Synthesis of 3-[(2,3-Dihydro-1,1,3-trioxo-1,2-benzisothiazol-2-yl)alkyl] 1,4-Dihydropyridine-3,5-dicarboxylate Derivatives as Calcium Channel Modulators

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1,4-Dihydropyridine (DHP) derivatives with a 1,2-benzisothiazol-3-one 1,1-dioxide group, linked through an alkylene bridge to the C-3 carboxylate of the DHP ring, with both vasoconstricting and vasorelaxant properties were obtained. In blocking Ca^{2+}-evoked contractions of K^+-depolarized rabbit aortic strips, compounds 12 and 41 were 10-fold more potent than nifedipine; 27 other compounds were 1–4-fold more potent. Their vascular versus cardiac selectivity was very pronounced; for instance, the selectivity index for compound 41 was 70-fold higher than that of nifedipine. This was also true for the vasoconstricting compound 22, which was as potent as Bay K 8644 in enhancing the Ca^{2+}-evoked contractions of rabbit aorta strips, yet it had poor inotropic activity in rabbit left atria. Oral adminstration of compounds 38, 40, 43, and 53 (20 mg/kg) caused a 35–37% decrease in systolic blood pressure in spontaneously hypertensive rats (SHR); these effects were similar to those of nifedipine. However, iv administration of these compounds to anesthetized SHR caused a decrease in blood pressure which was more pronounced and long-lasting than that of nifedipine. When administered iv at 100 μg/kg, the vasoconstricting compound 22 caused a 40% increase in systolic and diastolic blood pressure. Compound 22 exhibited an unusually interesting feature over the other five Ca^{2+} DHP agonists: it had diester substitutions at the C-3 and C-5 positions of the DHP ring. Overall, compounds possessing these properties might be useful in treating clinical cardiovascular conditions in which DHP Ca^{2+} antagonists or agonists are indicated.

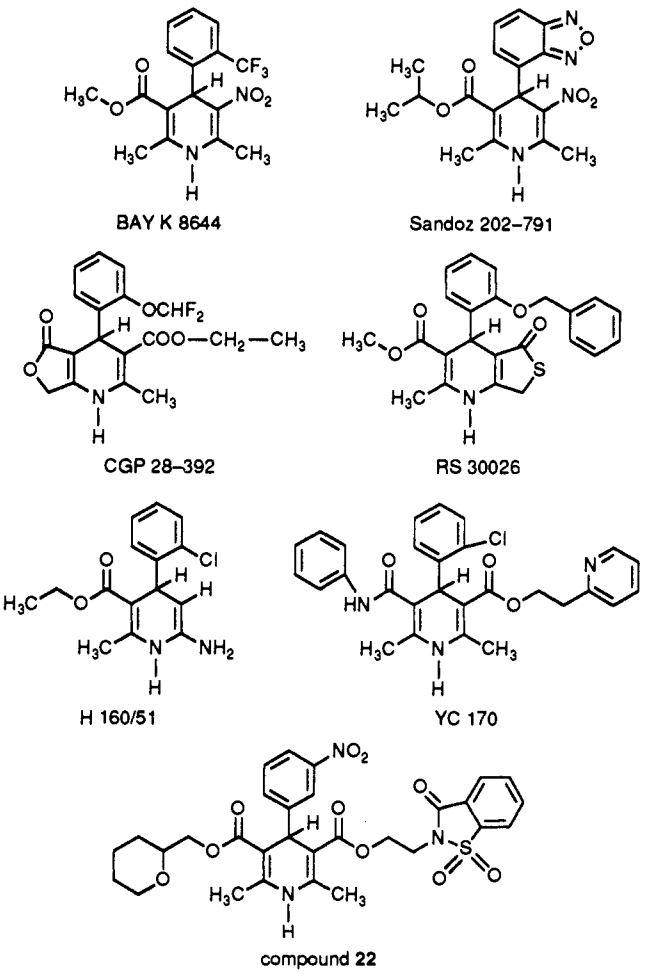
Introduction

The pharmacological modulation of the activity of voltage-sensitive slow Ca^{2+} channels has its origin in the pioneering finding of Fleckenstein[1] that verapamil decreased cardiac contractility and that excess Ca^{2+} counteracted such an effect. He coined the term Ca^{2+} antagonist to define drugs with these properties. Newer generation Ca^{2+} antagonists, 1,4-dihydropyridine (DHP) derivatives, are powerful arteriolar vasodilators with relatively few effects on the heart.[2] The synthesis of the nifedipine analogue Bay K 8644, which enhances rather than atteunates Ca^{2+} influx,[3] was the first of the Ca^{2+} channel activators. DHP Ca^{2+} channel activators described thus far, such as the 3-nitro derivatives Bay K 8644[3] and Sandoz 202-791,[4] the lactones CGP 28-392[5] and RS 30026,[6] the 2-amino derivative H 160/51,[7] and the amide YC 170,[8] constitute a structurally heterogeneous group with potent vasoconstrictor and cardiac positive inotropic effects (Chart I).

One of the desirable properties of novel Ca^{2+} channel antagonists and agonists as potential therapeutic tools is enhanced tissue selectivity. The discovery of various subtypes of Ca^{2+} channels with different tissue distribution[9] makes this goal attainable. For instance, DHP derivatives act preferentially on the vasculature, and because of this they can be used to lower blood pressure or to increase coronary blood flow without compromising the myocardium. However, the heart as well as skeletal muscle, bronchial muscle, and the brain are relatively insensitive to DHP in spite of the fact that these tissues contain high density of specific binding sites for these drugs.[10] Also, a selectivity for certain vascular beds might be desirable for treating pathological processes specifically affecting those vessels. This selectivity has been achieved for diltiazem, which blocks aortic muscle contracted with noradrenaline but not mesenteric arteries.[11]

In addition, DHP Ca^{2+} channel activators could have therapeutic potential as inotropic or vasoconstricting agents if true tissue selectivity could be found. However, this is not the case for the available compounds. Their

Chart I. Chemical Structure of Compound 22 and Six Other Known Ca^{2+} Agonists



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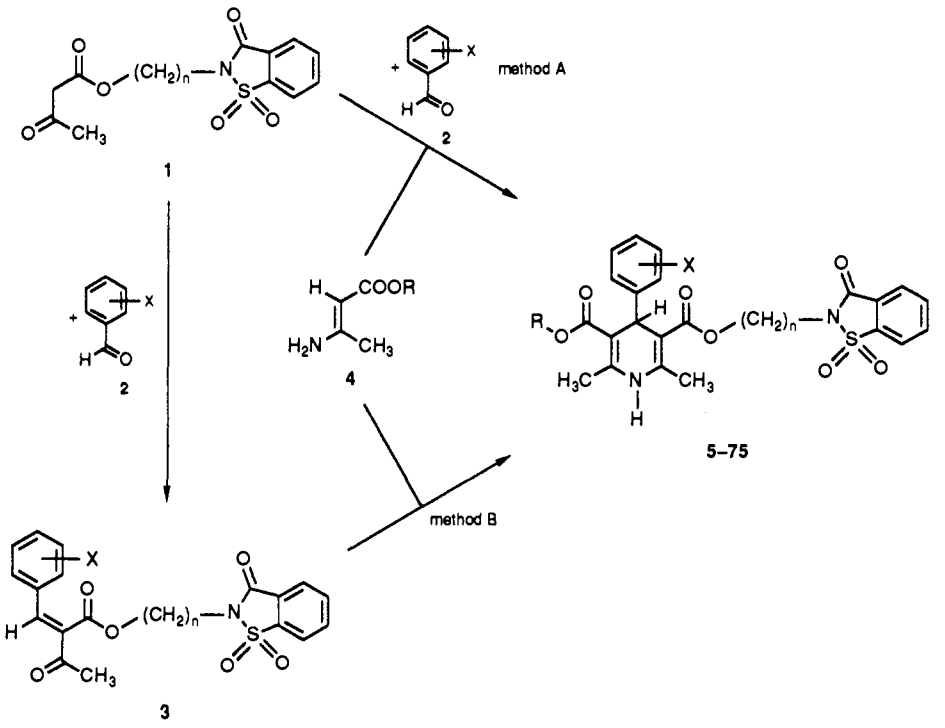
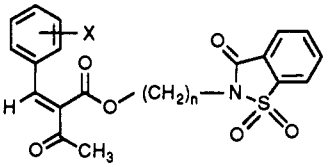
Scheme I. The Two Pathways (A and B) Used in This Study To Synthesize 1,4-Dihydropyridine (DHP) Derivatives with a 1,2-Benzisothiazol-3-one 1,1-Dioxide Group as Substituent on the C-3 Carboxylate Position of the DHP Ring



potent coronary vasoconstrictor effects[3,6] and their likely arrhythmogenic actions due to Ca^{2+} accumulation in cardiac fibers precludes their use as inotropic agents in cardiac heart failure or as vasoconstrictors in idiopathic hypotension or shock states. These problems have stimulated the continued synthesis of this type of compounds.

We have previously synthesized 4-alkyl-1,4-DHP derivatives with antithrombotic[12] or PAF-antagonist[13] (PAF, 1-*O*-hexadecyl/octadecyl-2-*O*-*sn*-glyceryl-3-phosphorylchlorine) activities. The 1,2-benzisothiazole-3-one 1,1-dioxide group was present as a substituent in the C-3 carboxylate position of the 4-alkyl-1,4-DHP derivatives with antithrombotic activity. On the basis that this functional group provides a diversity of actions, we reasoned that aryl-DHP derivatives with a saccharinic group in the C-3 position of the DHP ring could lead to compounds with interesting pharmacological properties on

Table I. Chemical Data for Benzylideneacetoacetates with a Substituted 1,2-Benzisothiazol-3-one 1,1-Dioxide Group



compd	X	*n*	% yield[a]	mp, °C	cryst solvent[b]	formula
3A	3-NO_2	2	85	145–7	A	$C_{20}H_{16}N_2O_8S$
3B	2-NO_2	1	65	140–1	A	$C_{19}H_{14}N_2O_8S$
3C	2-CF_3	2	87	135–7	B	$C_{21}H_{16}F_3NO_6S$
3D	2,3-Cl_2	2	95	114–6	B	$C_{20}H_{15}Cl_2NO_6S$
3E	2-Cl	2	92	163–5	B	$C_{20}H_{16}ClNO_6S$
3F	2-NO_2	3	75	112–3	B	$C_{21}H_{18}N_2O_8S$
3G	2-Cl	3	77	116–8	A	$C_{21}H_{18}ClNO_6S$
3H	2-CF_3	3	76	104–5	A	$C_{22}H_{18}F_3NO_6S$
3I	2,3-Cl_2	3	89	116–8	B	$C_{21}H_{17}Cl_2NO_6S$
3J	2-NO_2	2	85	161–3	C	$C_{20}H_{16}N_2O_8S$
3K	3-NO_2	1	65	175–7	A	$C_{19}H_{14}N_2O_8S$

[a] Refers to the recrystallized product. [b] Key: A, ethyl acetate; B, EtOH; C, MeOH. [c] Analytical data were within ±4% of the theoretical values.

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various cardiovascular parameters. This study reports the synthesis and cardiovascular profile of a series of 1,4-DHP derivatives whose common chemical feature is the presence of a 1,2-benzisothiazol-3-one 1,1-dioxide group, linked through an alkylene bridge to the C-3 carboxylate position of the DHP ring.

Chemistry

The 1,4-DHP derivatives were prepared by a modification of Hanztsch's synthesis as reported by Fax et al.[14] and illustrated in Scheme I (methods A and B).

Thus, compounds 1, obtained by procedures previously described,[15] were treated either with the appropiate aldehyde 2 to yield the benzylidene derivatives 3, which when condensed with the 3-aminocrotonate 4 yielded the corresponding 1,4-dihydropyridines 5–75. Alternatively, 1 was made to react in one step with the appropiate aldehydes 2 and 3-aminocrotonates 4.

3-Aminocrotonates of type 4 were synthesized according to literature methods,[16] unless commercially available.

Benzylidene compounds 3 were obtained according to the procedure described by Teller et al.,[17] in yields ranging from 65 to 95%. Chemical data of these compounds are shown in Table I.

The yields of 1,4-DHP 5–75 were in the range of 22–87%. Chemical data of these compounds are given in Table II.

Results and Discussion

Blockade of Calcium-Evoked Contractions in Depolarized Aortic Strips. In the presence of 35 mM K^+, the addition of 1.5 mM Ca^{2+} evoked contractile responses of rabbit aortic strips which were reproducible when repeated at 30-min intervals several times in the same preparation. Addition of increasing concentrations of nifedipine and most of the synthesized compounds evoked a progressive blockade of the Ca^{2+} response. Table II summarizes the chemical structures and the IC50's for blockage of aortic contractile responses.

Compounds 12 and 41 were 10-fold more potent than nifedipine, and compounds 13, 28, 38, 51, and 52 exhibited a 3–6-fold greater potency than the standard DHP. Approximately 22 additional molecules were equipotent or slightly superior to nifedipine. The remaining compounds exhibited a full range of potencies lower than the standard; four of them (compounds 22, 34, 50, and 62) were devoid of blocking effects.

The following order of activity was found for the aryl-substitutions: 2-CF_3 > 2,3-Cl_2 > 2-NO_2 > 2-Cl. It is clear that the longer the chain length (n) of the alkylenic bridge, the greater the activity. The six molecules referred to above have n = 2 or 3. However, compound 26 has n = 1, yet it is still twice as active as nifedipine. Finally, lower activity or even vasoconstricting activities were found when 2-methyltetrahydropyrane (THP) was a substituent in the C-5 carboxylate position of the DHP ring. This is the case for compound 22, which behaves as a potent vasoconstricting agent and increases blood pressure (see later discussion).

Cardiac versus Vascular Selectivity of Vasorelaxant DHP Derivatives. Though less cardiodepressant than the Ca^{2+} channel blockers benzothiazepine or dibenzylakylamine derivatives, 1,4-DHP derivatives have substantial negative inotropic effects. In rabbit isolated left atria, nifedipine depressed the basal contraction evoked by electrical field stimulation in a concentration-dependent manner. The IC_{50} needed to depress the heart beat was 113 nM (Table III); if divided by the IC_{50} needed to block Ca^{2+} contractions in rabbit aortic strips, a cardiac/vascular selectivity index of 39 was obtained. Compounds 27, 31, and 56 had a selectivity index 2–3-fold lower than nifedipine; compounds 27 and 56 were 2 times more potent than nifedipine in blocking vascular contractions, but compound 31 was a 10-fold poorer vascular blocker than nifedipine. Compounds 28 and 67 had indexes similar to that of nifedipine, but the other 14 compounds studied exhibited substantially higher selectivity indexes than the standard compound. Thus, compound 41 had an index of 2744, about 70-fold higher than nifedipine. In general, compounds exhibiting more potent vascular blocking effects had the higher selectivity index. The enhanced selectivity index of these compounds was accounted for by both a greater vasorelaxant effect and a poorer cardiodepressant effect, as illustrated by compounds 13, 38, 40, and 41. Thus, it seems that a true separation between both heart and vascular tissues as targets for DHP drugs can be achieved with these novel compounds.

Cardiac/vascular selectivity indexes for various Ca^{2+} channel blockers have been reported by Triggle and Janis[18] and by Ljund and Nordlander.[19] Verapamil has a selectivity index of 0.92 and diltiazem of 20; these are the two most cardiotropic Ca^{2+} antagonists known. The reported selectivity index for nifedipine (20) is almost the same as the one obtained in this study (39). Selectivity indexes for nitrendipine (80) and felodipine (103) are close to those of our compounds 15, 24, 42, 43, 53, 61, and 63. The most vasoselective compound reported so far, the DHP niludipine, exhibits a selectivity index (800) in the range of our compound 38 but still 4-fold lower than compound 41. To our knowledge, compound 41 exhibits the highest vascular over cardiac selectivity index (2744) thus far reported.

Separation of the Cardiac Positive Inotropic Effects from the Vasoconstricting Effects of 1,4-DHP Derivatives. Known Ca^{2+} antagonists and agonists exhibit poor vascular versus cardiac selectivity. For instance, the vasoconstricting relative potencies of Bay K 8644, CGP 28392, RS 30124, and RS 30026 to contract porcine coronary artery rings are parallel to their potencies to enhance the contractions of guinea pig papillary muscles.[6] This does not seem to be the case with some of the vasoconstricting compounds used in this study, which seem to be more vasculoselective than other reported compounds.

In rabbit aorta strips slightly depolarized with 10 mM K^+ in the absence of Ca^{2+}, the reintroduction of Ca^{2+} (1.5 mM) caused small contractions of the artery. Bay K 8644 (10^{-9}–10^{-6} M) dramatically enhanced the Ca^{2+} contractions in a concentration-dependent manner. Thus, at 10^{-6}M Bay K 8644 caused a contraction of 2570 mg (Table IV). Six of the compounds also had a pronounced vasoconstricting

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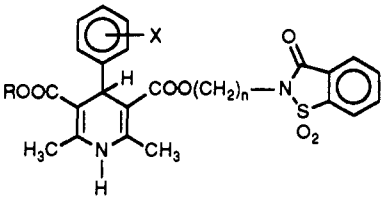
Table II. Chemical Structures and Blockade of Rabbit Aortic Contractions by 1,4-DHP Derivatives



compd	X	R[a]	n	method	time, h	% yield[b]	mp, °C	cryst solvent[c]	formula[d]	blockade of aortic contraction: IC_{50}[e]	relative potency[f]
5	3-NO_2	CH_3	1	A	10	43	187–8	A	$C_{24}H_{21}N_3O_9S$	15.1 (1–181)	0.2
6	3-NO_2	CH_3	2	A	10	67	179–82	A	$C_{25}H_{23}N_3O_9S$	39.8 (6–275)	0.1
7	3-NO_2	CH_3	3	A	8	70	185–8	A	$C_{26}H_{25}N_3O_9S\cdot{}^1/_2C_2H_6O$	2.6 (0.6–11)	1.1
8	3-NO_2	CH_3CH_2	1	A	8	40	186–8	A	$C_{25}H_{23}N_3O_9S$	3.3 (1–11)	0.1
9	3-NO_2	CH_3CH_2	2	A	10	87	89–91	A	$C_{26}H_{25}N_3O_9S$	2.6 (1–10)	1.1
10	3-NO_2	CH_3CH_2	3	A	8	55	143–5	A	$C_{27}H_{27}N_3O_9S$	1.9 (0.5–7)	1.5
11	3-NO_2	$CH_3(CH_2)_2$	1	A	16	76	164–5	C	$C_{26}H_{25}N_3O_9S$	64.5 (19–213)	<0.1
12	3-NO_2	$(CH_3)_2CH$	2	B	10	70	121–3	B	$C_{27}H_{27}N_3O_9S$	0.3 (0.1–0.7)	9.7
13	3-NO_2	$(CH_3)_2CH$	3	A	8	64	181–3	C	$C_{28}H_{29}N_3O_9S$	1.0 (0.3–4)	2.9
14	3-NO_2	$(CH_3)_3C$	1	B	12	70	207–9	A	$C_{27}H_{27}N_3O_9S$	7.8 (7–9)	0.4
15	3-NO_2	$(CH_3)_3C$	2	B	12	74	153–5	A	$C_{28}H_{29}N_3O_9S$	1.6 (1–2)	1.8
16	3-NO_2	$CH_3O(CH_2)_2$	2	B	10	51	126–8	D	$C_{27}H_{27}N_3O_{10}S$	28.8 (11–72)	0.1
17	3-NO_2	$CH_3O(CH_2)_2$	3	A	8	64	133–5	C	$C_{28}H_{29}N_3O_{10}S$	6.8 (3–17)	0.4
18	3-NO_2	2-THF-CH_2	1	A	10	72	157–9	A	$C_{28}H_{27}N_3O_{10}S$	25.1 (4–165)	0.1
19	3-NO_2	2-THF-CH_2	2	A	10	66	80–5	C	$C_{29}H_{29}N_3O_{10}S$	107.0 (21–537)	<0.1
20	3-NO_2	2-THF-CH_2	3	A	8	60	160–2	C	$C_{30}H_{31}N_3O_{10}S$	10.5 (5–23)	0.3
21	3-NO_2	2-THP-CH_2	1	B	10	65	129–32	C	$C_{29}H_{29}N_3O_{10}S$	13.3 (10–18)	0.2
22	3-NO_2	2-THP-CH_2	2	B	8	79	189–91	C	$C_{30}H_{31}N_3O_{10}S$	>1000	
23	2-NO_2	CH_3	1	B	10	75	242–4	C	$C_{24}H_{21}N_3O_9S$	30.9 (6–162)	0.1
24	2-NO_2	CH_3	3	B	10	62	191–3	A	$C_{26}H_{25}N_3O_9S$	1.5 (0.7–3)	1.9
25	2-NO_2	CH_3CH_2	3	B	16	60	166–8	A	$C_{27}H_{27}N_3O_9S$	2.6 (1–7)	1.1
26	2-NO_2	$(CH_3)_2CH$	1	B	10	79	176–8	A	$C_{28}H_{25}N_3O_9S$	1.4 (0.3–9)	2.1
27	2-NO_2	$(CH_3)_2CH$	3	B	12	60	177–9	C	$C_{28}H_{29}N_3O_9S$	1.2 (0.6–2.4)	2.4
28	2-NO_2	$(CH_3)_3C$	2	B	12	57	163–5	A	$C_{28}H_{29}N_3O_9S$	0.7 (0.6–0.8)	4.1
29	2-NO_2	$CH_3O(CH_2)_2$	1	B	10	67	180–2	C	$C_{28}H_{25}N_3O_{10}S$	5.1 (2–18)	0.6
30	2-NO_2	$CH_3O(CH_2)_2$	3	B	18	22	146–50	C	$C_{28}H_{29}N_3O_{10}S$	2.7 (1–7)	1.1
31	2-NO_2	2-THF-CH_2	1	B	10	63	168–70	A	$C_{28}H_{27}N_3O_{10}S$	38.0 (8–181)	0.1
32	2-NO_2	2-THF-CH_2	3	B	18	57	157–8	C	$C_{30}H_{31}N_3O_{10}S$	6.8 (2–19)	0.4
33	2-NO_2	2-THP-CH_2	1	B	12	44	141–3	A	$C_{29}H_{29}N_3O_{10}S\cdot C_2H_6O$	25.1 (9–59)	0.1
34	2-NO_2	2-THP-CH_2	2	B	24	35	143–6	A	$C_{30}H_{31}N_3O_{10}S\cdot{}^1/_2C_2H_6O$	>1000	
35	2-NO_2	2-THP-CH_2	3	B	12	64	170–2	C	$C_{31}H_{33}N_3O_{10}S$	11.5 (5–27)	0.3
36	2,3-Cl_2	CH_3	1	A	12	70	204–6	A	$C_{24}H_{20}Cl_2N_2O_7S\cdot{}^1/_2C_2H_6O$	5.1 (1–27)	0.6
37	2,3-Cl_2	CH_3	2	A	10	66	172–5	A	$C_{25}H_{22}Cl_2N_2O_7S$	17.3 (4–85)	0.2
38	2,3-Cl_2	CH_3	3	A	18	66	173–7	A	$C_{26}H_{24}Cl_2N_2O_7S$	0.8 (0.3–1.9)	3.6
39	2,3-Cl_2	CH_3CH_2	2	B	24	79	177–8	A	$C_{26}H_{24}Cl_2N_2O_7S$	4.0 (1–19)	0.7
40	2,3-Cl_2	CH_3-CH_2	3	B	24	41	172–4	C	$C_{27}H_{26}Cl_2N_2O_7S$	1.8 (0.8–4)	1.6
41	2,3-Cl_2	$(CH_3)_2CH$	2	B	20	79	194–5	A	$C_{27}H_{26}Cl_2N_2O_7S$	0.3 (0.1–1.1)	10.0
42	2,3-Cl_2	$(CH_3)_2CH$	3	B	24	70	165–7	A	$C_{28}H_{28}Cl_2N_2O_7S$	2.1 (0.7–6)	1.4
43	2,3-Cl_2	$(CH_3)_3C$	2	B	14	80	160–3	C	$C_{28}H_{28}Cl_2N_2O_7S$	2.3 (2–2.7)	1.3
44	2,3-Cl_2	$(CH_3)_3C$	3	B	20	58	201–3	A	$C_{29}H_{30}Cl_2N_2O_7S$	3.1 (3–4)	0.2
45	2,3-Cl_2	$CH_3O(CH_2)_2$	1	A	10	68	166–7	C	$C_{26}H_{24}Cl_2N_2O_8S$	2.9 (0.6–14)	1.0
46	2,3-Cl_2	$CH_3O(CH_2)_2$	2	B	20	46	153–6	A	$C_{27}H_{26}Cl_2N_2O_8S$	7.0 (1.7–29)	0.4
47	2,3-Cl_2	2-THF-CH_2	2	A	10	62	58–60	C	$C_{28}H_{28}Cl_2N_2O_8S$	2.1 (0.6–7)	1.4
48	2,3-Cl_2	2-THP-CH_2	2	B	18	66	177–9	A	$C_{30}H_{30}Cl_2N_2O_8S$	11.1 (8–16)	0.3
49	2,3-Cl_2	2-THP-CH_2	3	B	20	67	101–5	A	$C_{31}H_{32}Cl_2N_2O_8S$	4.0 (3–6)	0.7
50	2-CF_3	CH_3	2	B	20	78	195–7	A	$C_{26}H_{23}F_3N_2O_7S$	>1000	
51	2-CF_3	CH_3CH_2	2	B	20	76	147–8	A	$C_{27}H_{25}F_3N_2O_7S$	0.5 (0.1–1.9)	5.8
52	2-CF_3	CH_3CH_2	3	B	20	78	153–5	A	$C_{28}H_{27}F_3N_2O_7S$	0.8 (0.2–2)	3.6
53	2-CF_3	$(CH_3)_2CH$	2	B	20	77	146–7	C	$C_{28}H_{27}F_3N_2O_7S$	1.0 (0.3–3.8)	2.5
54	2-CF_3	$(CH_3)_2CH$	3	B	18	80	175–6	A	$C_{28}H_{29}F_3N_2O_7S$	2.7 (1–8)	1.1
55	2-CF_3	$CH_3O(CH_2)_2$	2	B	24	81	140–2	A	$C_{28}H_{27}F_3N_2O_8S$	1.4 (0.5–13)	2.1
56	2-CF_3	$CH_3O(CH_2)_2$	3	B	20	35	124–6	A	$C_{29}H_{29}F_3N_2O_8S$	1.4 (1–2)	2.1
57	2-CF_3	2-THF-CH_2	2	B	20	66	150–2	A	$C_{30}H_{29}F_3N_2O_8S$	2.5 (0.6–11)	1.2
58	2-CF_3	2-THF-CH_2	3	B	20	38	120–2	A	$C_{31}H_{31}F_3N_2O_8S$	3.7 (3–5)	0.8
59	2-CF_3	2-THP-CH_2	1	A	18	39	229–30	A	$C_{30}H_{29}F_3N_2O_8S$	3.6 (1–11)	0.8
60	2-CF_3	2-THP-CH_2	2	B	20	55	152–4	A	$C_{31}H_{31}F_3N_2O_8S$	25.5 (18–36)	0.1
61	2-CF_3	2-THP-CH_2	3	B	18	44	149–51	A	$C_{32}H_{33}F_3N_2O_8S$	1.4 (0.5–3.5)	2.1
62	2-Cl	CH_3	2	B	24	67	160–2	C	$C_{25}H_{23}ClN_2O_7S$	>1000	
63	2-Cl	CH_3	3	B	24	66	170–1	A	$C_{26}H_{25}ClN_2O_7S$	1.8 (0.9–4)	1.6
64	2-Cl	CH_3CH_2	2	B	24	65	168–70	A	$C_{26}H_{25}ClN_2O_7S$	10.0 (0.4–251)	0.3
65	2-Cl	CH_3CH_2	3	B	24	65	171–2	A	$C_{27}H_{27}ClN_2O_7S$	3.9 (1.7–9)	0.7
66	2-Cl	$(CH_3)_2CH$	2	B	24	75	186–7	C	$C_{27}H_{28}ClN_2O_7S$	3.1 (0.9–11.2)	0.9
67	2-Cl	$(CH_3)_2CH$	3	B	24	87	142–5	A	$C_{28}H_{29}ClN_2O_7S$	1.3 (1.1–1.6)	2.2
68	2-Cl	$(CH_3)_3C$	2	B	15	66	173–5	A	$C_{28}H_{29}ClN_2O_7S$	4.5 (4–6)	0.6
69	2-Cl	$CH_3O(CH_2)_2$	2	B	24	60	128–30	A	$C_{27}H_{27}ClN_2O_8S$	22.9 (2–281)	0.1
70	2-Cl	$CH_3O(CH_2)_2$	3	B	24	63	99–101	A	$C_{28}H_{29}ClN_2O_8S$	2.9 (2–3)	1.0
71	2-Cl	2-THF-CH_2	1	A	10	53	154–7	A	$C_{28}H_{27}ClN_2O_8S\cdot{}^1/_2C_2H_6O$	10.1 (4–25)	0.3

Table II (Continued)

compd	X	R[a]	*n*	method	time, h	% yield[b]	mp, °C	cryst solvent[c]	formula[d]	blockade of aortic contraction: IC_{50}[e]	relative potency[f]
72	2-Cl	2-THF-CH_2	2	B	24	67	139–41	C	$C_{28}H_{29}ClN_2O_6S$	3.5 (0.9–13.8)	0.8
73	2-Cl	2-THF-CH_2	3	B	24	41	128–30	A	$C_{30}H_{31}ClN_2O_6S$	7.1 (6–8)	0.4
74	2-Cl	2-THP-CH_2	2	B	24	40	106–8	A	$C_{30}H_{31}ClN_2O_6S \cdot {}^1/_2C_2H_6O$	348.5 (201–733)	<0.1
75	2-Cl	2-THP-CH_2	3	B	24	39	156–8	C	$C_{31}H_{33}ClN_2O_6S$	7.0 (6–9)	0.4
									nifedipine	2.9 (2.5–6.0)	1.0

[a] Key: THF = tetrahydrofuran; THP = tetrahydropyran. [b] Refers to the recrystallized product. [c] Key: A, EtOH; B, MeOH; C, ethyl acetate; D, ethyl acetate/*n*-hexane (1:1). [d] Analytical data were within ±0.4% of the theoretical values. [e] Nanomolar concentration required to block K^+-depolarized rabbit aorta by 50% (n = 8–10). 95% confidence limits in parentheses. [f] Nifedipine was used as the standard compound. Relative potency = IC_{50} of compound/IC_{50} of nifedipine.

Table III. Negative Inotropic Activity of Some Compounds and Their Selectivity Index

compd	cardiac inotropy: IC_{50}[a]	N[b]	selectivity index[c]
31	286.1 (122–123)	7	10
27	18.6 (9–39)	9	15
56	21.8 (16–29)	10	16
67	48.0 (33–71)	12	37
nifedipine	113.1 (67–192)	28	39
28	28.5 (19–43)	9	41
15	115.3 (68–197)	10	72
42	156.6 (64–386)	12	75
63	139.9 (115–289)	7	77
24	120.3 (58–248)	6	80
53	101.8 (43–238)	8	103
61	168.1 (77–369)	9	121
43	281.2 (178–455)	14	122
12	37.0 (4–380)	7	137
51	74.4 (32–172)	8	152
52	124.1 (63–243)	10	163
40	353.1 (108–1156)	10	200
13	209.7 (119–370)	8	205
38	521.0 (76–3590)	8	686
41	685.9 (100–4660)	19	2744

[a] Nanomolar concentration required to inhibit contraction of electrically stimulated rabbit left atria by 50%. 95% confidence limits are given in parentheses. [b] Number of experiments. [c] Selectivity index, IC_{50} of atrium/IC_{50} of aorta.

Table IV. Effects of 1,4-DHP Derivatives with Vasoconstrictor Activity, Compared with Bay K 8644[a]

compd	in vitro maximum contractile force, mg	N[b]	compd	in vitro maximum contractile force, mg	N[b]
Bay K 8644	2570 ± 100	9	19	570 ± 40	6
22	1940 ± 200	8	74	510 ± 50	6
34	990 ± 100	7	50	420 ± 170	6
62	610 ± 140	6			

[a] Rabbit aorta strips were slightly depolarized with 10 mM K^+ in the absence of Ca^{2+}. Then, contractions were elicited by adding 1.5 mM Ca. This procedure was repeated several times in the same preparation, in the presence of increasing concentrations of each compound. Usually, the maximum contractile activity was found at the concentration of 10^{-6} M. Data are means ± SEM of the contraction attained at 10^{-6} M. Number of strips used to study each compound.

effect under these experimental conditions. The most potent was compound 22 (Chart I), with a maximum contraction of 1940 mg, about 80% of the maximum contraction reached by Bay K 8644. In spite of this, the EC_{50} for Bay K 8644 was around 30 nM and for compound 22, around 10 nM.

In order to determine their cardiac positive inotropic effects, we compared the most potent vasoconstricting agent (compound 22) with Bay K 8644 in the electrically driven rabbit left atrium. Bay K 8644 enhanced the contractions in a concentration-dependent manner. At 10^{-6} M, this compound increased the basal atrial contractions

Table V. Effect of Compound 22 and Bay K 8644 on Electrically Driven Rabbit Left Atria

compd	N	contraction (% of initial)
vehicle (DMSO)	10	
1st addition		95.7 ± 4.5
2nd addition		95.7 ± 4.5
3rd addition		93.9 ± 6.7
22	6	
10^{-8} M		114.0 ± 5.4
10^{-7} M		129.3 ± 5
10^{-6} M		141.2 ± 5.1
Bay K 8644	6	
10^{-8} M		97.8 ± 3.8
10^{-7} M		232.2 ± 10.4
10^{-6} M		471.4 ± 11.3

Table VI. Recovery of the Initial Contraction of Rabbit Aortic Strips, Blocked by 10^{-7} M Concentrations of the Compounds (Mean ± SEM)

compd	% recovery[a]	N[b]	compd	% recovery[a]	N[b]
nifedipine	71.6 ± 5.0	5	53	0.5 ± 0.5	8
31	44.5 ± 3.8	4	27	0.2 ± 0.2	4
18	27.2 ± 2.4	4	28	0.2 ± 0.2	4
24	18.8 ± 4.7	8	15	0.0	4
56	18.3 ± 2.7	8	12	0.0	4
51	14.5 ± 0.8	4	38	0.0	6
63	6.05 ± 2.7	8	40	0.0	4
61	5.0 ± 4.2	4	41	0.0	14
13	1.6 ± 1.6	5	42	0.0	4
52	1.2 ± 0.9	4	43	0.0	4
69	0.5 ± 0.5	4			

[a] Percent recovery of the initial contraction. [b] Number of experiments.

by 471% (Table V). This was not the case for compound 22, which enhanced the initial basal contractions by only 41%.

From the chemical point of view, it is worth noting that vasoconstricting compounds differ from the Ca^{2+} channel agonists reported to date (see structural formulae in Chart I). Vasoconstricting compounds have diester substitutions in the C-3 and C-5 positions of the DHP ring; the most active compounds are those in which a heterocyclic group is substituted in C-5, particularly a tetrahydropyran, as in compounds 22 and 34. None of the compounds so far reported in the literature have this diester substitution. Therefore, Ca^{2+} agonists constitute a structurally heterogenous group to which a novel compound with a novel chemical structural feature is added. Theoretical calculations[20] and comparisons between crystal structures of some agonists with antagonists have led to hypotheses explaining the conformational differences between agonists and antagonists in terms of hydrogen bonding of the 1-NH

(20) Mahmoudian, M.; Richards, W. G. A conformational distinction between dihydropyridine calcium agonists and antagonists. *J. Chem. Soc. Chem. Commun.* 1986, 739–741.

Table VII. Rates of Reversal by Bay K 8644 of the Blockade of Rabbit Aortic Strips Produced by Nifedipine and 20 Selected Compounds[a]

compd	N^b	$T_{1/2}$, min	compd	N^b	$T_{1/2}$, min
53	7	43.4 ± 4.4	**31**	6	8.7 ± 0.4
41	8	36.8 ± 7.2	**27**	6	8.7 ± 1.0
28	6	20.5 ± 2.4	**12**	6	7.1 ± 0.2
38	8	17.0 ± 2.8	**63**	6	5.2 ± 0.3
43	6	12.6 ± 1.5	**24**	8	4.9 ± 0.2
40	6	12.0 ± 1.4	**67**	7	4.8 ± 0.7
51	7	11.2 ± 1.4	**13**	6	4.7 ± 0.3
42	8	11.1 ± 0.8	**61**	6	4.6 ± 0.4
18	6	9.6 ± 0.2	**56**	7	4.3 ± 0.6
52	6	9.3 ± 1.5	nifedipine	7	2.4 ± 0.3
15	6	8.8 ± 0.7			

[a] Blockade of contractions induced by 35 mM K^+ additions was achieved by adding 3×10^{-8} M of each compound to different aortic strips. Rates of contraction recovery are expressed as the time required to reach 50% of the initial contraction upon addition of Bay K 8644 (10^{-6} M). Data are means ± SEM. [b] Number of strips used for each compound.

group, hydrophobic fit, and ester group orientation.[21]

Slow Recovery of the Vasorelaxant or Vasoconstricting Effects. To test the duration of action of the most potent compounds found we performed in vitro experiments with rabbit aorta strips. Once stabilized, the initial contractions, evoked by Ca^{2+} reintroductions in tissues depolarized with 35 mM K^+, were blocked completely with 3×10^{-8} M of every compound tested. Then, the strips were washed at 30-min intervals with a 35 mM K^+ Ca^{2+}-free solution and their ability to contract upon Ca^{2+} additions retested. Table VI shows that after a 4-h washout, the response to Ca^{2+} of the tissues treated with nifedipine recovered by over 70%. Only four of the 20 compounds tested partially recovered the response to Ca^{2+} (18–44%). The remaining 16 compounds showed a persistent blockade of Ca^{2+} contractions even after 4 h of washing out with fresh K^+-enriched solution.

Because this washout procedure discriminated poorly between different compounds, another protocol was performed. Contractions evoked by addition of 35 mM K^+ were blocked by 3×10^{-8} M nifedipine or by 21 of the most potent vasorelaxing compounds (around 80–90% of the initial contraction). Then, Bay K 8644 (10^{-6} M) was added to each compound in order to reverse the blockade induced by each compound. After adding Bay K 8644, the tissues started to contract at different rates, depending on the compound, until reaching a stable plateau. Then, the time to reach 50% of the initial contraction in the absence of any drug was measured and taken as the $t_{1/2}$ of each molecule to reverse the blockade. Table VII shows the $t_{1/2}$ in decreasing order. Compound **24** exhibited a $t_{1/2}$ of 43.4 min, 18-fold longer than that of nifedipine (2.4 min). In general, a good correlation between $t_{1/2}$ and the potency to block the aortic contractions to Ca^{2+}, was found: the longer the $t_{1/2}$, the smaller the IC_{50}.

A similar experimental design was performed with compound **22** and Bay K 8644. This time the tissues were depolarized with only 10 mM K^+. The potentiation of the contractions evoked by Ca^{2+} (1.5 mM) were then elicited in the presence of 10^{-6} M of each of the vasoconstricting agents. Then, the tissues were thoroughly washed at 30-min intervals with a fresh solution containing 10 mM K^+ (ca^{2+} free) and the Ca^{2+} contractions retested. While the potentiation evoked by Bay K 8644 completely disappeared after 2 h of washing out, the potentiation evoked by compound **22** was still 80% of the initial contraction after 5 h of washing out.

The long duration of action of these compounds is unlikely to be associated either to the aryl substitutions or to the substitutions in the C-5 position of the 1,4-DHP ring. We suggest the presence of the saccharinic group as responsible for the long duration of the vasorelaxant and vasoconstricting effects of these compounds.

Table VIII. Effect of the Compounds on Systolic Blood Pressure After Administration of a Single Oral Dose (20 mg/kg) to Conscious SHR (mean ± standard error, n = 12)

compd	% SBP change[a]	compd	% SBP change[a]
53	37 ± 2.0*	**15**	25 ± 3.0*
43	36 ± 2.7*	**12**	20 ± 2.1*
40	36 ± 2.3*	**42**	11 ± 3.1
38	35 ± 2.6*	**41**	8 ± 3.7
nifedipine	35 ± 2.5*		

[a] SBP, systolic blood pressure. *$P < 0.05$, with respect to the initial basal values.

Table IX. Effective Dose and Duration of Action of Some Compounds and Nifedipine on Anesthetized SHR after Intravenous Administration (mean ± SEM)

compd	ED_{25}[a] SBP	ED_{25}[a] DBP	duration of action, min
38	12 ± 0.8	10 ± 0.4	>15
40	15 ± 1.4	12 ± 1.2	2
43	14 ± 1.5	11 ± 1.1	>15
53	9 ± 1.1	8 ± 1.2	>15
nifedipine	33 ± 2.1	31 ± 2.2	3

[a] ED_{25}, dose (μg/kg) reducing the blood pressure by 25%. SBP, systolic blood pressure. DBP, diastolic blood pressure.

Table X. Effects of Bay K 8644 and Compound 22 on Systolic (SBP) and Diastolic Blood Pressure (DBP) in Anesthetized Normotensive Rats[a]

	N^b	SBP[c]	DBP[c]
Bay K 8644	3	100.0 ± 5.0 (170 ± 3)	53.3 ± 2.9 (153 ± 6.5)
compound **22**	4	58.1 ± 12.9 (143 ± 9)	40 ± 6.8 (142 ± 9.3)

[a] The compounds were administered iv at a dose of 100 μg/kg, and the peak increase in blood pressure was recorded. Values are means ± SEM; they represent increases in mmHg above basal initial pressures. [b] Number of animals. [c] Percent increases of blood pressure are in parentheses.

Effects on Blood Pressure. When orally administered to spontaneously hypertensive rats (SHR) (20 mg/kg) some of the compounds tested produced a marked decrease in systolic blood pressure (SBP) measured in the tail of conscious animals. One hour after administration, compounds **38**, **40**, **43**, and **53** produced a 35–37% lowering of SBP, values similar to those obtained with nifedipine (35%). Compounds **12**, **15**, **41**, and **42** had lower effects than nifedipine (Table VIII). The duration of the antihypertensive action of some of these compounds is shown in Table IX. Anesthetized SHR were given various iv doses of each compound to estimate their ED_{25} to lower SBP and diastolic blood pressures (DBP). The four compounds exhibiting higher potency to lower blood pressure when orally administered (**38**, **40**, **43**, and **53**) had lower ED_{25} values and substantially longer duration of hypotensive actions than nifedipine.

Administration of 100 μg/kg iv of each compound caused an increase in both the SBP and the DBP (Table X). However, there is a distinction between the increases produced by Bay K 8644 and by compound **22**. While the former increased the SBP (70% above control to a greater

(21) Langs, D. A.; Triggle, D. J. Conformational features of calcium channel agonist and antagonist analogs of nifedipine. *Mol. Pharmacol.* 1985, *27*, 544–548.

extent than the DBP (53%), the latter similarly increased both pressures (around 40%). The lesser increase in SBP by compound 22 is probably related to its scarce inotropic effects (see Table V).

In conclusion, the cardiovascular profile of 1,4-DHP derivatives with a 1,2-benzisothiazol-3-one 1,1-dioxide group has been evaluated. Some compounds behave as Ca^{2+} agonists and others act as potent Ca^{2+} antagonists, possessing pronounced vasorelaxing effects and a poor negative inotropic effect; therefore, their vascular over cardiac selectivity is very high. Compound 22 is a potent vasoconstricting agent in peripheral vessels, yet it produces very little coronary vasoconstriction. Both types of compounds, antagonists and agonists, show a long duration of action. Compounds 22 (agonist) and 38 (antagonist) are being subjected to further development.

Experimental Section

Chemistry. All melting points were determined in a Büchi 510 open capillary melting point apparatus and were uncorrected. The structures of all compounds were documented by IR and NMR spectra. IR spectra were obtained with a Perkin-Elmer 881 spectrometer and NMR spectra were recorded on a Varian T-60 spectrometer. All elemental analyses were within ±0.4%.

General Procedure for Preparation of (1,1,3-Trioxo-2,3-dihydro-1,2-benzisothiazol-2-yl)alkyl Benzylideneacetoacetate (3). A mixture of equimolecular amounts of compounds 1 and 2 in isopropyl alcohol (650 mL/mol of the chemical intermediate) was prepared in the presence of acetic acid and piperidine (0.06 mol/mol of the intermediate) and was stirred at 40 °C for 1 h, at room temperature for 16 h, and at 0 °C for 2 h. Finally, the reaction mixture was left standing at −10 °C overnight. The compound thus obtained was filtered and purified by recrystallization in the appropriate solvent as shown in Table I.

General Procedure for Preparation of (1,1,3-Trioxo-2,3-dihydro-1,2-benzisothiazol-2-yl)alkyl 5-(Alkoxycarbonyl)-4-aryl-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (5–75). The compounds can be obtained by two methods, A and B. The reactions must be carried out in the dark to protect the dihydropyridine ring against oxidation.

Method A. Equimolar amounts of compounds 1, 2, and 4 were mixed in absolute ethanol (1 L/mol of reagent) and stirred under reflux for the periods of time given in Table II. Afterward, the reaction mixture was treated in one of three ways: (1) cooled and filtered, (2) concentrated under reduced pressure, and (3) concentrated to dryness and addition of the adequate solvent, generally ethyl acetate, to achieve the product crystallization. The products were recrystallized from the appropiate solvent as shown in Table I.

Method B. A mixture composed by equimolar amounts of compounds 3 and 4 in absolute ethanol (1 L/mol of reagent) was stirred under reflux for the periods of time given in Table I. The resulting reaction mixture was processed the same way as that indicated in method A.

Biological Methods. Contractions of Rabbit Aorta. Male New Zealand rabbits weighing 1.5–2.5 kg were sacrificed, and the thoracic aortas removed and placed in Krebs-bicarbonate buffer. Excess fat and tissue were removed, adn the aorta was cut in helicoidal strips.[22] Strips were mounted in organ baths under a 2.5-g preload in a Krebs-bicarbonate solution, at 37 °C and bubbled with 95% O_2 + 5% CO_2 (final pH 7.4). Equilibration for 1 h was allowed. The aorta was washed every 20 min to avoid interference of metabolites. Afterward a depolarization was induced by adding 35 mmol K^+ (without osmotic adjustments), and 10 min later 1.5 mmol Ca^{2+} was added to evoke contractions. This process was repeated until a reproducible response was achieved. Strips were thereafter exposed to increasing concentrations of compounds or nifedipine 20 min before and during the Ca^{2+} addition period. Responses in the presence of each DHP concentration were recorded and normalized with respect to initial recorded tensions. IC_{50} values were determined from concentration–response curves by the method of Finney.[23]

When calcium-agonism or vasoconstrictor properties were studied once the equilibrium period with Krebs Ca^{2+}-free buffer was accomplished, the buffer was changed to one containing 10 mM K^+, and repeated pulses of 1.5 mM Ca^{2+} were given until reproducible contractions were achieved. Thereafter, the first dose of the assay compound was given, and after 10 min, contractions were induced by another pulse of 1.5 mM Ca^{2+}. Other doses were then administered in a similar way. The maximum contractile force in milligrams was calculated as the vasoconstrictor effect of the compound. The length of the control contraction was measured in millimeters and compared with those achieved after the different doses of the compounds, and the percent potentiation was calculated in comparison with the highest, which was considered to be 100%. Then, an effective concentration (EC_{50}) was calculated as above.

Isolated Rabbit Atria. Male New Zealand rabbits (2–2.5 kg body weight) were sacrificed by cervical dislocation. After thoracotomy the hearts were immediately removed, and the atria separated from the ventricles and mounted in organ baths (40 mL) containing bicarbonated Krebs–Henseleit buffer bubbled with 95% O_2 + 5% CO_2 at 32 °C. Inotropic effects were recorded from electrically driven left atria by Grass S-9 stimulator (1 Hz frequency, 10 ms duration, and voltage double than threshold).

Left atria were allowed to equilibrate for 1–1.5 h before starting the experiment, and thereafter compounds were added to the bath in cumulative concentrations (10^{-10}–10^{-6} M). Stock solutions of compounds and nifedipine (10^{-2} M) were prepared in DMSO and then diluted in Krebs–Henseleit buffer. The final concentration of DMSO in the organ bath was always <0.1%.

Isometric contractions were measured continuously by using a LETICA LE 5700 force-displacement transducer and recorded on a GRAPHIC 1002 LLOYD instrument recorder. The IC_{50} values were calculated from contractile force changes and expressed with respect to control, initial contractile values.

Washout Studies. The washout recovery percentage was recorded on isolated rabbit aorta prepared as above. After blockade by each compound (10^{-7} M) of Ca^{2+}-evoked contractions of depolarized rabbit aortic strips, the preparations were washed out at 30-min intervals with drug-free Krebs solution containing 35 mM K^+ but no Ca^{2+}. Contractions were subsequently evoked, at 30-min intervals, with Ca^{2+} additions. The percent recovery of the initial contraction was recorded. The washout period was extended for 4 h.

Reversal by Bay K 8644 of the Blockade of K^+-Evoked Aortic Contractions. Helical strips of albino rabbit thoracic aorta were prepared and mounted as described above. After the initial equilibration, they were contracted by adding 35 mM K^+ to the organ bath, without osmotic adjustments. The stable response (usually upon the third or fourth addition) was termed 100%.

Then, contractions were blocked around 80–90% by adding 3×10^{-6} M of a given compound for 1 h; reversal of blockade was achieved by adding 10^{-6} M Bay K 8644 to each compound, until reaching a plateau. The $t_{1/2}$ for recovery was defined as the time elapsed from the addition of Bay K 8644 until the recovery of 50% of the initial contraction obtained in the absence of any compound.

Studies with Anesthetized Rats. In vivo measurements of changes in arterial blood pressure were carried out in male Wistar *anesthetized* rats (normotensive rats) or Okamoto strain rats (spontaneously hypertensive rats, SHR), weighing 290 ± 30 g. Animals were prepared for iv administration by anesthetization with sodium pentobarbitone (70 mg/kg, ip). After cannulation of the trachea, the arterial blood pressure was recorded from the left carotid artery, and the compounds were administered intravenously through the right femoral vein. The peripheral electrocardiogram (DII) was analyzed by subcutaneous electrodes. A 7758B Hewlett-Packard physiopolygraph was used for final measurements.

(22) Furchgott, R. F.; Bhadrakon, S. Reactions of strips of rabbit aorta to epinephrine isopropyl acterenol, sodium nitrate and other drugs. *J. Pharmacol. Exp. Ther.* **1953**, *10*, 129–143.

(23) Finney, D. J.; Statistical Methods in Biological Assay, 3rd ed.; Charles Griffin and Co.: London, 1978.

Once the blood pressure was stabilized, the control values were taken and the compounds were injected intravenously (bolus) in cumulative and increasing doses. The effects were recorded continuously during 15 min. The dosages were 1, 3, 6, 10, 20, and 30 μg/kg when antihypertensive effects were recorded and 0.1 mg/kg when the hypertensive potency was evaluated. The compounds were dissolved in polyethylene glycol 400 (PEG-400; Merck, Germany) (0.1 mL/kg).

In *conscious* animals, systolic blood pressure was monitored by tail plethysmography. The animals were trained during 2 weeks prior to the monitoring procedure. On the day of the experiment the rats were placed at 31 ± 1 °C for 2 h, and blood pressure was recorded.

The measurement was the average of five determinations each time. Only animals with basal values over 170 mmHg systolic blood pressure were considered as hypertensive. After 24 h of fasting, the selected hypertensive animals were administered the compound and/or vehicle [20% PEG-400 in 0.5% aqueous methylcellulose (BDH) (10 mL/kg)] orally by gastric gavage. Data were expressed as percent change at 2 h post-treatment. Statistical analysis was performed by means of SPSS programs (ANOVA and Student's *t* test).

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Inhibition of N^8-Acetylspermidine Deacetylase by Active-Site-Directed Metal Coordinating Inhibitors

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A number of substrate analogues of N^8-acetylspermidine (N^8-AcSpd) (16) and chemical modifying agents containing metal coordinating ligands were assayed as inhibitors of the cytoplasmic enzyme N^8-AcSpd deacetylase from rat liver. The enzyme is inhibited by metal chelators, several ω-amino-substituted carboxylic acids, and some thiol reagents. Inhibition by diisopropyl fluorophosphate was observed only at high concentrations. These results suggest that the catalytic mechanism of the enzyme requires a transition state metal and free sulfhydryl groups for activity. The most potent inhibitor synthesized 6-[(3-aminopropyl)amino]-*N*-hydroxyhexanamide (15), has an apparent K_i of 0.001 μM. It binds to the target enzyme 11 000 times tighter than the substrate (apparent K_m = 11 μM). These compounds and a previously reported series of compounds (Dredar, S. A.; Blankenship, J. W.; Marchant, P. E.; Manneh, V. A.; Fries, D. S. *J. Med. Chem.* **1989**, *32*, 984–989) are useful in mapping the active site and determining the physiological function of N^8-AcSpd deacetylase.

The naturally occurring polyamines, putrescine, spermidine, and spermine, play important roles in the regulation of DNA and RNA function and thereby affect cell growth and differentiation.[1-3] In previous publications from our laboratory,[4,5] the biochemical pathways of polyamine metabolism and interconversion were discussed. The focus of our work is the nuclear enzymatic acetylation of spermidine selectively at the N8 position[6,7] and the cytoplasmic deacetylation of this product to regenerate spermidine.[8,9] Polyamines, especially spermine and spermidine, are known to bind to and to stabilize DNA and chromosomal structure.[10-12] N-Acetylated polyamines have a reduced number of positive charges and are less efficient at stabilizing DNA than their nonacetylated precursors.[11] Thus, a simple hypothesis, that is depicted by the model shown in Figure 1, has been proposed to explain the function of the enzymatic nuclear acetylation and cytoplasmic deacetylation of spermidine. Inhibitors of the acetyltransferase and deacetylase enzymes are required to test the hypothesis. In this paper we describe the design, synthesis, and in vitro activity of potent inhibitors of N^8-acetylspermidine (N^8-AcSpd) deacetylase.

Care should be taken not to confuse the nuclear acetylation of spermidine with the cytoplasmic acetyltransferase which acetylates spermidine at the N^1 position.[13,14] N^1-Acetylspermidine is acted upon by polyamine oxidase to produce putrescine and 3-aminopropanal,[15,16] while N^8-AcSpd is deacetylated.

Table I. In Vitro Inhibition of N^8-AcSpd Deacetylase from Rat Liver Cytosol

no.	structure or name	app K_i (μM)[a]
1	diisopropyl fluorophosphate	33000
2	*p*-(hydroxymercurio)benzoate	150
3	*N*-ethylmaleimide	860
4	iodoacetamide	NI[b]
5	EDTA	3500
6	2,2′-dipyridyl	1900
7	1,10-phenanthroline	1300
8	4,7-phenanthroline	NI[b]
9	sodium butyrate	38500
10	$H_2N(CH_2)_3COOH$	36500
11	$H_2N(CH_2)_4COOH$	4100
12	$H_2N(CH_2)_5COOH$	50
13	$H_2N(CH_2)_4NHCOCH_3$	800
14	$H_2N(CH_2)_3HN(CH_2)_5COOH$	11
15	$H_2N(CH_2)_3HN(CH_2)_5CONHOH$	0.001
16	$H_2N(CH_2)_3HN(CH_2)_4NHCOCH_3$ (substrate)	11 (K_m)

[a] The apparent K_i values were determined from Dixon plots. [b] NI = no inhibition to 10^{-3} M.

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