Synthesis and Quantitative Structure–Activity Relationships of *N*-(3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines as Na/H Exchange Inhibitors

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***N*-(3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines 4 were prepared and tested for Na/H exchange inhibitory activities in order to clarify the structure–activity relationship (SAR). Quantitative SAR (QSAR) analysis of 6-carbonylguanidines 4 indicated that the length of the 4-substituent was parabolically related to activity and that the calculated optimum 4-substituents were propyl, ethyl and isopropyl groups. This SAR was similar to the SAR of the 2- and 4-substituents of 7-carbonylguanidine derivatives 3, although the position relative to the essential guanidinocarbonyl group was different. Larger 2-substituents, such as a phenyl group were unfavorable. The most potent derivative in this series was *N*-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidine 4g, with an IC_{50} value of 0.12 μM. The methanesulfonate salt (KB-R9032) of 4g had excellent water-solubility and showed anti-arrhythmia activity against a rat acute myocardial infarction model. KB-R9032 was selected for further investigation as a therapy for ischemia-reperfusion induced injury.**

Key words Na/H exchange inhibitor; ischemia-reperfusion injury; KB-R9032; quantitative structure–activity relationship

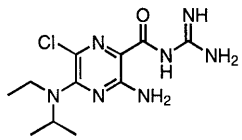
The Na/H exchanger is a major Na^+ entry pathway in many types of cell and plays an important role for regulation of cell volume and ion concentration, and is rapidly activated at post-ischemic reperfusion and causes a Na^+ overload. In myocardial cells, a Na^+ overload induces a Ca^{2+} overload, which is known to be associated with cellular dysfunction, damage and necrosis. Therefore, a Na/H exchange inhibitor is a potentially useful candidate for improvement of ischemia-reperfusion induced injury.[1)]

EIPA **1**[2)] and HOE-694 **2a**[3)] are known to be Na/H exchange inhibitors (Chart 1), but there are no inhibitors used clinically. After our project was initiated, HOE-642[4)] was reported to be undergoing clinical trials in the United States and Europe. In our previous paper, we investigated the structural requirements of bicyclic aroylguanidines as potent Na/H exchange inhibitors.[5)] Based on these results, we designed *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine **3a**.[6)] The optimization of **3a** was carried out using quantitative structure–activity relationship (QSAR) analysis. As expected from the structural requirements, the length of the substituent R^3 at the 4-position of the 2*H*-benzo[1,4]oxazine ring was parabolically related to activity. Furthermore, the QSAR results also indicated that the length of the 2-substituent was parabolically related to activity.

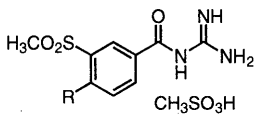
Among *N*-(2*H*-benzo[1,4]oxazine-5, 6, 7, or 8-carbonyl)-guanidines, the 6-carbonylguanidines **4** were also expected to have potent Na/H exchange inhibitory activity like the 7-carbonylguanidines **3**, because they both have a structure in which *meta*- and *para*-positions to the guanidinocarbonyl group are cyclized.[5)] The relative positions of the 2- and 4-substituents to the guanidinocarbonyl group are different between the 6- and 7-carbonylguanidines **4** and **3**. Therefore, we became interested in the Na/H exchange inhibitory activities of the 6-carbonylguanidines **4** in order to further understand the SAR of our series of Na/H exchange inhibitors.

In this paper, we describe the synthesis, evaluation and structure–activity relationships (SAR) of the Na/H exchange inhibitory activities of *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]-oxazine-6-carbonyl)guanidines **4**, and we also report the anti-arrhythmia activity of the methanesulfonate of the 4-isopropyl-2,2-dimethyl derivative **4g**, the most potent derivative in this series.

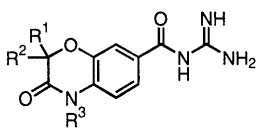
Chemistry *N*-(3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines **4** listed in Table 1 were synthesized by the route in Chart 2. Methyl 3-amino-4-hydroxybenzoate **5** was allowed to react with a 2-haloacid halide to obtain methyl 3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carboxylates **6**. These were then alkylated to give *N*-alkyl derivatives **7**. The 6-carbonylguanidines **4** were synthesized from **6** or **7** by refluxing with an excess amount of guanidine in methanol (MeOH) for several hours. The physical data are listed in Tables 1—4.



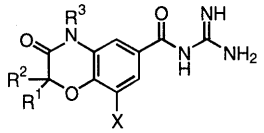
EIPA **1**



HOE-694 **2a** R=piperidinyl
HOE-642 **2b** R=iso-Pr



N-(2*H*-benzo[1,4]oxazine-7-carbonyl)guanidines **3**
3a: R^1=R^2=R^3=H
3b: R^1=Et, R^2=H, R^3=iso-Pr



N-(2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines **4**
4g: R^1=R^2=Me, R^3=iso-Pr, X=H

Chart 1. Na/H Exchange Inhibitors

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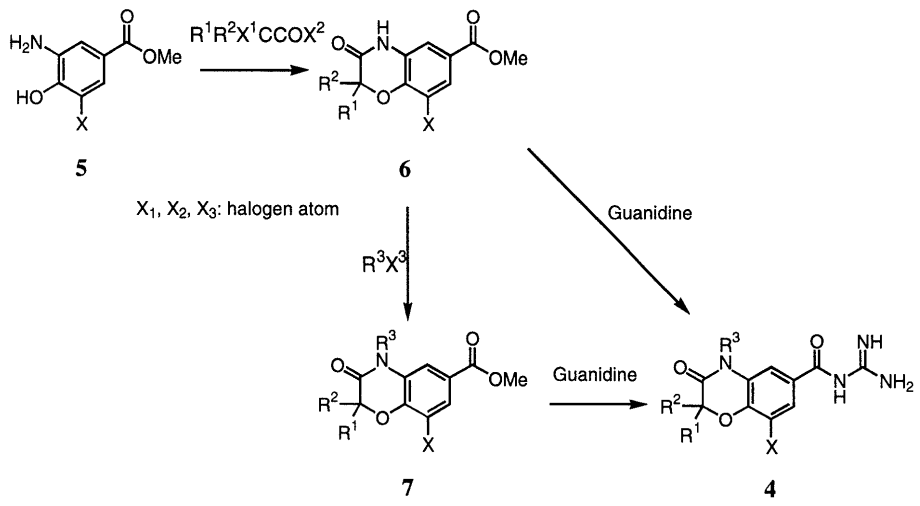
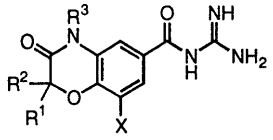


Chart 2. Synthesis of *N*-(2*H*-Benzo[1,4]oxazine-6-carbonyl)guanidines **4**

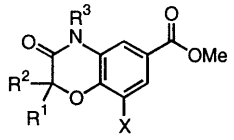
Table 1. Physical Data for *N*-(2*H*-Benzo[1,4]oxazine-6-carbonyl)guanidines **4**



No.	R^1	R^2	R^3	X	Note	Yield (%)	mp (°C) (Recryst. solvent)	Formula	Analysis Calcd (Found)		
									C	H	N
4a	H	H	H	H		6	>280 (MeOH–H_2O)	$C_{10}H_{10}N_4O_3 \cdot 1/4CH_3OH$	50.83 (51.15)	4.58 4.29	23.13 22.99)
4b	H	H	Et	H		60	180—183 (AcOEt)	$C_{12}H_{14}N_4O_3 \cdot 3/4H_2O$	52.26 (52.52)	5.67 5.31	20.32 20.27)
4c	H	H	iso-Pr	H		56	159—161 (MeCN)	$C_{13}H_{16}N_4O_3$	56.51 (56.54)	5.84 5.70	20.28 20.48)
4d	H	Me	Et	H		53	208—211 (MeCN)	$C_{13}H_{16}N_4O_3$	56.51 (56.42)	5.84 5.87	20.28 20.42)
4e	H	Et	iso-Pr	H		62	217—220 (MeCN)	$C_{15}H_{20}N_4O_3$	59.20 (59.23)	6.62 6.49	18.41 18.29)
4f	H	Ph	iso-Pr	H		31	195—198 (Et_2O–MeCN)	$C_{19}H_{20}N_4O_3$	64.76 (64.61)	5.72 5.74	15.90 15.89)
4g	Me	Me	iso-Pr	H		6	212—214 (MeOH)	$C_{15}H_{20}N_4O_3$	59.20 (59.02)	6.62 6.67	18.41 18.45)
4h	Me	Me	H	H	HCl[a)]	15	>280 (EtOH–H_2O)	$C_{12}H_{14}N_4O_3 \cdot HCl$	48.25 (48.05)	5.06 4.98	18.76 18.86)
4i	Me	Me	Me	H	HCl[a)]	64	248—250 (EtOH)	$C_{13}H_{16}N_4O_3 \cdot HCl$	49.92 (49.82)	5.48 5.41	17.91 18.01)
4j	Me	Me	Et	H		51	182—185 (AcOEt)	$C_{14}H_{18}N_4O_3$	57.92 (57.93)	6.25 6.26	19.30 19.30)
4k	Me	Me	Pr	H		49	175—177 (MeCN)	$C_{15}H_{20}N_4O_3$	59.20 (59.21)	6.62 6.46	18.41 18.61)
4l	Me	Me	Butyl	H	HCl[a)]	50	217—219 (EtOH)	$C_{16}H_{22}N_4O_3 \cdot HCl$	54.16 (54.14)	6.53 6.58	15.79 15.65)
4m	Me	Me	$(CH_2)_2OEt$	H	HCl[a)]	37	179—180 (MeCN)	$C_{16}H_{22}N_4O_4 \cdot HCl$	51.82 (52.03)	6.25 6.24	15.10 15.25)
4n	Me	Me	Hexyl	H	HCl[a)]	67	174—176 (MeCN)	$C_{18}H_{26}N_4O_3 \cdot HCl \cdot 1/10H_2O$	56.20 (55.92)	7.13 7.02	14.56 14.50)
4o	H	H	Me	Cl		79	267—269 (EtOH)	$C_{11}H_{11}ClN_4O_3$	46.74 (46.86)	3.92 3.99	19.82 20.01)
4p	H	H	Et	Cl		76	211—213 (AcOEt)	$C_{12}H_{13}ClN_4O_3$	48.58 (48.53)	4.42 4.48	18.88 18.65)
4q	H	H	iso-Pr	Cl	HCl[a)]	28	269—271 (EtOH)	$C_{13}H_{15}ClN_4O_3 \cdot HCl$	44.97 (44.83)	4.65 4.66	16.14 16.17)
4r	H	H	Me	OMe		48	246—248 (MeCN)	$C_{12}H_{14}N_4O_4$	51.80 (51.82)	5.07 5.03	20.13 20.00)
4s	H	H	Et	OMe		18	204—207 (MeCN)	$C_{13}H_{16}N_4O_4$	53.42 (53.41)	5.52 5.50	19.17 19.35)
4t	Me	Me	iso-Pr	OMe		61	222—224 (MeCN)	$C_{16}H_{22}N_4O_4$	57.47 (57.52)	6.63 6.60	16.76 16.99)

a) Hydrochloride.

Table 2. Physical Data for Methyl 2*H*-Benzo[1,4]oxazine-6-carboxylates **6** and **7**



No.	R^1	R^2	R^3	X	Yield (%)	mp (°C) (Recryst. solvent)	Formula	Analysis Calcd (Found)		
								C	H	N
6a	H	H	H	H	75	193—194 (AcOEt)	$C_{10}H_9NO_4$	57.97 (58.03	4.38 4.37	6.76 6.77)
6b	H	Me	H	H	71	156—159 (MeCN)	$C_{11}H_{11}NO_4$	59.72 (59.73	5.01 4.96	6.33 6.41)
6c	H	Et	H	H	79	147—149 (MeOH)	$C_{12}H_{13}NO_4$	61.27 (61.28	5.57 5.50	5.95 5.80)
6d	H	Ph	H	H	91	178—180 (MeCN)	$C_{16}H_{13}NO_4$	67.84 (67.87	4.63 4.70	4.94 4.90)
6e	Me	Me	H	H	91	199—200 (MeCN)	$C_{12}H_{13}NO_4$	61.27 (61.64	5.57 5.54	5.95 5.96)
6f	H	H	H	Cl	49	238—240 (MeOH)	$C_{10}H_8ClNO_4$	49.71 (49.63	3.34 3.34	5.80 5.59)
6g	H	H	H	OMe	91	229—231 (MeOH)	$C_{11}H_{11}NO_5$	55.70 (55.70	4.67 4.66	5.90 5.74)
6h	Me	Me	H	OMe	79	205—207 (MeOH)	$C_{13}H_{15}NO_5$	58.86 (58.86	5.70 5.67	5.28 4.99)
7a	H	H	Et	H	87	100—102 (AcOEt–hexane)	$C_{12}H_{13}NO_4$	61.27 (61.20	5.57 5.58	5.95 5.95)
7b	H	H	iso-Pr	H	28	81—84 (AcOEt–hexane)	$C_{13}H_{15}NO_4$	62.64 (62.71	6.07 6.08	5.62 5.55)
7c	H	Me	Et	H	80	Oil	$C_{13}H_{15}NO_4$	62.64 (62.50	6.07 6.14	5.62 5.62)
7d	H	Et	iso-Pr	H	36	Oil	$C_{15}H_{19}NO_4$	64.97 (64.93	6.91 6.88	5.05 4.87)
7e	H	Ph	iso-Pr	H	19	Oil	$C_{19}H_{19}NO_4$	70.14 (69.92	5.89 5.72	4.31 4.34)
7f	Me	Me	Me	H	93	89—91 (AcOEt–hexane)	$C_{13}H_{15}NO_4$	62.64 (62.57	6.07 6.05	5.62 5.54)
7g	Me	Me	Et	H	95	76—78 (AcOEt–hexane)	$C_{14}H_{17}NO_4$	63.87 (63.92	6.51 6.46	5.32 5.24)
7h	Me	Me	Pr	H	72	Oil	$C_{15}H_{19}NO_4$	64.97 (65.02	6.91 6.88	5.05 4.96)
7i	Me	Me	iso-Pr	H	27	75—77 ($MeOH–H_2O$)	$C_{15}H_{19}NO_4$	64.97 (64.86	6.91 6.88	5.05 4.91)
7j	Me	Me	Butyl	H	83	Oil	$C_{16}H_{21}NO_4$	65.96 (65.86	7.26 7.26	4.81 4.77)
7k	Me	Me	$(CH_2)_2OEt$	H	73	Oil	$C_{15}H_{19}NO_5$	62.53 (62.28	6.89 6.81	4.56 4.56)
7l	Me	Me	Hexyl	H	75	Oil	$C_{18}H_{25}NO_4$	67.69 (67.56	7.89 7.88	4.39 4.34)
7m	H	H	Me	Cl	63	165—168 (AcOEt–hexane)	$C_{11}H_{10}ClNO_4$	51.68 (51.68	3.94 3.97	5.48 5.35)
7n	H	H	Et	Cl	14	122—125 (AcOEt–Et2O)	$C_{12}H_{12}ClNO_4$	53.44 (53.62	4.49 4.55	5.19 5.08)
7o	H	H	iso-Pr	Cl	3	127—128 (AcOEt–hexane)	$C_{13}H_{14}ClNO_4$	55.04 (55.01	4.97 4.96	4.94 4.89)
7p	H	H	Me	OMe	80	156—158 (MeCN)	$C_{12}H_{13}NO_5$	57.37 (57.40	5.22 5.20	5.58 5.52)
7q	H	H	Et	OMe	45	132—134 (MeCN)	$C_{13}H_{15}NO_5$	58.86 (58.82	5.70 5.69	5.28 5.27)
7r	Me	Me	iso-Pr	OMe	30	125—127 (MeCN)	$C_{16}H_{21}NO_5$	62.53 (62.67	6.84 6.93	4.56 4.56)

Results and Discussion

The Na/H exchange inhibitory activities of *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines **4** were tested by their ability to inhibit platelet swelling induced by sodium propionate, in accordance with the method of Rosskopf *et al.*[7] The results are given in Table 5.

N-(3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidine **4a** was expected to be as active as the 7-carbonylguanidine derivative **3a**, since the hydrophobicity (clogP) of the bicyclic ring moiety is proportionally related to the activity,[5] and **3a** and **4a** were calculated to have equal clogP values (−0.36).[6] Compound **4a** showed Na/H ex-

Table 3. ^{1}H-NMR Spectral Data for *N*-(2*H*-Benzo[1,4]oxazine-6-carbonyl)guanidines **4**

No.	Spectral data (DMSO-d_6)
4a	(250 MHz) δ 4.59 (2H, s), 6.65 (2H, br), 6.90 (1H, d, J=8 Hz), 7.62 (1H, d, J=1 Hz), 7.68 (1H, dd, J=8, 1 Hz), 7.90 (2H, br), 10.77 (1H, br).
4b	(60 MHz) δ 1.30 (3H, t, J=7 Hz), 4.17 (2H, q, J=7 Hz), 4.71 (2H, s), 7.13 (1H, d, J=8 Hz), 7.45 (4H, br), 8.08 (1H, dd, J=8, 2 Hz), 8.14 (1H, d, J=2 Hz).
4c	(60 MHz) δ 1.52 (6H, d, J=7 Hz), 4.51 (2H, s), 4.45—4.97 (1H, m), 6.98 (1H, d, J=8 Hz), 7.30 (4H, br), 7.84 (1H, dd, J=8, 2 Hz), 8.05 (1H, d, J=2 Hz).
4d	(60 MHz) δ 1.20 (3H, t, J=7 Hz), 1.83 (3H, d, J=8 Hz), 3.85 (2H, q, J=7 Hz), 4.58 (1H, q, J=8 Hz), 6.85 (1H, d, J=8 Hz), 7.35 (4H, br), 7.69 (1H, dd, J=8, 1 Hz), 7.76 (1H, d, J=1 Hz).
4e	(60 MHz) δ 1.00 (3H, t, J=7 Hz), 1.50 (6H, d, J=7 Hz), 1.50—2.08 (2H, m), 4.38 (1H, dd, J=7, 8 Hz), 4.50—4.90 (1H, m), 6.96 (1H, d, J=8 Hz), 7.25 (4H, br) ,7.80 (1H, dd, J=8, 2 Hz), 8.00 (1H, d, J=2 Hz).
4f	(60 MHz) δ 1.69 (6H, d, J=7 Hz), 4.60—5.10 (1H, m), 5.79 (1H, s), 7.08 (1H, d, J=8 Hz), 7.25 (4H, br), 7.42 (5H, s), 7.87 (1H, dd, J=8, 2 Hz), 8.12 (1H, d, J=2 Hz).
4g	(60 MHz) δ 1.47 (6H, s), 1.60 (6H, d, J=6 Hz), 4.70—5.10 (1H, m), 7.12 (1H, d, J=7 Hz), 7.90 (1H, dd, J=7, 1 Hz), 8.10 (1H, d, J=1 Hz), 8.82 (4H, br).
4h	(300 MHz) δ 1.34 (6H, s), 7.11 (1H, d, J=8 Hz), 7.55 (1H, d, J=2 Hz), 7.89 (1H, dd, J=8, 2 Hz), 8.51 (2H, br), 8.70 (2H, br), 10.97 (1H, br), 11.82 (1H, br).
4i	(60 MHz) δ 1.51 (6H, s), 3.49 (3H, s), 7.24 (1H, d, J=8 Hz), 8.00 (1H, dd, J=8, 2 Hz), 8.21 (1H, d, J=2 Hz), 8.85 (4H, br).
4j	(60 MHz) δ 1.21 (3H, t, J=6 Hz), 1.50 (6H, s), 4.10 (2H, q, J=6 Hz), 7.05 (1H, d, J=7 Hz), 7.90 (1H, dd, J=7, 2 Hz), 7.97 (1H, d, J=2 Hz), 8.20 (4H, br).
4k	(60 MHz) δ 0.94 (3H, t, J=7 Hz), 1.43 (6H, s), 1.31—1.92 (2H, m), 3.93 (2H, t, J=7 Hz). 6.95 (1H, d, J=8 Hz), 7.30 (4H, br), 7.72 (1H, dd, J=8, 2 Hz), 7.75 (1H, d, J=2 Hz).
4l	(60 MHz) δ 0.94 (3H, t, J=7 Hz), 1.22—1.90 (10H, m), 4.12 (2H, t, J=7 Hz). 7.14 (1H, d, J=8 Hz), 8.01 (1H, dd, J=8, 2 Hz), 8.09 (1H, d, J=2 Hz), 8.10 (2H, br), 9.10 (2H, br).
4m	(60 MHz) δ 1.08 (3H, t, J=7 Hz), 1.50 (6H, s), 3.40—3.95 (4H, m), 4.36 (2H, t, J=7 Hz), 7.20 (1H, d, J=8 Hz), 8.04 (1H, dd, J=8, 2 Hz), 8.30 (1H, d, J=2 Hz), 8.78 (2H, br), 8.98 (2H, br).
4n	(60 MHz) δ 0.75—1.85 (17H, m), 4.10 (2H, t, J=7 Hz), 7.18 (1H, d, J=8 Hz), 8.01 (1H, dd, J=8, 2 Hz), 8.10 (1H, d, J=2 Hz), 8.10 (2H, br), 8.95 (2H, br).
4o	(60 MHz) δ 3.37 (3H, s), 4.82 (2H, s), 8.78 (1H, d, J=2 Hz), 8.93 (1H, d, J=2 Hz), 8.20 (4H, br).
4p	(60 MHz) δ 1.20 (3H, t, J=7 Hz), 3.96 (2H, q, J=7 Hz), 4.76 (2H, s), 7.34 (4H, br), 7.77 (1H, d, J=2 Hz), 7.90 (1H, d, J=2 Hz).
4q	(60 MHz) δ 1.51 (6H, d, J=7 Hz), 4.71 (2H, s), 4.54—5.04 (1H, m), 7.09 (2H, s), 8.63 (4H, br).
4r	(60 MHz) δ 3.32 (3H, s), 3.88 (3H, s), 4.64 (2H, s), 7.38 (4H, br), 7.59 (2H, s).
4s	(60 MHz) δ 1.25 (3H, t, J=7 Hz), 3.88 (3H, s), 4.00 (2H, q, J=7 Hz), 4.60 (2H, s), 7.38 (4H, br), 7.65 (2H, s).
4t	(60 MHz) δ 1.39 (6H, s), 1.52 (6H, d, J=7 Hz), 3.85 (3H, s), 4.43—4.93 (1H, m), 7.25 (4H, br), 7.55 (1H, d, J=2 Hz), 7.70 (1H, d, J=2 Hz).

change inhibitory activity with an IC_{50} value of 10 μM, which was slightly weaker than the 7-carbonylguanidine derivative **3a** (IC_{50}=2.70 μM). However, these two IC_{50} values were in the 95% confidence interval range of the extrapolated value (around 13 μM) from the QSAR equation.[6] It was assumed that the 6- and 7-carbonylguanidines **4** and **3** interact with the same region of the Na/H exchanger, because the potencies of **3a** and **4a** were explained by the same QSAR equation.

In order to investigate the SAR of the 2-position of the 2*H*-benzo[1,4]oxazine ring, compounds **4b**—**g** were synthesized. These compounds have an ethyl or an isopropyl group at the 4-position. The activity of the 2-phenyl derivative **4f** was the lowest among six compounds **4b**—**g**. The other five compounds **4b**—**e** and **4g**, which have smaller substituents such as a hydrogen atom, a methyl group or an ethyl group, showed almost the same potency. Therefore, smaller substituents seemed to be tolerable at the 2-position. The chirality at the 2-position was thought not to affect activity, based on the fact that the 2,2-dimethyl derivative **4j** was almost as effective as 2-methyl (**4d**) and the 2-non-substitued derivative (**4b**). Next, we investigated the SAR of the 4-position fixing the 2,2-dimethyl moiety at the 2-position, because **4g** was the most active among **4b**—**g**. Compounds **4h**—**n** having various alkyl groups at the 4-position were synthesized. The most potent compound was the 4-isopropyl derivative **4g**.

Next, the SAR of the substituent at the benzene ring was investigated. A chloro atom and a methoxy group were incorporated at the 8-position (**4o**—**t**), since the starting compounds **5** were easily available. The substituent at the 8-position did not affect the activity.

QSAR analysis of 6-carbonylguanidines **4** was carried out by the Hansch–Fujita method, and the statistically significant equations 1 and 2 were obtained.

$$pIC_{50}=1.42(\pm0.57)L(R^3)-0.15(\pm0.06)L(R^3)^2-2.87(\pm1.36) \quad (1)$$
$$n=20,\ r=0.806,\ s=0.361,\ F=15.72,\ L(R^3)_{opt}=4.81$$

$$pIC_{50}=1.55(\pm0.53)B_4(R^3)-0.24(\pm0.08)B_4(R^3)^2-2.05(\pm0.86) \quad (2)$$
$$n=20,\ r=0.840,\ s=0.331,\ F=20.04,\ B_4(R^3)_{opt}=3.33$$

In Eqs. 1 and 2, L and B_4 are Verloop's STERIMOL parameters[8] and are assumed to be in an extended conformation, the number in parentheses is the 95% confidence interval, n is the number of data points used in deriving the equation, r is the correlation coefficient, s is the standard deviation, and F is the F-ratio between the variances of calculated and observed activities. $L(R^3)_{opt}$ and $B_4(R^3)_{opt}$ are the calculated optimum values of $L(R^3)$ and $B_4(R^3)$, respectively.

Equation 1 indicates that the length of the substituent R^3 is parabolically related to inhibitory activity and the calculated optimum substituents are propyl, ethyl and isopropyl group. This agreed with the observation that the most potent derivative was the 4-isopropyl derivative **4g**. Equation 2, which indicates that the widest width B_4 of the substituent R^3 is parabolically related to activity, was also formulated. The reason for the statistical significance of $B_4(R^3)$ was the high correlation (r=0.98) with $L(R^3)$. Nonetheless, we concluded that the length L is significant to the activity, because L of the 2- and 4-substituents of the 7-carbonylguanidines **3** was also para-

Table 4. ^{1}H-NMR Spectral Data for Methyl 2*H*-Benzo[1,4]oxazine-6-carboxylates **6** and **7**

No.	Spectral data
6a	(60 MHz, $CDCl_3$) δ 3.85 (3H, s), 4.61 (2H, s), 6.91 (1H, d, J=8 Hz), 7.59 (1H, d, J=2 Hz), 7.60 (1H, dd, J=8, 2 Hz), 10.55 (1H, br).
6b	(60 MHz, $CDCl_3$) δ 1.65 (3H, d, J=6 Hz), 3.85 (3H, s), 4.68 (1H, q, J=6 Hz), 6.98 (1H, d, J=8 Hz), 7.61 (1H, d, J=1 Hz), 7.72 (1H, dd, J=8, 1 Hz), 10.57 (1H, br).
6c	(60 MHz, $CDCl_3$) δ 1.10 (3H, t, J=7 Hz), 1.30—1.80 (2H, m), 3.90 (3H, s), 4.60 (1H, t, J=7 Hz), 6.98 (1H, d, J=8 Hz), 7.60 (1H, d, J=2 Hz), 7.68 (1H, dd, J=8, 2 Hz), 9.50 (1H, br).
6d	(60 MHz, DMSO-d_6) δ 3.80 (3H, s), 5.62 (1H, s), 6.97 (1H, d, J=8 Hz), 7.30 (5H, s), 7.59 (1H, d, J=2 Hz), 7.60 (1H, dd, J=8, 2 Hz), 10.81 (1H, br).
6e	(60 MHz, DMSO-d_6) δ 1.50 (6H, s), 3.75 (3H, s), 6.96 (1H, d, J=8 Hz), 7.50 (1H, d, J=1 Hz), 7.60 (1H, dd, J=8, 1 Hz), 10.55 (1H, br).
6f	(60MHz, DMSO-d_6) δ 3.85 (3H, s), 4.79 (2H, s), 7.48 (1H, d, J=2 Hz), 7.59 (1H, d, J=2 Hz), 11.10 (1H, br).
6g	(60 MHz, DMSO-d_6) δ 3.95 (6H, s), 4.61 (2H, s), 7.23 (2H, s), 10.71 (1H, br).
6h	(60 MHz, DMSO-d_6) δ 1.49 (6H, s), 3.88 (6H, s), 7.29 (2H, s), 10.25 (1H, br).
7a	(60 MHz, $CDCl_3$) δ 1.29 (3H, t, J=7 Hz), 3.90 (3H, s), 4.02 (2H, q, J=7 Hz), 4.62 (2H, s), 6.97 (1H, d, J=8 Hz), 7.63 (1H, d, J=2 Hz), 7.65 (1H, dd, J=8, 2 Hz).
7b	(60 MHz, $CDCl_3$) δ 1.50 (6H, d, J=7 Hz), 3.83 (3H, s), 4.46 (2H, s), 4.25—4.75 (1H, m), 6.90 (1H, d, J=8 Hz), 7.68 (1H, dd, J=8, 2 Hz), 7.82 (1H, d, J=2 Hz).
7c	(60 MHz, $CDCl_3$) δ 1.29 (3H, t, J=8 Hz), 1.59 (3H, d, J=7 Hz), 3.92 (3H, s), 4.08 (2H, q, J=8 Hz), 4.74 (1H, q, J=7 Hz), 7.04 (1H, d, J=8 Hz), 7.70 (1H, d, J=1 Hz), 7.85 (1H, dd, J=8, 1 Hz).
7d	(60 MHz, $CDCl_3$) δ 1.03 (3H, t, J=7 Hz), 1.55 (6H, d, J=7 Hz), 1.60—2.05 (2H, m), 3.90 (3H, s), 4.27 (1H, t, J=7 Hz), 4.60—5.10 (1H, m), 6.98 (1H, d, J=8 Hz), 7.65 (1H, d, J=2 Hz), 7.75 (1H, dd, J=8, 2 Hz).
7e	(60 MHz, $CDCl_3$) δ 1.52 (3H, d, J=7 Hz), 1.64 (3H, d, J=7 Hz), 3.89 (3H, s), 4.65—5.05 (1H, m), 5.59 (1H, s), 6.99 (1H, d, J=8 Hz), 7.29 (5H, s), 7.66 (1H, d, J=2 Hz), 7.75 (1H, dd, J=8, 2 Hz).
7f	(60 MHz, $CDCl_3$) δ 1.50 (6H, s), 3.40 (3H, s), 3.91 (3H, s), 7.00 (1H, d, J=8 Hz), 7.65 (1H, d, J=2 Hz), 7.76 (1H, dd, J=8, 2 Hz).
7g	(60 MHz, $CDCl_3$) δ 1.30 (3H, t, J=6 Hz), 1.53 (6H, s), 3.92 (3H, s), 4.05 (2H, q, J=6 Hz), 7.00 (1H, d, J=9 Hz), 7.68 (1H, d, J=1 Hz), 7.75 (1H, dd, J=9, 1 Hz).
7h	(60 MHz, $CDCl_3$) δ 0.97 (3H, t, J=7 Hz), 1.40—1.95 (2H, m), 1.50 (6H, s), 3.89 (3H, s), 3.91 (2H, t, J=7 Hz), 6.96 (1H, d, J=8 Hz), 7.63 (1H, d, J=2 Hz), 7.71 (1H, dd, J=8, 2 Hz).
7i	(60 MHz, $CDCl_3$) δ 1.50 (6H, s), 1.60 (6H, d, J=6 Hz), 3.98 (3H, s), 4.50—5.10 (1H, m), 7.06 (1H, d, J=7 Hz), 7.82 (1H, dd, J=7, 1 Hz), 7.87 (1H, d, J=1 Hz).
7j	(60 MHz, $CDCl_3$) δ 0.80—1.81 (7H, m), 1.50 (6H, s), 3.90 (3H, s), 3.95 (2H, t, J=7 Hz), 6.96 (1H, d, J=8 Hz), 7.61 (1H, d, J=2 Hz), 7.70 (1H, dd, J=8, 2 Hz).
7k	(60 MHz, $CDCl_3$) δ 1.25 (3H, t, J=7 Hz), 1.55 (6H, s), 3.40—3.95 (4H, m), 3.80 (3H, s), 4.39 (2H, t, J=7 Hz), 7.00 (1H, d, J=8 Hz), 7.80 (1H, dd, J=8, 2 Hz), 7.95 (1H, d, J=2 Hz).
7l	(300 MHz, $CDCl_3$) δ 0.89 (3H, t, J=7 Hz), 1.25—1.32 (6H, m), 1.42 (6H, s), 2.62—2.75 (2H, m), 3.92 (3H, s), 3.94 (2H, t, J=7 Hz), 6.98 (1H, d, J=8 Hz), 7.64 (1H, d, J=2 Hz), 7.71 (1H, dd, J=8, 2 Hz).
7m	(60 MHz, $CDCl_3$) δ 3.42 (3H, s), 3.94 (3H, s), 4.77 (2H, s), 7.67 (1H, d, J=2 Hz), 7.75 (1H, d, J=2 Hz).
7n	(60 MHz, $CDCl_3$) δ 1.25 (3H, t, J=7 Hz), 3.86 (3H, s), 4.00 (2H, q, J=7 Hz), 4.70 (2H, s), 7.52 (1H, s), 7.72 (1H, s).
7o	(60 MHz, $CDCl_3$) δ 1.58 (6H, d, J=7 Hz), 3.94 (3H, s), 4.64 (2H, s), 4.55—4.95 (1H, m), 7.80 (2H, s).
7p	(60 MHz, $CDCl_3$) δ 3.40 (3H, s), 3.90 (6H, s), 4.70 (2H, s), 7.39 (2H, s).
7q	(60 MHz, $CDCl_3$) δ 1.32 (3H, t, J=7 Hz), 3.96 (6H, s), 4.07 (2H, q, J=7 Hz), 4.72 (2H, s), 7.42 (2H, s).
7r	(60 MHz, $CDCl_3$) δ 1.53 (6H, s), 1.58 (6H, d, J=7 Hz), 3.97 (6H, s), 4.64—5.00 (1H, m), 7.50 (1H, d, J=2 Hz), 7.64 (1H, d, J=2 Hz).

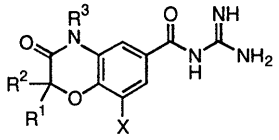
bolically related,[6] besides the width B_4 is not highly significant.

The electronic (σ) and the hydrophobic (π) factors of the 8-substituent are not included in Eq. 1, as was expected from the observation that the 8-hydrogen (**4b**), 8-chloro (**4q**) and 8-methoxy (**4s**) derivatives showed almost the same inhibitory potency. The physicochemical parameters (L, B_{1-4}, σ, π, *etc.*) of the substituents R^1, R^2 at the 2-position of the 2*H*-benzo[1,4]oxazine ring were not significant, although we concluded that a larger substituent is unfavorable for the 2-position from the fact that the 2-phenyl derivative **4f** exhibited the weakest activity among compounds **4b**—**g**.

The SARs of *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines **4** are summarized in Chart 3, together with the SARs of the 7-carbonylguanidines **3**.[6] Compounds **3** and **4** are thought to interact with the same region of the Na/H exchanger, since the potencies of **3a** and **4a** are explained by the same equation. The aroylguanidine moiety is thought to be essential for Na/H exchange inhibitory activity. We previously found that the SARs of the 2- and 4-substituents of the 7-carbonylguanidines **3** were similar (the parabolical relationship between activity and length). The relative positions of the 2-substituents R^1 and R^2 and the 4-substituent R^3 to the guanidinocarbonyl group are different from each other between **3** and **4**. However, it was clarified that the SAR of the 4-substituent of 6-carbonylguanidines **4** is also similar to that of **3**. On the other hand, the SAR of the 2-substituents of **4** is somewhat different from that of **3**. With respect to compounds **4**, smaller substituents are favorable for activity, while larger substituents are unfavorable. When the essential guanidinocarbonyl groups of **3** and **4** are overlapped, the 4-positions of **3** and **4** can not to be superimposed. This suggests that the 4-position of **4** does not correspond to the 4-position of **3**. From this study, it was revealed that the 2- and 4-substituents are important to increase the Na/H exchange inhibitory activity, although the relative position to the essential guanidinocarbonyl group is not different.

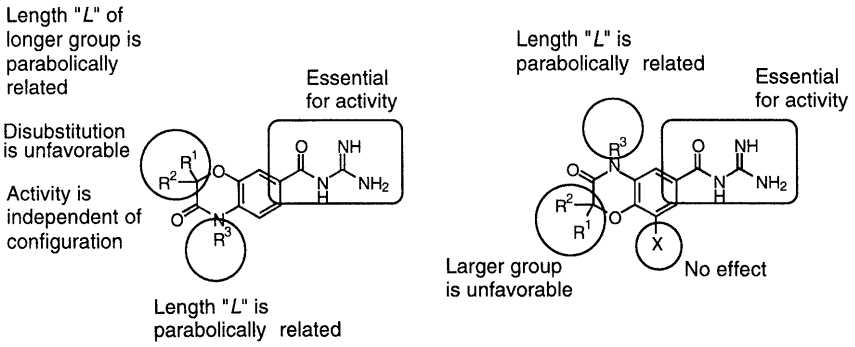
The most potent 6-carbonylguanidine derivative **4** was *N*-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2*H*-benzo-[1,4]oxazine-6-carbonyl)guanidine **4g**, with an IC_{50} value of 0.12 μM. The methanesulfonate of **4g** (**4g**·MsOH) showed excellent water-solubility (about 27 mg/ml). Therefore, **4g**·MsOH was subjected to *in vivo* investigation. Anti-arrhythmia activity of **4g**·MsOH was tested in terms of the du-

Table 5. Na/H Exchange Inhibitory Activity of *N*-(2*H*-Benzo[1,4]oxazine-6-carbonyl)guanidines **4**



No.	R^1	R^2	R^3	X	Note	$L(R^3)$	$B_4(R^3)$	Na/H exchange inhibitory activity			
								IC_{50} (μM)	pIC_{50}		
									Obsd.	Eq. 1	Eq. 2
4a	H	H	H	H		2.06	1.00	10	−1.00	−0.57	−0.73
4b	H	H	Et	H		4.11	2.97	0.33	0.48	0.48	0.48
4c	H	H	iso-Pr	H		4.11	3.16	0.25	0.60	0.48	0.50
4d	H	Me	Et	H		4.11	2.97	0.29	0.54	0.48	0.48
4e	H	Et	iso-Pr	H		4.11	3.16	0.17	0.76	0.48	0.50
4f	H	Ph	iso-Pr	H		4.11	3.16	1.0	0.00	0.48	0.50
4g	Me	Me	iso-Pr	H		4.11	3.16	0.12	0.92	0.48	0.50
4h	Me	Me	H	H	HCl[a]	2.06	1.00	7.4	−0.87	−0.57	−0.73
4i	Me	Me	Me	H	HCl[a]	3.00	2.04	0.81	0.09	0.06	0.14
4j	Me	Me	Et	H		4.11	2.97	0.38	0.42	0.48	0.48
4k	Me	Me	Pr	H		5.05	3.49	0.74	0.13	0.55	0.49
4l	Me	Me	Butyl	H	HCl[a]	6.17	4.42	1.3	−0.11	0.29	0.20
4m	Me	Me	$(CH_2)_2OEt$	H	HCl[a]	6.99	4.82	5.6	−0.75	−0.13	−0.05
4n	Me	Me	Hexyl	H	HCl[a]	8.22	5.87	3.7	−0.57	−1.13	−1.08
4o	H	H	Me	Cl		3.00	2.04	0.33	0.48	0.06	0.14
4p	H	H	Et	Cl		4.11	2.97	0.22	0.66	0.48	0.48
4q	H	H	iso-Pr	Cl	HCl[a]	4.11	3.16	0.16	0.80	0.48	0.50
4r	H	H	Me	OMe		3.00	2.04	0.50	0.30	0.06	0.14
4s	H	H	Et	OMe		4.11	2.97	0.27	0.57	0.48	0.48
4t	Me	Me	iso-Pr	OMe		4.11	3.16	0.37	0.43	0.48	0.50

a) Hydrochloride.

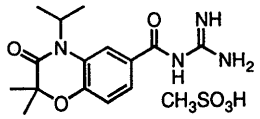


N-(2*H*-benzo[1,4]oxazine-7-carbonyl)guanidines **3**

N-(2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines **4**

Chart 3. SARs of Compounds **3** and **4**

Table 6. Na/H Exchange Inhibitory Activity and Duration of Ventricular Fibrillation of Compound **4g**·MsOH

No.	Structure	Na/H exchange inhibitory activity IC_{50} (μM)	Duration (s) of ventricular fibrillation[a] (mean±S.E.)
Control	—	—	171.0±5.8
4g·MsOH (KB-R9032)		0.12 (0.086—0.19)	68.7±39.9*

a) i.v. administration of the compound 0.1 mg/kg, reperfusion induced arrhythmias in anesthetized rats (n=4—6). Significantly different from the control group *: $p<0.05$ (Mann–Whitney U-test).

ration of ventricular fibrillation (VF) time using a rat acute myocardial infarction model, in accordance with the method of Tagliavini *et al.*[9] The duration of VF time for the control group was 171±5.8 s (mean±standard error). The administration of **4g**·MsOH (0.1 mg/kg, i.v.) before occlusion significantly reduced the duration of VF (68.7±39.9 s). In addition, **4g**·MsOH showed interesting pharmacokinetic properties.[10] Therefore, **4g**·MsOH (KB-R9032) was selected for further investigation.

Conclusion

In the course of the study of Na/H exchange inhibitors, *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines **4** were prepared in order to investigate the SAR, since we earlier found a parabolic relationship between the length of the 2- and 4-substituents and activity in 7-carbonylguanidines **3**.[6] The SAR of the 4-substituent of **4** was similar to that of **3** (Chart 3), however, the SAR of the 2-substituent of **4** was somewhat different from that of **3**.

The most potent compound in this new series was *N*-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2*H*-benzo[1,4]-oxazine-6-carbonyl)guanidine **4g**; its methanesulfonate salt (KB-R9032) had excellent water-solubility, and showed anti-arrhythmia activity against a rat acute myocardial infarction model. Therefore, KB-R9032 was selected for further investigations.

Experimental

Melting points were measured with a capillary melting point apparatus (Yamato MP-21) and are uncorrected. ^{1}H-NMR spectra were taken on Bruker AM-300 NMR (300 MHz), Bruker DPX-250 NMR (250 MHz) and Hitachi R-24B NMR (60 MHz) spectrometers with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given as δ values (ppm). Elemental analysis was performed with a Yanagimoto CHN-CORDER MT-5.

General Procedure for the Preparation of Methyl 3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carboxylates 6 A 2-haloacid halide (10 mmol) was added to a mixture of methyl 3-amino-4-hydroxybenzoate **5** (10 mmol), sodium hydrogencarbonate (11 mmol), ethyl acetate (AcOEt) (10 ml), and water (10 ml) at room temperature. The mixture was stirred at the same temperature for 30 min, and separated. The organic phase was washed with water, dried over anhydrous magnesium sulfate ($MgSO_4$), and the solvent removed *in vacuo*. Potassium carbonate (10 mmol) was added to a solution of the residue in *N*,*N*-dimethylformamide (DMF) (10 ml) at room temperature. The mixture was stirred at the same temperature overnight, then water was added to the mixture. The precipitate was collected by filtration, dried, and recrystallized to give **6**. Physical data are listed in Tables 2 and 4.

General Procedure for the Preparation of Methyl 3-Oxo-4-substituted-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carboxylates 7 A (substituted)alkyl halide (15 mmol) was added to a mixture of methyl 3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carboxylate 6 (10 mmol), sodium hydride (12 mmol) and DMF (10 mmol) at room temperature. The mixture was stirred at 60 °C for several hours, then water was added and the whole was extracted with AcOEt. The extract was washed with water, and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*. The residue was chromatographed on silica gel (hexane–AcOEt) to give **7**. Physical data are listed in Tables 2 and 4.

General Procedure for the Preparation of *N*-(3-Oxo-(4-substituted)-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines 4 Guanidine hydrochloride (55 mmol) was added to a sodium methoxide solution, prepared from sodium (50 mmol) and MeOH (25 ml), and the mixture refluxed for 30 min, and filtered. A methyl 3-oxo-(4-substituted-)3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carboxylate **6** (or **7**) (5 mmol) was then added to the filtrate. The mixture was refluxed for several hours, diluted with water, and extracted with AcOEt. The extract was washed with water and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*. The residue was chromatographed on silica gel (chloroform–MeOH) to give **4**. Physical data are listed in Tables 1 and 3.

***N*-(4-Isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidine Methanesulfonate 4g·MsOH** Compound **4g** (18.7 g, 61.4 mmol) was dissolved in hot isopropanol (200 ml), then methanesulfonic acid (6.00 g, 62.4 mmol) was added to the solution. After cooling, the precipitate was collected by filtration and recrystallized from isopropanol to give methanesulfonate of **4g** (13.7 g, 34.2 mmol, yield 56%). mp: 192—194 °C. ^{1}H-NMR (250 MHz, DMSO-d_6) δ; 1.41 (6H, s), 1.50 (6H, d, J=7 Hz), 2.39 (3H, s), 4.60—4.80 (1H, m), 7.20 (1H, d, J=8 Hz), 7.65 (1H, dd, J=8, 2 Hz), 7.75 (1H, d, J=2 Hz), 8.38 (4H, br), 11.22 (1H, s). *Anal.* Calcd for $C_{15}H_{20}N_4O_3 \cdot CH_3SO_3H$: C, 47.99; H, 6.04; N, 14.00. Found: C, 47.82; H, 6.06; N, 13.92. This compound was further recrystallized from ethanol (11.0 g, 27.5 mmol, yield 80%). mp: 203—206 °C.

Na/H Exchange Inhibitory Activity Na/H exchange inhibitory activity was determined based on the ability to inhibit sodium propionate-induced swelling of platelets in accordance with the method of Rosskopf *et al.*[7] Platelet-rich plasma was prepared as described by Mammen and coworkers.[11] Wistar male rats (190—420 g) were anesthetized with ether, and blood was taken from the abdominal aorta. To inhibit blood coagulation, acid citrate dextrose (ACD) solution (a mixture of 65 mM citric acid, 85 mM sodium citrate, and 11 mM dextrose) was added to the blood and this treated blood was centrifuged at 90×***g*** for 10 min. The supernatant was separated to prepare platelet-rich plasma. A solution of a test compound in dimethylsulfoxide (DMSO) was then added to 140 mM sodium propionate buffer solution. To this mixture was added the platelet-rich plasma prepared above and the decrease in optical density was recorded at 37 °C using a platelet aggregometer (turbidimeter) and an X–Y recorder [decrease rate in optical density in the presence of the test compound (*D*)]. As the control, the solvent DMSO alone was used instead of a solution of test compound, and the decrease in optical density was recorded in a similar manner [control (*C*)]. The swelling inhibitory rate (Na/H exchange inhibitory rate, %) was calculated using Eq. 3.

$$\text{Swelling inhibitory rate (Na/H exchange inhibitory rate, \%)} = (1 - D/C) \cdot 100 \quad (3)$$

The concentration of the test compound which causes 50% inhibition (IC_{50}) was calculated by the least-squares method.

Inhibitory Activity against Reperfusion-Induced Arrhythmia Using a rat acute myocardial infarction model, compound **4g**·MsOH was tested for inhibitory activity against reperfusion arrhythmia in accordance with the method of Tagliavini *et al.*[9] Male Sprague-Dawley rats (310—400 g) were anesthetized by intraperitoneal administration of sodium pentobarbital (50 mg/kg). The rats were cannulated *via* the trachea and the cannula was linked to a respirator. An electrocardiogram (the lead II) was obtained from electrodes attached to limbs using a bioelectric amplifier, while maintaining body temperature at 37 °C. The chest of the rats were opened at the fifth intercostal space and the pericardium was cut open to reveal the heart. A solution of the test compound in a mixed solvent of polyethylene glycol 400, ethanol and physiological saline [3 : 3 : 14 (v/v)] was then administered into the femoral vein. Ten minutes after administration of the compound, the origin of the left coronary was occluded. After occlusion for 5 min, reperfusion was carried out for 10 min and the duration (seconds) of ventricular fibrillation was assessed in accordance with the guidelines of the Lambeth Convention.[12] The mixed solvent of polyethylene glycol 400, ethanol and physiological saline [3 : 3 : 14 (v/v)] was used as a control and the duration (seconds) of ventricular fibrillation was measured in a similar manner.

References and Notes

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