	17
2	H	CH_3	H	$C_{10}H_{10}N_2O_3$			*f*		8.75	(2)	93	16	−8	3.45	138
3	6-OCH_3	CH_3	H	$C_{10}H_{10}N_2O_2$	CHN[g]	232–236 dec	Q	A	3.75	(2)	111	18	−10	1.32	
4	6-Cl	CH_3	H	$C_9H_7ClN_2O$	CHN	286–288	Q	A	8.75	(1)	85	33	−21	1.80	
5	6-$N(CH_3)_2$	CH_3	H	$C_{11}H_{13}N_3O$	CHN	278–281	Q	B	8.75	(4)	90	23	−23	1.20	38
6		CH_3	H	$C_{13}H_{15}N_3O_2$·H_2O	CHN	233 dec	O	C	0.875	(2)	59	13	−10	0.70	
7		CH_3	H	$C_{14}H_{17}N_3O$·$^1/_2H_2O$	CHN[h]	130 dec	O	D	3.75	(1)	116	35	−21	1.13	
8		CH_3	H	$C_{14}H_{18}N_4O$·H_2O	CHN[i]	263–265	O	C	1.875	(1)	10	6	0	>1.87	300
9	6,7-Cl_2	CH_3	H	$C_9H_6Cl_2N_2O$			*f*		8.75	(1)	10	2	1	>8.75	
10	6,7-$(OCH_3)_2$	CH_3	H				*f*		8.75	(3)	90	19	−35	2.42	100
11	5,7-$(OCH_3)_2$	CH_3	H	$C_{11}H_{12}N_2O_3$·$^1/_4H_2O$	CHN	245–248 dec	R	A	8.75	(3)	97	25	−40	2.08	25
12	5,8-$(OCH_3)_2$	CH_3	H	$C_{11}H_{12}N_2O_3$	CHN	214–215	N	D	8.75	(3)	54	13	−6	8.75	129
13	6,8-$(OCH_3)_2$	CH_3	H	$C_{11}H_{12}N_2O_3$·$^1/_2H_2O$	CHN[j]	254–256	N	D	8.75	(2)	60	13	−6	7.45	190
14	5,6-$(OH)_2$	CH_3	H	$C_9H_8N_2O_3$·HBr	CHN	324–326	T	E	8.75	(1)	39	0	8	>8.75	18
15	5,6-(OCH_2O)	CH_3	H	$C_{10}H_8N_2O_3$	CHN	267–270	O	F	1.875	(2)	71	13	−14	0.69	
16	6,7-(OCH_2O)	CH_3	H	$C_{10}H_8N_2O_3$	CHN	310–312	Q	G	8.75	(2)	66	10	1	5.65	
17	5,6,7-$(OCH_3)_3$	CH_3	H	$C_{12}H_{14}N_2O_4$	CHN	229–230	R	F	1.875	(1)	11	0	−9	>1.87	
18	6,7,8-$(OCH_3)_3$	CH_3	H	$C_{12}H_{14}N_2O_4$	CHN	208–210	O	C	1.875	(1)	10	3	−4	>1.87	
19	5,6-$(OCH_3)_2$	H	H	$C_{10}H_{10}N_2O_3$	CHN	242–244	N	C	1.875	(3)	101	10	−9	1.64	
20	5,6-$(OCH_3)_2$	C_2H_5	H	$C_{12}H_{14}N_2O_3$·$^1/_4H_2O$	CHN	224–226	S	D	1.875	(1)	112	20	9	0.51	18
21	6,7-$(OCH_3)_2$	C_2H_5	H				*k*		8.75	(1)	82	13	−28	3.4	
22	6,7-$(OCH_3)_2$	$CH(CH_3)_2$	H	$C_{13}H_{16}N_2O_3$	CHN	238–240	R	H	8.75	(3)	112	21	−27	1.39	43
23	6,7-$(OCH_3)_2$	$CH_2C_6H_5$	H				*k*		1.875	(1)	33	3	30	>1.87	13
24	6,7-$(OCH_3)_3$	c-C_5H_9	H	$C_{15}H_{18}N_2O_3$	CHN	196–197	R	C	8.75	(1)	71	11	−19	>8.75	22
25	6,7-$(OCH_3)_2$	C_6H_5	H				*k*		8.75	(1)	106	7	−25	4.1	14
26	6,7-$(OCH_3)_2$	CF_3	H	$C_{11}H_9N_2F_3O_3$·$^1/_2H_2O$	CHN	266–268	R	D	8.75	(1)	23	0	−15	>8.75	100
27	5,6-$(OCH_3)_2$	CH_3	CH_3	$C_{12}H_{14}N_2O_3$·$^1/_2H_2O$	CHN	172–174	U	H	1.875	(1)	31	5	6	>1.87	
amrinone									1.875	(6)	134	12	−10	0.38	60 (50)[l]

[a] Recrystallization solvents: A, acetone wash; B, acetone; C, not recrystallized; D, ethanol; E, ether wash; F, methanol; G, chloroform; H, 2-propanol. [b] Maximum dose administered. [c] Inotropic response produced by the maximum dose administered. [d] Effective dose of compound (mg/kg, iv) that produces a 50% increase in myocardial contractile force (CF) above control. ED_{50} values were obtained from three to four point dose–response curves using either graphic analysis (n = 1, 2) or linear regression analysis ($n \geq 3$) where n = number of animals. [e] IC_{50} = micromolar inhibitor concentration required to inhibit 50% of the enzyme activity, cardiac PDE-fraction III. The IC_{50} values are the result of at least two separate determinations at several inhibitor concentrations and are reported with a precision of ±15% (SEM) or better. [f] Cited in ref 10. [g] Anal. Calcd: N, 14.72. Found: N, 14.04. [h] Anal. Calcd: N, 16.65. Found: N, 16.16. [i] Anal. Calcd: C, 60.85. Found: C, 60.35. [j] Anal. Calcd: $C_{11}H_{12}N_2O_3$·$^1/_2H_2O$: C, 57.63; H, 5.71; N, 12.21. Found: C, 57.18; H, 5.64; N, 11.49. [k] Cited in ref 7. [l] Values cited in ref 1.

Table II. Physical Data of Intermediates



no.	R_1	R_2	R_3	R_4	R_5	R_6	formula	anal.	mp, °C	solvent[a]	meth
28		NO_2	H	H	OCH_3	OCH_3	$C_{11}H_{13}NO_6$	CHN	74–76	A	A
29		NH_2	H	H	OCH_3	OCH_3	$C_{11}H_{15}NO_4$	*b*	78–80	A	B
30		$NHCO_2C_2H_5$	H	H	OCH_3	OCH_3	$C_{14}H_{19}NO_6$	CHN	95–96	A	C
31	CHO	$NHCO_2C_2H_5$	H	H	OCH_3	OCH_3	$C_{12}H_{15}NO_5$	CHN	86–88	A	D
32	$COCH(CO_2C_2H_5)_2$	NO_2	H	H	OCH_3	OCH_3	$C_{16}H_{19}NO_9$	CHN	73–74	B	E
33	$COCH_3$	NO_2	H	H	OCH_3	OCH_3	$C_{10}H_{11}NO_5$	CHN	66–67	C	F
34	$COCH_3$	NH_2	H	H	OCH_3	OCH_3	$C_{10}H_{13}NO_3$	CHN	60–61	C	G
35	$COCH_3$	$NHCO_2C_2H_5$	H	H	OCH_3	OCH_3	$C_{13}H_{17}NO_5$	CHN	63–65	D	C
36	$COCH_3$	NH_2	H	H	OCH_3	H	$C_9H_{11}NO_2$	*b*	115		G[c]
37	$COCH_3$	$NHCO_2C_2H_5$	H	H	OCH_3	H	$C_{12}H_{15}NO_4$	CHN	90–92	A	C
38	$COCH_3$	$NHCO_2C_2H_5$	H	H	Cl	H	$C_{11}H_{12}ClNO_3$	CHN	63–64	A	C[d]
39	$COCH_3$	NO_2	H	H	$N(CH_3)_2$	H	$C_{10}H_{12}N_2O_3$	CHN	148–150	E	H
40	$COCH_3$	NH_2	H	H	$N(CH_3)_2$	H	$C_{10}H_{14}N_2O$	CHN	68–71	A	J
41	$COCH_3$	$NHCO_2C_2H_5$	H	H	$N(CH_3)_2$	H	$C_{13}H_{18}N_2O_3 \cdot {}^3/_4H_2O$	CHN[e]	161–163	E	C
42	$COCH_3$	NO_2	H	H		H	$C_{12}H_{14}N_2O_4$	CHN	139–141	E	H
43	$COCH_3$	NH_2	H	H		H	$C_{12}H_{16}N_2O_2$	CHN	104–106	F	J
44	$COCH_3$	NH_2	H	H		H	$C_{13}H_{18}N_2O$	CHN	64–65	G	J[f]
45	$COCH_3$	NO_2	H	H		H	$C_{13}H_{17}N_3O_3$	CHN	67–69	E	H
46	$COCH_3$	NH_2	H	H		H	$C_{13}H_{19}N_3O \cdot H_2O$	CHN[g]	90–92	G	J
47	H	$NHCONHCOCH_3$	H	OCH_3	H	OCH_3	$C_{11}H_{14}N_2O_4$	CHN	218–220	H	K
48	$COCH_3$	NO_2	OCH_3	H	H	OCH_3	$C_{10}H_{11}NO_5$	CHN	72–74		L
49	$COCH_3$	NH_2	OCH_3	H	H	OCH_3	$C_{10}H_{13}NO_3$	CHN	63–65		I
50	$COCH_3$	$NHCO_2C_2H_5$	OCH_3	H	H	OCH_3	$C_{13}H_{17}NO_5$	CHN	94–96	C	C
51	$COCH_3$	NO_2	OCH_3	H	OCH_3	H	$C_{10}H_{11}NO_5$	CHN	118–120	H	L
52	$COCH_3$	NH_2	OCH_3	H	OCH_3	H	$C_{10}H_{13}NO_3$	CHN	71–72	B	I
53	$COCH_3$	$NHCO_2C_2H_5$	OCH_3	H	OCH_3	H	$C_{13}H_{17}NO_5$	CHN	94–95	J	C
54	$COCH_3$	NO_2	H	H	5,6-(OCH_2O)		$C_9H_7NO_5$	*b*			L[h]
55	$COCH_3$	NH_2	H	H	5,6-(OCH_2O)		$C_9H_9NO_3$	*b*			J
56	$COCH_3$	NH_2	H	4,5-(OCH_2O)		H	$C_9H_9NO_3$	CHN	158–161	I	G[i]
57	$COCH_3$	$NHCO_2C_2H_5$	H	4,5-(OCH_2O)		H	$C_{12}H_{13}NO_5$	CHN	161–163	E	C
58	$COCH_3$	NH_2	OCH_3	OCH_3	OCH_3	H	$C_{11}H_{15}NO_4$	*b*			B[j]
59	H	$NHCONHCOCH_3$	H	OCH_3	OCH_3	OCH_3	$C_{12}H_{16}N_2O_5$	CHN	177–179	H	K
60	H	$NHCONHCOCH(CH_3)_2$	H	H	OCH_3	OCH_3	$C_{13}H_{18}N_2O_4$	*b*	176–179	H	K
61	H	NHCONHCO-c-C_5H_9	H	H	OCH_3	OCH_3	$C_{15}H_{20}N_2O_4$	*b*	155–159	H	K
62	H	$NHCONHCOCF_3$	H	H	OCH_3	OCH_3	$C_{11}H_{11}F_3N_2O_4$	*b*	190–193	H	K
63	CN	NO_2	H	H	OCH_3	OCH_3	$C_9H_8N_2O_4$	CHN	165–166	B	M
64	CN	NH_2	H	H	OCH_3	OCH_3	$C_9H_{10}N_2O_2$	*b*	*k*		I
65	CN	$NHCO_2CH_3$	H	H	OCH_3	OCH_3	$C_{11}H_{12}N_2O_4$	CHN	143–144	B	C

[a] Recrystallization solvents: A, hexane; B, methanol; C, 2-propanol; D, 2-propanol–hexane; E, water wash; F, ether; G, ether–hexane; H, acetone wash; I, ethyl acetate; J, ethyl acetate–hexane. [b] Characterized by TLC, NMR, and mass spectroscopy. [c] Intermediate NO_2 cited in ref 11. [d] Precursor amine cited in ref 12. [e] Anal. Calcd: C, 59.18. Found: C, 58.73. [f] Precursor nitro cited in ref 13. [g] Anal. Calcd: H, 8.43. Found: H, 7.98. [h] Prepared from acetophenone intermediate cited in ref 14. [i] Prepared from nitro precursor as cited in ref 15. [j] Intermediate nitro cited in ref 16. [k] Red oil.

esthetized open-chest dogs.[4,8] The effects of intravenously infused compound on myocardial contractile force, arterial blood pressure, and heart rate are reported in Table I. Cardiotonic activity is described as the dose required to increase contractile force by 50% over controls (ED_{50}). Canine cardiac fraction III cyclic AMP phosphodiesterase inhibition was assayed according to literature methods[9] and is expressed as IC_{50} values. The activity of the series is described in Table I.

Comparison of the observed cardiotonic activity for a series of 27 2(1*H*)-quinazolinones resulted in the following observation concerning structure–activity relationships

(8) Alousi, A. A.; Farah, A. I.; Lesher, G. Y.; Opalka, C. J. *Circ. Res.* **1979**, *45*, 666.

(9) Thompson, W. J.; Terasaki, W. L.; Epstein, P. M.; Strada, S. J. *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 69.

(SAR). Optimal potency for the series was observed with 1. The presence of a proton at the 1-position was preferred since replacement by methyl in analogue **27** resulted in reduced potency. A study of substituent effects at the 4-position revealed a small hydrophobic binding area and a limited bulk tolerance in that area. Optimal activity resided in the methyl (1), ethyl (**20**), and isopropyl (**22**) analogues while replacement with the larger benzyl (**23**), cyclopentyl (**24**), and phenyl (**25**) substituents reduced activity. Aromatic substitution studies indicated that a variety of 6-substituted derivatives possessed cardiotonic activity (**3–8**). Maximal efficacy resided with the 6-methoxy analogue **3**. Of the alkoxy-substituted analogues prepared, the 5,6-dimethoxy (1) and 5,6-methylenedioxy (**15**) derivatives were found to be the most potent cardiotonics of the series. Dimethoxy ring substituted analogues isomeric to 1 were much less active (**10–13**).

Using molecular modeling, Bristol and co-workers[1] reported a hypothetical five-point model for positive inotropic activity. This included a strong dipole at one end of the molecule adjacent to an acidic proton, a small hydrophobic pocket suitable for a methyl group at the 4-position region, flat topography, and a basic or hydrogen bond acceptor site opposite the dipole. We have found that the observed SAR for a series of 2(1*H*)-quinazolinones is in general agreement with the model proposed previously. However, the contribution of the small, methyl-specific binding region was not well defined for the present series of quinazolines. When substitution at the 4-position was varied in size, a limited contribution by smaller alkyl groups such as methyl and ethyl was noted in analogues **1**, **19**, **20** and **10**, **21**, **22**. Bulk tolerance in that area was also tolerated to a limited extent since some activity was retained when cyclopentyl or phenyl groups were present (**24**, **25**).

A study of the possible relationship between cardiotonic and PDE-III enzyme inhibition for the present series was undertaken. Generally, similar structural requirements were noted for both activities (Table I). These include the flat ring nucleus, hydrogen bond acceptor substitution, a dipole region at the opposite end of the molecule, and a somewhat limited hydrophobic "alkyl" pocket. The most potent cardiotonic in the series, 1, was one of the better enzyme inhibitors in the series. While PDE-III inhibition did not clearly predict the best cardiotonic in the series, it did indicate potential for activity since poor enzyme inhibitors tested were also found to be only weakly active cardiotonics.

On the basis of its inotropic potency and activity, further studies were undertaken with 1. These investigations showed that 1 was active in vitro as a positive inotropic agent and displayed selectivity for force over rate. Propranolol pretreatment did not block the activity of 1. Oral administration of 1 (2.5–10 mg/kg) to conscious instrumented dogs produced dose-related increases in dP/dt_{max} of 28–87% above control accompanied by increases in heart rate (15–39%) and modest reductions in mean arterial blood pressure (0% to −5%). Onset of activity occurred within 15 min and the duration exceeded 3 h at the 10 mg/kg dose.

Due to its favorable pharmacologic profile, 1 is currently under development as bemarinone hydrochloride (USAN), an orally effective cardiotonic agent. The complete description of the pharmacologic profile of 1 will be reported at a later date.

Experimental Section

Melting point determinations were done on either a Mel-Temp or Thomas-Hoover capillary apparatus and are uncorrected. All compounds reported had IR, UV, NMR, and mass spectra compatible with their structures and were homogeneous by TLC. Combustion analysis gave results within 0.4% of theory.

The procedures for the preparations of the reported compounds are listed as methods A–U and may be considered as general methods of preparation. The reported yields for the products obtained were not maximized.

Method A. 2,3-Dimethoxy-6-nitrobenzaldehyde Ethylene Acetal (28). A mixture of 2,3-dimethoxy-6-nitrobenzaldehyde (17) (16.0 g, 75 mmol), ethylene glycol (64 g, 103 mmol), and *p*-toluenesulfonic acid monohydrate (0.2 g) in benzene (750 mL) was heated to reflux with a Dean–Stark apparatus for 48 h. The solution was then poured into H_2O (1 L). The organic phase was washed with aqueous, saturated $NaHCO_3$ (2 × 20 mL), dried over Na_2SO_4, and filtered, and the solvent was removed in vacuo. The crude product was recrystallized from hexane (2 L) to give **28** (15.2 g, 78.2%), mp 74–76 °C.

Method B. 6-Amino-2,3-dimethoxybenzaldehyde Ethylene Acetal (29). A solution of **28** (39.12 g, 0.153 mol) in EtOAc (350 mL) was treated with NaOAc (1.2 g) and PtO_2 (2.45 g) and hydrogenated on a Parr apparatus at 50 psi for 1 day. The reaction mixture was filtered and the filtrate was concentrated to a brown syrup, 33.2 g (96.4%), which was crystallized from hexane to give **29** as a tan solid, mp 78–80 °C. This material was converted to the carbamate **30** without additional purification.

Method C. 6-(*N*-Carbethoxyamino)-2,3-dimethoxybenzaldehyde Ethylene Acetal (30). Ethyl chloroformate (1.9 g, 17.5 mmol) was added with stirring to **29** (1.6 g, 7.1 mmol) dissolved in THF (50 mL). An exothermic reaction occurred and a solid formed instantly. A solution of sodium hydroxide (0.72 g in 3.5 mL of H_2O) was added and the solution stirred for 2 h at room temperature. The THF was removed in vacuo and the residue extracted with $CHCl_3$ (2 × 100 mL), dried over Na_2SO_4, and filtered, and the solvent was removed in vacuo. The crude product was recrystallized from hexane to afford **30** (1.2 g, 57.1%), mp 95–96 °C.

Method D. 6-(*N*-Carbethoxyamino)-2,3-dimethoxybenzaldehyde (31). Compound **30** (5.0 g, 16.8 mmol) was dissolved in acetone (36 mL) and concentrated HCl (3 mL). The mixture was stirred at room temperature for 4 h. The solvent was removed in vacuo to give a yellow solid (3.9 g). Recrystallization from hexane gave pure **31** as a yellow solid, 3.6 g (84.7%), mp 86–88 °C.

Method E. Diethyl 2-(2,3-Dimethoxy-6-nitrobenzoyl)-propanedioate (32). 2,3-Dimethoxy-6-nitrobenzoic acid[18] (25 g, 0.11 mol) was added to $SOCl_2$ (50 mL) and the mixture was heated to reflux for 3 h. Excess $SOCl_2$ was evaporated in vacuo and the acid chloride was dissolved in toluene (40 mL) and Et_2O (40 mL) for the next step.

Grignard-quality magnesium (3.04 g, 0.125 mol) was treated with absolute EtOH (3.1 mL) and CCl_4 (0.5 mL), and Et_2O (100 mL) was added once vigorous reaction began. A solution of diethyl malonate (23.4 g, 0.146 mol) in Et_2O (18 mL) and EtOH (14 mL) was added over 1 h to maintain reflux. The mixture was heated to reflux an additional 3 h. The reaction solution was then diluted with toluene (40 mL) and Et_2O (80 mL).

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The acid chloride solution from above was added to the magnesium salt solution over a period of 0.5 h at reflux and that the resultant mixture was heated an additional 3 h. After the mixture was cooled to 15 °C, the precipitate was collected by filtration; this solid was then added to 10° H_2SO_4 (125 mL) and the resultant aqueous solution was extracted with CH_2Cl_2. The CH_2Cl_2 layers were dried over $MgSO_4$ and concentrated to give diester **32**, 53.8 g (95%), mp 71–73 °C. An analytical sample of **32** was prepared from MeOH, mp 73–74 °C.

Method F. 2,3-Dimethoxy-6-nitroacetophenone (33). Diester **32** (53.8 g, 0.105 mol) was heated to reflux in 60% aqueous HOAc (60 mL) containing H_2SO_4 (4.25 mL) for 7 h. Aqueous NaOH (4 g in 8 mL of H_2O) was added slowly with cooling and the solution was concentrated to an oil. This residue was dissolved in CH_2Cl_2, decolorized with charcoal, evaporated, and recrystallized from 2-PrOH to give the acetophenone **33**, 22.5 g (100%), mp 66–67 °C.

Method G. 6-Amino-2,3-dimethoxyacetophenone (34). Nitro ketone **33** (20.0 g, 0.089 mol) dissolved in MeOH (100 mL) containing 10% Pd–C catalyst (0.5 g) was hydrogenated with cooling in a Parr apparatus. The catalyst was removed by filtration, the filtrate concentrated, and the residue recrystallized from 2-PrOH to give **34**, 16.2 g (94%), mp 60–61 °C.

Method H. 5-Morpholino-2-nitroacetophenone (42). A mixture of 5-chloro-2-nitroacetophenone[12] (16.9 g, 85.1 mmol), morpholine (22.7 g, 261 mmol), and DMF (75 mL) was heated at 110 °C for 4.5 h. The solution was cooled to room temperature and poured into an ice–water mixture (500 mL). The resultant precipitate was collected by filtration and washed with H_2O and dried to yield 19.8 g (93.3%) of pure **42**, mp 139–141 °C.

Method I. 2-Amino-5-(dimethylamino)acetophenone (40). A mixture containing **39** (75.5 g, 363 mmol), glacial HOAc (491 mL), and H_2O (491 mL) was heated to 90–95 °C. Iron powder (154 g) was added portionwise over 0.5 h with vigorous stirring and the resultant reaction mixture was heated for an additional 2 h. Water (544 mL) was added to the cooled mixture, and the insoluble solids were removed by filtration. The aqueous filtrate was extracted with $CHCl_3$, dried over $MgSO_4$, and evaporated in vacuo to give a brown oil, which solidified upon standing. Crude **40** was recrystallized from hexane, giving 28.7 g (44.5%) of analytically pure yellow solid, mp 68–71 °C.

Method J. 2-Amino-5-morpholinoacetophenone (43). The nitroacetophenone **42** (18.5 g, 74.0 mmol) was dissolved in hot ethanol (250 mL) and added with stirring to a hot solution of $FeSO_4 \cdot 7H_2O$ (144 g, 518 mmol) in H_2O (750 mL). After the mixture was refluxed for 10 min, concentrated NH_4OH (175 mL) was added slowly over a 0.5-h period. The mixture was filtered through Celite and the pad was washed with EtOH. The aqueous solution was extracted with EtOAc (3 × 1 L), which was dried over $MgSO_4$ and evaporated in vacuo to give a residual solid, which was recrystallized from Et_2O to afford 12.6 g (77.0%) of pure **43**, mp 104–106 °C.

Method K. 1-Acetyl-3-(3,5-dimethoxyphenyl)urea (47). A mixture of 3,5-dimethoxyphenyl isocyanate (6.65 g, 37.1 mmol) and acetamide (2.19 g, 37.1 mmol) was fused under N_2 at 130–135 °C for 3 h. The initial clear melt solidified after 30 min. After cooling to room temperature, acetone was added and the resultant slurry was stirred for 2 h. The solid material was collected by filtration to give 7.90 g (89.4%) of pure **47**, mp 218–220 °C.

Method L. 3,6-Dimethoxy-2-nitroacetophenone (48). 2,5-Dimethoxyacetophenone (150 g, 832 mmol) was added dropwise to concentrated HNO_3 (750 mL) cooled to −20 °C. The reaction mixture was stirred at −20 °C for an additional 1.5 h and poured onto an ice–water mixture (8.0 L). The crude nitration product was collected by filtration, washed with H_2O (300 mL), and dried, giving 174 g (92%) of crude solid, which was purified by column chromatography (SiO_2 20% EtOAc/cyclohexane) to yield 133 g (71%) of pure **48**, mp 72–74 °C, as a yellow crystalline solid.

Method M. 2,3-Dimethoxy-6-nitrobenzonitrile (63). 2,3-Dimethoxy-6-nitrobenzaldehyde[17] (15.0 g, 0.071 mol) and hydroxylamine hydrochloride (6.42 g, 0.097 mol) were mixed in formic acid (200 mL) and heated to reflux for 2 h. The mixture was cooled to room temperature and quenched with of ice–H_2O (1.5 L). Solid KOH was added to alkalinity and the precipitate was collected by filtration, washed with H_2O, and air-dried to give the crude product (14.2 g, 96%).

A sample (2.2 g) of **63** was recrystallized from MeOH to give pure material, (1.90 g), mp 165–166 °C.

Method N. 5,6-Dimethoxy-2(1*H*)-quinazolinone (19). Ammonia was bubbled into EtOH (1 L) cooled to dry ice bath temperature for 1 h. Aldehyde **31** (45.1 g, 0.178 mol) was added and the resultant solution was heated to 130 °C in an autoclave for 6 h. The brown solution was treated with charcoal and filtered, and the filtrate was concentrated to 300 mL. A yellow solid precipitated upon cooling, which was collected by filtration to give **19**, 19.6 g (53.5%), mp 242–244 °C.

Method O. 5,6-Dimethoxy-4-methyl-2(1*H*)-quinazolinone Hydrochloride (1). Aminoacetophenone **34** (100 g, 0.513 mol) was dissolved in glacial HOAc (2 L) and treated with a filtered solution of potassium cyanate (50 g) in H_2O (200 mL) over 1 h with cooling to maintain the reaction temperature <25 °C. The mixture was stirred overnight and the precipitate was collected by filtration and washed with H_2O until the filtrates were colorless and acetone until the filtrate was colorless. Drying in vacuo gave a crude light yellow solid, 107 g (95%).

This solid was dissolved in concentrated HCl (1.1 L) at 35 °C, filtered, diluted with H_2O (6.2 L), and stored at 5 °C for 12 h. The yellow solid was collected by filtration, washed with acetone (600 mL), and air-dried to give **1** as a monohydrate 118 g (94%), mp 208–210 °C.

Method P. 5,6-Dimethoxy-4-methyl-2(1*H*)-quinazolinone Hydrochloride (1). Carbamate **(35)** (65.9 g, 0.246 mol) was placed in a flask and covered with NH_4OAc (365 g) and the mixture was heated in an oil bath until a clear melt was obtained. Stirring and heating of the melt at 125–130 °C for 2 h followed by quenching of the molten reaction in H_2O (2 L) gave 40 g (74%) of product after the filtered precipitate was dried. The material was converted to the hydrochloride salt as described above.

Method Q. 6-Chloro-4-methyl-2(1*H*)-quinazolinone (4). Dry ammonia gas was passed for 3 h through a solution of carbamate **38** (48.5 g, 0.20 mmol) and NH_4OAc (485 g) in DMF (200 mL) maintained at 155–160 °C. The reaction mixture was cooled and poured into ice–H_2O (1 L). The tan precipitate was filtered, washed with acetone, and dried to give 33.1 g (85.0%), mp 286–288 °C, of pure **4**.

Method R. 5,7-Dimethoxy-4-methyl-2(1*H*)-quinazolinone (11). A suspension of urea **47** (5.58 g, 23.4 mmol) in polyphosphoric acid (100 g) was heated at 140 °C for 3 h with stirring. The resultant clear red solution was quenched by the addition of ice–H_2O (400 g) and adjustment of the pH to 7.6 with concentrated NH_4OH. The yellow solid that formed was collected by filtration, washed with H_2O, and dried to give 4.80 g (93.0%) of **11**, mp 245–248 °C dec. The solid product was obtained in analytical purity by trituration with acetone, yielding 3.80 g (73.6%), mp 245–248 °C dec.

Method S. 5,6-Dimethoxy-4-ethyl-2(1*H*)-quinazolinone (20). EtMgBr (30.9 mL of a 2 N solution in THF, 0.061 mol) was added via syringe to dry THF (150 mL) and the solution was cooled in an ice bath. Carbamate **65** (7.3 g, 0.031 mol) in dry THF (100 mL) was added dropwise to the cooled Grignard reagent. The reaction mixture was warmed to room temperature for 2 h and recooled and ice–H_2O (50 mL) was added. H_2SO_4 (1 N, 10 mL) was added and the mixture was stirred for 3 days. Solvent was removed in vacuo without heat and the aqueous residue was extracted with Et_2O and with $CHCl_3$. The organic layers were dried over Na_2SO_4. The aqueous layer was neutralized with 5% aqueous sodium bicarbonate and the resultant solid was collected by filtration and combined with the dried organic extracts. Concentration and recrystallization of the solid from EtOH gave **20** as a yellow solid, 0.80 g (11.1%), mp 224–226 °C.

Method T. 5,6-Dihydroxy-4-methyl-2(1*H*)-quinazolinone Hydrobromide (14). A solution of **1** (0.813 g, 3.69 mmol) in 50 mL of 48% HBr and 50 mL of HOAc was heated at reflux for 16 h. The reaction mixture was cooled and the resulting precipitate that formed was collected by filtration. The product, **14**, was washed with Et_2O and dried, giving the analytically pure **14** as an orange solid, yield 0.691 g (68.4%), mp 324–326 °C.

Method U. 5,6-Dimethoxy-1,4-dimethyl-2(1*H*)-quinazolinone (27). To a mixture of 1.30 g (5.90 mmol) of **1** in THF (250 mL) and DMF (100 mL) were added NaH (0.14 g, 5.90 mmol, 60% dispersion in oil) and methyl iodide (0.64 g, 5.9 mmol).

The resultant suspension was heated at 50 °C for 6 h. The solvents were removed in vacuo, and the brown residue obtained was extracted with CCl_4. Evaporation of the extract gave crude **27**, which was recrystallized from 2-PrOH, giving **27** as a pale yellow solid (0.50 g, 35.2%), mp 172–174 °C.

Cardiotonic activity of the substituted quinazolines was determined according to the following general procedure.

Adult mongrel dogs are anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and artificially respired. Mean arterial pressure (MAP) is recorded from a cannulated femoral artery and drugs are infused into a cannulated femoral vein. The arterial pressure pulse is used to trigger a cardiotachometer for determination of heart rate (HR). Left ventricular pressure is measured with a Miller catheter and dP/dt_{max} is derived. A right thoracotomy is performed and myocardial contractile force (CF) is measured with a Walton Brodie strain gauge sutured to the right ventricle. A lead II EKG is also recorded. A standard dose of dopamine is administered to determine myocardial responsiveness.

Test compounds were solubilized in a small volume of DMF diluted to a final concentration of 10% in physiological saline. Alternatively, where possible, a soluble hydrochloride salt was prepared by addition of 0.1 N HCl diluted in physiological saline. Vehicles were tested in appropriate volumes and found to exert less than a 5% effect on contractile force. Compounds were administered by infusion pump (one drug per animal) at rates of 0.58–2.2 mL/min in three to four stepwise increasing doses. Each dose was infused over 5 min immediately after the effect of the previous dose peaked. MAP, HR, and CF responses were continuously monitored on a Beckman or Gould recorder and expressed as a percent change from predrug control values vs. the cumulative dose of drug administered. Quantitation of the inotropic potency was obtained by calculation of the contractile force 50% effective dose (CF ED_{50}). This is defined as the dose of compound that produces a 50% increase in myocardial contractile foce and is a means of comparing potencies among compounds. The value was obtained from three to four point dose–response curves using either graphic estimation (where $n = 1, 2$) or linear regression analysis (where $n \geq 3$).

For oral evaluations, a Konigsberg transducer was implanted into the left ventricle of dogs as well as arterial and venous catheters, using sterile technique. MAP, HR, and the peak of the first derivative of left ventricular pressure (LV dP/dt_{max}) was obtained as an index of contractility. ORF 16600 was administered as a hydrochloride salt solution in distilled H_iO by gavage tube.

Dog Heart Nucleotide Phosphodiesterase Fraction III (PDE-III). The PDE-III enzyme preparation was obtained from dog heart by the method described by Thompson.[9]

Briefly, crude supernatent is chromatographed on a DEAE-cellulose column equilibrated with a NaOAc buffer (pH 6.5) containing 30% ethylene glycol. The third fraction eluted from the column is isolated by a 70–1000 mM NaOAc gradient. The enzyme is dialyzed and stored, until use, at −20 °C in 70 mM NaOAc buffer (pH 6.5) containing 30% ethylene glycol. Potential inhibitors are tested at several concentrations in the presence of cyclic AMP (0.25 μM, containing 0.2 μCi of [^{3}H]cyclic AMP), enzyme, and 0.05 M Tris·HCl buffer (pH 7.4) containing 5 mM $MgCl_2$. The reaction is stopped by heating at 100 °C for 1 min. After cooling, 0.10 mL of a solution containing snake venom (1 mg/mL) is added to convert the enzymatic product, 5′-AMP, to adenosine. After 30 min, 1.0 mL of 33% Dowex AGIX8 slurry is added to remove unreacted excess cyclic AMP. An aliquot (0.2 mL) is removed from the resulting supernatant and [^{3}H]adenosine is quantitated by liquid scintillation spectrometry.

Activity of the inhibitor is expressed as the IC_{50} or the micromolar concentration of compound required to inhibit 50% of the PDE-III activity. The IC_{50} values are the result of at least two separate determinations at inhibitor concentrations ranging from 0.01 to 1000 μM and are reported with a precision of ±15% (SEM) or better.

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gem-Diphosphonate and *gem*-Phosphonate–Phosphate Compounds with Specific High Density Lipoprotein Inducing Activity

Lân M. Nguyen, Eric Niesor, and Craig L. Bentzen*

Symphar S.A., 6, chemin des Carpières, 1219 Le Lignon, Geneva, Switzerland. *Received July 23, 1986*

New diphosphonate compounds and related derivatives[1] were synthesized and investigated for their activity in specifically inducing plasma high density lipoproteins (HDL) and high density lipoprotein cholesterol (HDL-C) in normal rats. The screening of numerous compounds has permitted the determination of the structural variations leading to optimal plasma lipid altering activity, indicating antiatherosclerotic potential. Among the compounds observed to be the most active, dimethyl α-(dimethoxyphosphinyl)-*p*-chlorobenzyl phosphate (**20**, SR-202, mifobate) was selected for further pharmacological and subsequent clinical development.

In spite of the well-established relationship between high levels of plasma cholesterol and coronary heart disease, the treatment of hyperlipoproteinemia has remained a controversial issue, mainly because of a W.H.O. study[2] where clofibrate failed to produce the evidence for an overall beneficial effect. More recently however, the Lipid Research Clinics Program[3] provided conclusive results demonstrating the efficacy and safety of cholestyramine, a nonabsorbable bile acid sequestering resin, in reducing mortality and morbidity due to coronary heart disease. The study also clearly established the direct correlation between the increase in plasma high density lipoprotein cholesterol (HDL-C) concentration due to treatment and the protection from coronary heart disease.

Screening programs have been established by research groups with the purpose of identifying compounds that would increase HDL levels in humans.[4] This activity, however, has been evaluated by using cholesterol-fed rats in which it is well known that the HDL-C (α-cholesterol) levels are markedly depressed and are inversely correlated with dietary cholesterol intake.[3a] Under these conditions of cholesterol feeding, either a drug-induced reduction in cholesterol absorption or a decrease in plasma low and very

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