Quantitative Structure–Activity Relationship Study of *N*-(3-Oxo-3,4-dihydro-2*H*-benzo[1,4]thiazine-6-carbonyl)guanidines as Potent Na/H Exchange Inhibitors

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We have previously reported that *N*-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl)guanidine (4b) methanesulfonate salt (KB-R9032) is a potent and highly water-soluble Na/H exchange inhibitor. In a series of studies on Na/H exchange inhibitors, we designed and synthesized *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazine-6-carbonyl)guanidines (5) as more potent inhibitors with high water-solubility. The design strategy for 5 was based on a quantitative structure–activity relationship (QSAR) study, involving the proportional relationship between the biological activity and hydrophobicity of the ring structure of compounds 4. As expected, compounds 5 showed more potent activity than 4. It was found by using the QSAR analysis that 5 were about five-fold more potent than 4. The increase in potency of compounds 5 well agreed with our previous QSAR analysis result. The most potent derivative was the methanesulfonate salt 5d of the 4-isopropyl derivative (IC_{50}=0.0091 μM). And in addition to the *in vitro* study, 5d showed significant protective activity against a rat acute myocardial infraction model.

Key words Na/H exchange inhibitor; quantitative structure–activity relationship; *N*-(2*H*-benzo[1,4]thiazine-6-carbonyl)guanidine

Changes in intracellular pH have been implicated in the pathophysiology of essential hypertension, myocardial ischemia, postischemic dysfunction and cellular death. However, the cells have some mechanisms which control and regulate intracellular pH. These mechanisms become vital, *e.g.*, for the correction of intracellular acidosis during and following a period of myocardial ischemia. One of the major alkalizing exchangers that exist in the myocardial cell is the Na/H exchanger which extrudes protons by the countertransport of Na^+ ions. There are many reports that the Na/H exchanger plays a key role in the pathophysiology of cardiac ischemia and reperfusion.[1)]

Although activation of the Na/H exchanger is essential for the restoration of normal intracellular pH, it results in a deleterious Na^+ overload after a prolonged period of ischemia. Due to the coupling via a Na/Ca exchanger, this causes a cellular Ca^{2+} overload, and finally, serious contractile dysfunction, arrhythmia, and cellular death. Recently, inhibition of the Na/H exchange has been experimentally shown to be a useful approach in limiting Ca^{2+} influx and avoiding its serious consequences during ischemia and reperfusion.[2)]

Some monocyclic aroylguanidines, EIPA (**1**),[3)] HOE-694 (**2a**)[4)] and HOE-642 (**2b**),[5)] are known to be typical Na/H exchange inhibitors (Chart 1), but there may still be some limitation for wide clinical application, because of their low water-solubility. Thus, we have started a new research project to discover more potent and highly water-soluble inhibitors. We have already reported the structural requirements for potent Na/H exchange inhibitors using the quantitative structure–activity relationship (QSAR) analysis of EIPA (**1**) and its derivatives.[6)] Namely, the structural requirements necessary for potent activity were as follows: 1) bicyclic aroylguanidines inhibited the Na/H exchanger, 2) the activity was proportionally related to the hydrophobicity of the bicyclic ring structure, and 3) a substituent having an appropriate length at the 5-position of 2-naphthoylguanidine enhanced the activity. In the series of study on Na/H exchange inhibitor design, we discovered *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine (**3a**) as a lead compound for potent and highly water-soluble inhibitors.[7)] In this studies, we designed some compounds having moderate hydrophobicity (clog*P*[8)]), around 1.5—2, based on an optimum value necessary for good bioavailability.[9)] The QSAR analysis of **3** indicated that the lengths of the 2- and 4-substituents were parabolically related to the inhibitory activity, independently (Chart 2). The 2-ethyl-4-ethyl (or isopropyl) derivatives **3b** or **3c** (clog*P*=1.33 or 1.65) were biologically potent in *in vitro* study and highly water-soluble from a physicochemical view point.

Recently, we found that *N*-(2*H*-benzo[1,4]oxazine-6-carbonyl)guanidines **4** also had potent Na/H exchange inhibitory activity.[10)] The SAR of the 4-substituent of compounds **4** was similar to that of compounds **3**, although the relative positions of the guanidinocarbonyl group essential for potent activity were different from each other (Chart 2). With regard to the 2-substituent of compounds **3** and **4**, the SARs seemed to be somewhat different. Shorter and/or smaller substituents were tolerated, while longer and/or larger substituents reduced the activity. The most potent compound among **4** was the 2,2-dimethyl-4-isopropyl derivative **4b** (clog*P*=1.65). The methanesulfonate salt of **4b** (KB-R9032) showed high water-solubility and good bioavailability, so it was under further investigation as a promising Na/H exchange inhibitor candidate.

In addition, the QSAR analysis of **4** predicted that the isopropyl group of **4b** was significant for enhancement of the biological potency. However, it was not actually indicated that the 2,2-dimethyl moiety contributed to the increase of the activity in an *in vitro* assay, although this hydrophobic moiety would be important for adjustment of the clog*P* value

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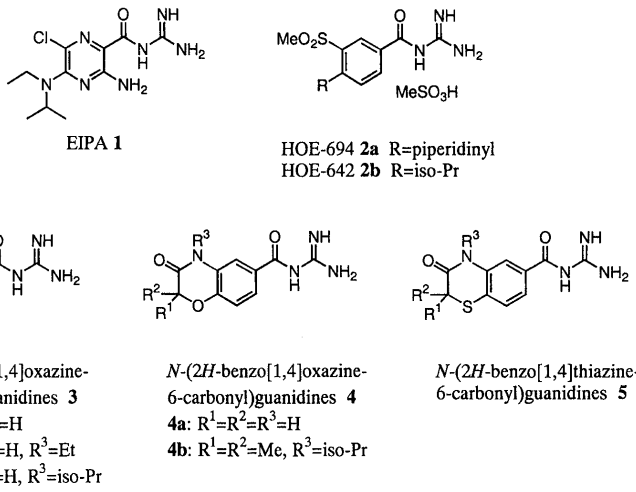


Chart 1. Na/H Exchange Inhibitors

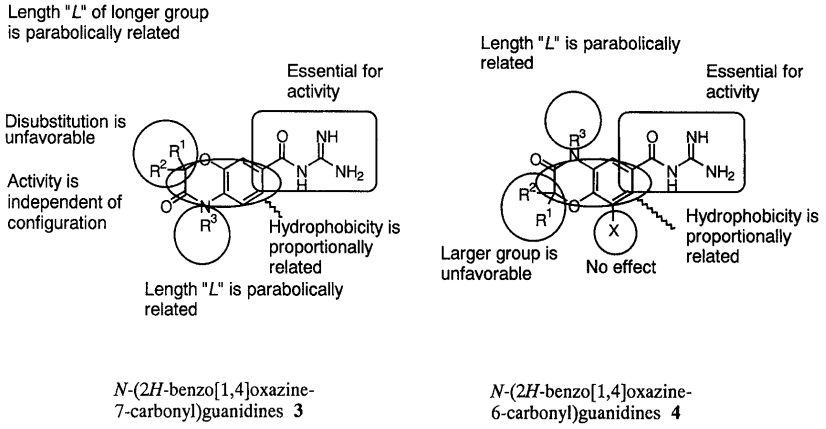


Chart 2. Structure–Activity Relationships of Benzoxazine Derivatives **3** and **4**

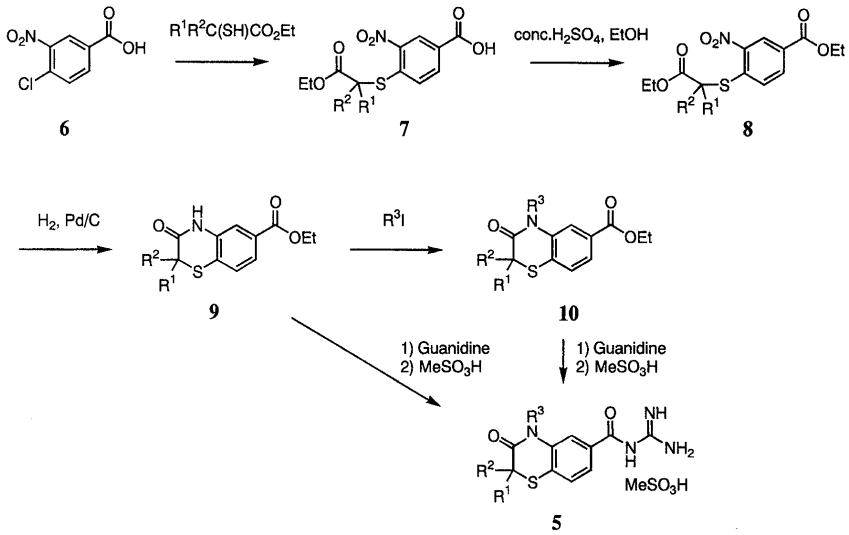
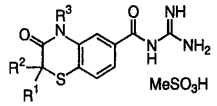


Chart 3. Synthesis of *N*-(2*H*-Benzo[1,4]thiazine-6-carbonyl)guanidines **5**

to the optimum range necessary for good bioavailability (around 1.5—2). As described before, hydrophobic fused-rings would be favorable for potent activity.[6] These results prompted us to design a more hydrophobic bicyclic ring by removal of the dimethyl moiety of **4b**. Consequently, we have designed *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazine-6-carbonyl)guanidines **5a** as the new lead compound, with a clog*P* value appropriate for optimized compounds. The benzothiazine derivative **5a** was expected to be more potent than the benzoxazine derivative **4a**, because the clog*P* value of **5a** (0.20) was higher than that of **4a** (−0.36).[8]

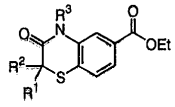
In this paper, we describe the synthesis and SAR study of

Table 1. Physical Properties of *N*-(2*H*-Benzo[1,4]thiazine-6-carbonyl)guanidines **5**



No.	R^1	R^2	R^3	Yield (%)	mp (°C) (Recryst. solvent)	Formula	Analysis Calcd (Found)		
							C	H	N
5a	H	H	H	5	263—264 (MeOH–H_2O)	$C_{10}H_{10}N_4O_2S \cdot CH_3SO_3H$	38.14 (38.14	4.07 4.23	16.18 16.28)
5b	H	H	Me	13	231—233 (MeOH–H_2O)	$C_{11}H_{12}N_4O_2S \cdot CH_3SO_3H$	39.99 (39.93	4.47 4.57	15.50 15.59)
5c	H	H	Et	7	232—234 (MeOH–H_2O)	$C_{12}H_{14}N_4O_2S \cdot CH_3SO_3H$	41.70 (41.68	4.85 4.88	14.96 15.09)
5d	H	H	iso-Pr	36	188—190 (EtOH)	$C_{13}H_{16}N_4O_2S \cdot CH_3SO_3H$	43.29 (43.07	5.19 5.28	14.42 14.36)
5e	H	H	Pr	44	228—230 (EtOH)	$C_{13}H_{16}N_4O_2S \cdot CH_3SO_3H$	43.29 (43.16	5.19 5.21	14.42 14.38)
5f	H	H	Butyl	41	193—195 (EtOH)	$C_{14}H_{18}N_4O_2S \cdot CH_3SO_3H$	44.76 (44.67	5.51 5.48	13.92 13.90)
5g	H	H	Pentyl	39	239—240 (EtOH)	$C_{15}H_{20}N_4O_2S \cdot CH_3SO_3H$	46.14 (46.02	5.81 5.85	13.45 13.51)
5h	H	Me	Et	28	169—171 (EtOH)	$C_{13}H_{16}N_4O_2S \cdot CH_3SO_3H$	43.29 (43.28	5.19 5.19	14.42 14.45)
5i	H	Me	iso-Pr	35	183—185 (EtOH)	$C_{14}H_{18}N_4O_2S \cdot CH_3SO_3H$	44.76 (44.68	5.51 5.48	13.92 13.93)

Table 2. Physical Properties of Methyl 2*H*-Benzo[1,4]thiazine-6-carboxylates **10**



No.	R^1	R^2	R^3	Yield (%)	mp (°C) (Recryst. solvent)	Formula	Analysis Calcd (Found)		
							C	H	N
10a	H	H	Me	63	110—112 (IPE)	$C_{12}H_{13}NO_3S$	57.35 (57.35	5.21 5.20	5.57 5.64)
10b	H	H	Et	65	56—57 (Hexane)	$C_{13}H_{15}NO_3S$	58.85 (58.80	5.70 5.60	5.28 5.25)
10c	H	H	iso-Pr	50	Oil	$C_{14}H_{17}NO_3S$	60.19 (60.17	6.13 6.24	5.01 5.04)
10d	H	H	Pr	37	Oil	$C_{14}H_{17}NO_3S$	60.19 (60.58	6.13 6.37	5.01 4.84)
10e	H	H	Butyl	62	Oil	$C_{15}H_{19}NO_3S$	61.41 (61.45	6.53 6.65	4.77 4.64)
10f	H	H	Pentyl	44	Oil	$C_{16}H_{21}NO_3S$	62.52 (62.75	6.89 7.08	4.56 4.44)
10g	H	Me	Et	79	Oil	$C_{14}H_{17}NO_3S$	60.19 (60.45	6.13 6.26	5.01 4.95)
10h	H	Me	iso-Pr	83	Oil	$C_{15}H_{19}NO_3S$	61.41 (61.36	6.53 6.61	4.77 4.50)

Na/H exchange inhibitory activity of *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazine-6-carbonyl)guanidines **5** in order to find potent Na/H exchange inhibitors.

Chemistry *N*-(2*H*-Benzo[1,4]thiazine-6-carbonyl)guanidines **5** listed in Table 1 were synthesized by the route shown in Chart 3. 4-Chrolo-3-nitrobenzoic acid (**6**) was allowed to react with ethyl mercaptoacetate or ethyl 2-mercaptopropionate to obtain 4-ethoxycarbonylmethyl (or 4-[(1-ethoxycarbony)ethyl])sulfanyl-3-nitrobenzoic acids **7**, which were converted to esters **8** by reflux with concentrated sulfuric acid in ethanol (EtOH). Compounds **8** were hydrogenated with palladium–charcoal (Pd–C), and the hydrogenated intermediates spontaneously cyclized to give ethyl 3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazine-6-carboxylates **9**, which were alkylated to give *N*-alkyl derivatives **10**. *N*-(2*H*-Benzo[1,4]thiazine-6-carbonyl)guanidines **5** were prepared from **9** or **10** by reflux with an excess amount of guanidine in dimethyl sulfoxide (DMSO) for several hours. The physical data for **5** and **10** are

Table 3. ^{1}H-NMR Spectral Data for *N*-(2*H*-Benzo[1,4]thiazine-6-carbonyl)guanidines **5**

No.	Spectral data (DMSO-d_6) δ
5a	2.40 (3H, s), 3.57 (2H, s), 7.52—7.60 (3H, m), 8.35 (4H, br), 10.77 (1H, br), 11.20 (1H, br)
5b	2.43 (3H, s), 3.43 (3H, s), 3.61 (2H, s), 7.60—7.71 (3H, m), 8.43 (4H, br), 11.35 (1H, br)
5c	1.18 (3H, t, *J*=7 Hz), 2.42 (3H, s), 3.58 (2H, s), 4.06 (2H, q, *J*=7 Hz), 7.59—7.68 (2H, m), 7.73 (1H, d, *J*=1 Hz), 8.43 (4H, br), 11.35 (1H, br)
5d	1.51 (6H, d, *J*=7 Hz), 2.45 (3H, s), 3.44 (2H, s), 4.58—4.64 (1H, m), 7.64—7.76 (3H, m), 8.42 (4H, br), 11.37 (1H, br)
5e	0.85 (3H, t, *J*=7 Hz), 1.52—1.64 (2H, m), 2.39 (3H, s), 3.61 (2H, s), 4.03 (2H, q, *J*=7 Hz), 7.61 (1H, dd, *J*=8, 2 Hz), 7.70 (1H, d, *J*=8 Hz), 7.73 (1H, d, *J*=2 Hz), 8.40 (4H, br), 11.31 (1H, br)
5f	0.88 (3H, t, *J*=7 Hz), 1.20—1.35 (2H, m), 1.46—1.58 (2H, m), 2.40 (3H, s), 3.61 (2H, s), 4.06 (2H, q, *J*=7 Hz), 7.62 (1H, dd, *J*=8, 1 Hz), 7.70 (1H, d, *J*=8 Hz), 7.75 (1H, d, *J*=1 Hz), 8.42 (4H, br), 11.33 (1H, br)
5g	0.84 (3H, t, *J*=7 Hz), 1.12—1.33 (4H, m), 1.46—1.60 (2H, m), 2.41 (3H, s), 3.61 (2H, s), 4.05 (2H, q, *J*=7 Hz), 7.62 (1H, d, *J*=8 Hz), 7.70 (1H, d, *J*=8 Hz), 7.75 (1H, s), 8.42 (4H, br), 11.34 (1H, br)
5h	1.18 (3H, t, *J*=7 Hz), 1.34 (3H, d, *J*=7 Hz), 2.41 (3H, s), 3.78 (1H, q, *J*=7 Hz), 4.07 (2H, q, *J*=7 Hz), 7.63 (1H, dd, *J*=8, 1 Hz), 7.69 (1H, d, *J*=8 Hz), 7.74 (1H, d, *J*=1 Hz), 8.44 (4H, br), 11.38 (1H, br)
5i	1.29(3H, d, *J*=7 Hz), 1.45 (3H, d, *J*=7 Hz), 1.46 (3H, d, *J*=7 Hz), 2.42 (3H, s), 3.57 (1H, q, *J*=7 Hz), 4.53—4.69 (1H, m), 7.59—7.67 (2H, m), 7.75 (1H, s), 8.43 (4H, br), 11.38 (1H, br)

Table 4. ^{1}H-NMR Spectral Data for Methyl 2*H*-Benzo[1,4]thiazine-6-carboxylates **10**

No.	Spectral data ($CDCl_3$) δ
10a	(DMSO-d_6) 1.40 (3H, t, *J*=7 Hz), 3.43 (2H, s), 3.49 (3H, s), 4.39 (2H, q, *J*=7 Hz), 7.41 (1H, d, *J*=8 Hz), 7.69 (1H, dd, *J*=8, 2 Hz), 7.75 (1H, d, *J*=2 Hz)
10b	(DMSO-d_6) 1.30 (3H, t, *J*=7 Hz), 1.40 (3H, t, *J*=7 Hz), 3.40 (2H, s), 4.10 (2H, q, *J*=7 Hz), 4.39 (2H, q, *J*=7 Hz), 7.41 (1H, d, *J*=8 Hz), 7.67 (1H, dd, *J*=8, 2 Hz), 7.81 (1H, d, *J*=2 Hz)
10c	1.39 (3H, t, *J*=7 Hz), 1.53 (6H, d, *J*=7 Hz), 3.31 (2H, s), 4.42 (2H, q, *J*=7 Hz), 4.63—4.74 (1H, m), 7.43 (1H, d, *J*=8 Hz), 7.67 (1H, dd, *J*=8, 1 Hz), 7.89 (1H, d, *J*=1 Hz)
10d	0.94 (3H, t, *J*=7 Hz), 1.42 (3H, t, *J*=7 Hz), 1.60—1.79 (2H, m), 3.41 (2H, s), 4.04 (2H, t, *J*=7 Hz), 4.39 (2H, q, *J*=7 Hz), 7.42 (1H, d, *J*=8 Hz), 7.68 (1H, dd, *J*=8, 1 Hz), 7.80 (1H, d, *J*=1 Hz)
10e	0.95 (3H, t, *J*=7 Hz), 1.36—1.42 (2H, m), 1.40 (3H, t, *J*=7 Hz), 1.60—1.64 (2H, m), 3.40 (2H, s), 4.05 (2H, t, *J*=7 Hz), 4.39 (2H, q, *J*=7 Hz), 7.42 (1H, d, *J*=8 Hz), 7.68 (1H, dd, *J*=8, 1 Hz), 7.81 (1H, d, *J*=1 Hz)
10f	0.88 (3H, t, *J*=7 Hz), 1.31—1.38 (4H, m), 1.41 (3H, t, *J*=7 Hz), 1.60—1.67 (2H, m), 3.40 (2H, s), 4.04 (2H, t, *J*=7 Hz), 4.38 (2H, q, *J*=7 Hz), 7.42 (1H, d, *J*=8 Hz), 7.68 (1H, dd, *J*=8, 2 Hz), 7.80 (1H, d, *J*=2 Hz)
10g	1.29 (3H, t, *J*=7 Hz), 1.41 (3H, t, *J*=7 Hz), 1.45 (3H, d, *J*=7 Hz), 3.49 (1H, q, *J*=7 Hz), 4.09 (2H, q, *J*=7 Hz), 4.40 (2H, q, *J*=7 Hz), 7.41 (1H, d, *J*=8 Hz), 7.68 (1H, dd, *J*=8, 2 Hz), 7.80 (1H, d, *J*=2 Hz)
10h	1.41 (3H, t, *J*=7 Hz), 1.43 (3H, d, *J*=7 Hz), 1.51 (3H, d, *J*=7 Hz), 1.54 (3H, d, *J*=7 Hz), 3.36 (1H, q, *J*=7 Hz), 4.39 (2H, q, *J*=7 Hz), 4.62—4.78 (1H, m), 7.43 (1H, d, *J*=8 Hz), 7.68 (1H, dd, *J*=8, 2 Hz), 7.88 (1H, d, *J*=8 Hz)

listed in Tables 1—4.

Biological Results and Discussion

The Na/H exchange inhibitory activities of *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazine-6-carbonyl)guanidines **5** were tested by their ability to inhibit platelet swelling induced by sodium propionate, in accordance with the method of Rosskopf *et al.*[11] The results are given in Table 5.

As expected from the clog*P* values, **5a** was actually about five-fold more potent than **4a** in *in vitro* assay. Prompted by this result, we optimized the lead compound **5a**. Several substituents were introduced to the 2- and/or 4-positions of the benzothiazine ring. All the 4-substituted compounds **5** showed more potent activity than the corresponding 4-substituted benzoxazine derivatives **4**, and their activities were considered to be parabolically related to the length of the 4-substituent. On the other hand, the 2-substituent did not much affect the activity. In order to confirm the SAR in greater detail, compounds **5** were subjected to QSAR analysis. Eq. 1 was obtained as the best equation.

$$pIC_{50}=2.10(\pm1.25)L(R^3)-0.21(\pm0.13)L(R^3)^2-3.63(\pm2.78) \quad (1)$$

$$n=9,\ r=0.861,\ s=0.396,\ F=8.61,\ L(R^3)_{opt.}=4.91$$

In Eq. 1, L is Verloop's STERIMOL parameter[12] and is 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. $L(R^3)_{opt.}$ is the calculated optimum values of $L(R^3)$.

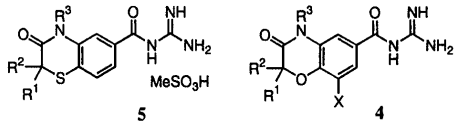
Equation 1 suggested a parabolic relation between activity and the length $L(R^3)$ of the 4-substituent. This QSAR result was identical to the SAR (Eq. 2) of the benzoxazine derivastives **4**.[7]

$$pIC_{50}=1.42(\pm0.57)L(R^3)-0.15(\pm0.06)L(R^3)^2-2.87(\pm1.36) \quad (2)$$

$$n=20,\ r=0.806,\ s=0.361,\ F=15.72,\ L(R^3)_{opt.}=4.81$$

The optimum $L(R^3)$s are almost equal in these two equations. This result was reasonable, because compounds **5** and **4** would interact with the same region of the Na/H exchanger protein. However, the constants are different in spite of similar optimum $L(R^3)$s. Therefore, we supposed that the constants depended differences in the hydrophobicity between the ring structure of **5** and **4**. In order to confirm this hypothesis, compounds **5** and **4** were subjected to QSAR analysis using the indicator variable D, which takes one for compounds **5** and takes zero for **4**. And as a result, Eq. 3 was obtained.

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



No.	R^1	R^2	R^3	X	$L(R^3)^{a)}$	Na/H exchange inhibitory activity				
						IC_{50} (μM)	pIC_{50}			
							Obsd.	Eq. 1	Eq. 2[b)]	Eq. 3
5a	H	H	H	—	2.06	2.1	−0.32	−0.20	—	0.10
5b	H	H	Me	—	3.00	0.19	0.72	0.75	—	0.83
5c	H	H	Et	—	4.11	0.051	1.29	1.40	—	1.32
5d	H	H	iso-Pr	—	4.11	0.0091	2.04	1.40	—	1.32
5e	H	H	Pr	—	5.05	0.086	1.07	1.53	—	1.41
5f	H	H	Butyl	—	6.17	0.11	0.96	1.20	—	1.15
5g	H	H	Pentyl	—	8.22	0.19	0.72	0.50	—	0.61
5h	H	Me	Et	—	4.11	0.070	1.15	1.40	—	1.32
5i	H	Me	iso-Pr	—	4.11	0.018	1.74	1.40	—	1.32
4a	H	H	H	H	2.06	10	−1.00	—	−0.57	−0.71
4b	H	H	Et	H	4.11	0.33	0.48	—	0.48	0.51
4c	H	H	iso-Pr	H	4.11	0.25	0.60	—	0.48	0.51
4d	H	Me	Et	H	4.11	0.29	0.54	—	0.48	0.51
4e	H	Et	iso-Pr	H	4.11	0.17	0.76	—	0.48	0.51
4f	H	Ph	iso-Pr	H	4.11	1.0	0.00	—	0.48	0.51
4g	Me	Me	iso-Pr	H	4.11	0.12	0.92	—	0.48	0.51
4h[c)]	Me	Me	H	H	2.06	7.4	−0.87	—	−0.57	−0.71
4i[c)]	Me	Me	Me	H	3.00	0.81	0.09	—	0.06	0.02
4j	Me	Me	Et	H	4.11	0.38	0.42	—	0.48	0.51
4k	Me	Me	Pr	H	5.05	0.74	0.13	—	0.55	0.61
4l[c)]	Me	Me	Butyl	H	6.17	1.3	−0.11	—	0.29	0.34
4m[c)]	Me	Me	$(CH_2)_2OEt$	H	6.99	5.6	−0.75	—	−0.13	−0.11
4n[c)]	Me	Me	Hexyl	H	8.22	3.7	−0.57	—	−1.13	−1.20
4o	H	H	Me	Cl	3.00	0.33	0.48	—	0.06	0.02
4p	H	H	Et	Cl	4.11	0.22	0.66	—	0.48	0.51
4q[c)]	H	H	iso-Pr	Cl	4.11	0.16	0.80	—	0.48	0.51
4r	H	H	Me	OMe	3.00	0.50	0.30	—	0.06	0.02
4s	H	H	Et	OMe	4.11	0.27	0.57	—	0.48	0.51
4t	Me	Me	iso-Pr	OMe	4.11	0.37	0.43	—	0.48	0.51

a) See reference 12. *b*) See reference 10. *c*) Hydrochloride.

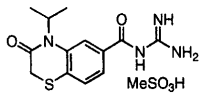
$$pIC_{50} = 1.60(\pm 0.48)L(R^3) - 0.16(\pm 0.05)L(R^3)^2 + 0.81(\pm 0.30)D - 3.31(\pm 1.13) \quad (3)$$

$$n=29,\ r=0.875,\ s=0.367,\ F=27.26,\ L(R^3)_{opt.}=4.89$$

In Eq. 3, the indicator variable D is significant. The coefficient 0.81(±0.30) of D is statistically equal to the calculated factor (0.58) based on the difference of clog*P*s between **5a** (0.20) and **4a** (−0.36).[13] Thus, our hypothesis was confirmed and it was clarified that the benzothiazine derivatives **5** were about five-fold more potent than the benzoxazine derivatives **4**. In addition, the proportional relationship between the activity and the hydrophobicity of the ring structure was re-confirmed.

The most potent derivative was the methanesulfonate salt of the 4-isopropyl derivative **5d**, with an IC_{50} value of 0.0091 μM. The clog*P* (1.15) of the free base of **5d** was around the optimum value (around 1.5—2) for good bioavailability. Compound **5d** also showed good water-solubility. Therefore, **5d** was subjected to *in vivo* investigation. Anti-arrhythmia activity of **5d** was tested in terms of the duration of ventricular fibrillation (VF) using a rat acute myocardial infarction model, in accordance with the method of Tagliavini *et al.*[14] The duration of VF time for the control group was 171±5.8 s (mean±standard error). The administration of **5d** (0.3 mg/kg, i.v.) before occlusion significantly reduced the duration of VF (5.1±5.1 s).

Table 6. Na/H Exchange Inhibitory Activity and Duration of Ventricular Fibrillation of Compound **5d**

No.	Structure	Na/H exchange inhibitory activity IC_{50} (μM)	Duration(s) of ventricular fibrillation[a)] (Mean±S.E.)
Control	—	—	171.0±5.8
5d		0.0091	5.1±5.1[b)]

a) i.v. administration of compound 0.3 mg/kg, reperfusion induced arrhythmia in anesthetized rats (n=5—7). Significantly different from the control group. *b*) $p<0.01$ (Mann–Whitney U-test).

Conclusion

In order to find potent Na/H exchange inhibitors, we designed and synthesized *N*-(3-oxo-3,4-dihydro-2*H*-benzo-

[1,4]thiazine-6-carbonyl)guanidines **5** and evaluated their inhibitory activities. The result of QSAR analysis indicated that the activity was parabolically related to the length of the 4-substituent. As expected from our previous SAR study, compounds **5** were about five-fold more potent than the benzoxazine derivatives **4**. The most potent derivative was the methanesulfonate salt of the 4-isopropyl derivative **5d** (IC_{50}=0.0091 μM). The hydrophobicity (clog$P$=1.15) of the free base of **5d** was at the optimum value (around 1.5—2) for good bioavailability. Compound **5d** also showed significant protective activity against a rat acute myocardial infarction model. This SAR-based methodology of drug design would be useful for the rapid optimization of lead candidates.

Experimental

Melting points were measured with a capillary melting point apparatus (Yamato MP-21) and are uncorrected. ^{1}H-NMR spectra were taken on a Bruker DPX-250 NMR (250 MHz) spectrometer 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.

4-Ethoxycarbonylmethylsulfanyl-3-nitrobenzoic Acid (7a) A mixture of 4-chloro-3-nitrobenzoic acid (**6**, 100 g, 496 mmol), ethyl mercaptoacetate (72 g) and pyridine (250 ml) was refluxed for 8 h. After cooling, the reaction mixture was acidified with diluted hydrochloric acid. The precipitates were collected by filtration and washed with water to give **7a** (121 g, 424 mmol, yield 85%). The analytical sample was obtained by recrystallization from EtOH, mp 159—161 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, t, J=7 Hz), 4.12 (2H, q, J=7 Hz), 4.17 (2H, s), 7.70 (1H, d, J=9 Hz), 8.15 (1H, dd, J=9, 2 Hz), 8.63 (1H, d, J=2 Hz), 13.46 (1H, br). *Anal.* Calcd for $C_{11}H_{11}NO_6S$: C, 46.31; H, 3.89; N, 4.91. Found: C, 46.37; H, 3.95; N, 4.51.

4-(1-Ethoxycarbonylethylsulfanyl)-3-nitrobenzoic Acid (7b) A mixture of 4-chloro-3-nitrobenzoic acid (**6**, 62.6 g, 311 mmol), ethyl 2-methylmercaptoacetate (50 g) and pyridine (150 ml) was refluxed for 20 h. After cooling, the reaction mixture was acidified with diluted hydrochloric acid. The precipitates were collected by filtration and washed with water and diisopropyl ether (IPE) to give **7b** (78 g, 261 mmol, yield 84%). The analytical sample was obtained by recrystallization from IPE, mp 89—91 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7 Hz), 1.67 (3H, t, J=7 Hz), 4.07—4.28 (3H, m), 7.73 (1H, d, J=9 Hz), 8.24 (1H, dd, J=9, 2 Hz), 8.88 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{12}H_{13}NO_6S$: C, 48.16; H, 4.38; N, 4.68. Found: C, 48.22; H, 4.40; N, 4.60.

Ethyl 4-Ethoxycarbonylmethylsulfanyl-3-nitrobenzoate (8a) A mixture of **7a** (30.0 g, 105 mmol), sulfuric acid (5 ml) and EtOH (300 ml) was refluxed for 5 h and concentrated *in vacuo*. Water was added to the residue. The precipitates were collected by filtration and washed with water to give **8a** (33.0 g, 105 mmol, yield 100%). The analytical sample was obtained by recrystallization from IPE, mp 66—67 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7 Hz), 1.42 (3H, t, J=7 Hz), 3.78 (2H, s), 4.23 (2H, q, J=7 Hz), 4.42 (2H, q, J=7 Hz), 7.58 (1H, d, J=9 Hz), 8.18 (1H, dd, J=9, 2 Hz), 8.85 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{13}H_{15}NO_6S$: C, 49.83; H, 4.83; N, 4.47. Found: C, 49.81; H, 4.83; N, 4.22.

Ethyl 4-(1-Ethoxycarbonylethylsulfanyl)-3-nitrobenzoate (8b) A mixture of **7b** (73.0 g, 244 mmol), sulfuric acid (7.3 ml) and EtOH (730 ml) was refluxed for 22 h and concentrated *in vacuo*. Aqueous sodium carbonate was added to the residue. The mixture was extracted with ethyl acetate (AcOEt). The extract was concentrated *in vacuo* to give **8b** (74.0 g, 226 mmol, yield 93%) as an oil. The analytical sample was obtained by column chromatography on silica gel with chloroform–MeOH (10 : 1, v/v). ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.66 (3H, d, J=7 Hz), 4.09 (1H, q, J=7 Hz), 4.12—4.26 (2H, m), 4.42 (2H, q, J=7 Hz), 7.68 (1H, d, J=9 Hz), 8.16 (1H, dd, J=9, 2 Hz), 8.79 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{14}H_{17}NO_6S$: C, 51.37; H, 5.23; N, 4.28. Found: C, 51.16; H, 5.17; N, 4.31.

Ethyl 3-Oxo-3,4-dihydro-2*H*-benzo[1,4]thiazine-6-carboxylate (9a) A mixture of **8a** (31.0 g, 98.9 mmol), 10% Pd–C (3.1 g) and EtOH (300 ml) was stirred at 50 °C under a hydrogen atmosphere (4 kg/cm^2) for 2 h. The mixture was filtered. The filtrate was evaporated *in vacuo*. The residue was column chromatographed on silica gel with chloroform and triturated with hexane–AcOEt to give **9a** (7.80 g, 32.9 mmol, yield 33%). The analytical sample was obtained by recrystallization from hexane–AcOEt, mp 159—160 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7 Hz), 3.46 (2H, s), 4.37 (2H, q, J=7 Hz), 7.37 (1H, d, J=8 Hz), 7.50 (1H, d, J=2 Hz), 7.69 (1H, dd, J=8, 2 Hz), 7.92 (1H, br). *Anal.* Calcd for $C_{11}H_{11}NO_3S$: C, 55.68; H, 4.67; N, 5.90. Found: C, 55.76; H, 4.71; N, 5.75.

Ethyl 2-Methyl-3-oxo-3,4-dihydro-2*H*-benzo[1,4]thiazine-6-carboxylate (9b) A mixture of **8b** (40.0 g, 122 mmol), 10% Pd–C (4.0 g) and EtOH (400 ml) was stirred at 40 °C under a hydrogen atmosphere (4 kg/cm^2) for 16 h. The mixture was filtered. The filtrate was evaporated *in vacuo*. The residue was washed with IPE and recrystallized from AcOEt–IPE to give **9b** (18.85 g, 75.0 mmol, yield 61%), mp 155—157 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7 Hz), 1.51 (3H, d, J=7 Hz), 3.59 (1H, q, J=7 Hz), 4.39 (2H, q, J=7 Hz), 7.37 (1H, d, J=8 Hz), 7.56 (1H, d, J=2 Hz), 7.68 (1H, dd, J=8, 2 Hz), 8.38 (1H, br). *Anal.* Calcd for $C_{12}H_{13}NO_3S$: C, 57.35; H, 5.21; N, 5.57. Found: C, 57.46; H, 5.23; N, 5.38.

General Procedure for the Preparation of Methyl 3-Oxo-4-substituted-3,4-dihydro-2*H*-benzo[1,4]thiazine-6-carboxylates 10 An alkyl iodide (20 mmol) was added to a mixture of **9** (10 mmol), potassium *tert*-butoxide (15 mmol) and tetrahydrofuran (24 ml) at room temperature. The mixture was stirred at 60 °C for several hours. Water was added to the mixture and the whole was extracted with AcOEt. The extract was washed with water and dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo*. The residue was chromatographed on silica gel with hexane–AcOEt or recrystallized 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]thiazine-6-carbonyl)guanidine Methanesulfonates 5 A mixture of **9** or **10** (5 mmol), guanidine hydrochloride (55 mmol), sodium methoxide (50 mmol), and DMSO (25 ml) was stirred at 120 °C for several hours, then diluted with water. The precipitates were collected by filtration, washed with water and dissolved in MeOH. Methanesulfonic acid was added to the solution. After concentration *in vacuo*, the residue was recrystallized to give **5**. Physical data are listed in Tables 1 and 3.

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.*[11] Platelet-rich plasma was prepared as described by Mammen and coworkers.[15] Male Wistar rats (230—300 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 the test compound in 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 a 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. 4.

$$\text{Swelling inhibitory rate (Na/H exchange inhibitory rate, \%)} = (1 - D/C) \times 100 \quad (4)$$

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 **5d** was tested for inhibitory activity against reperfusion arrhythmia in accordance with the method of Tagliavini *et al.*[14] Male Sprague–Dawley rats (310—510 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 (lead II) was obtained from electrodes attached to limbs using a bioelectric amplifier, while body temperature was maintained at 37 °C. The chests 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.[16] The mixed solvent of polyethylene glycol 400, ethanol and physio-

logical saline [3 : 3 : 14 (v/v)] was used as a control, and the duration (seconds) of ventricular fibrillation was measured in a similar manner.

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