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Design, Synthesis and Quantitative Structure–Activity Relationship Study of *N*-(3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine Derivatives as Potent Na/H Exchange Inhibitors

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Inhibition of the Na/H exchanger is a promising approach for treating ischemia-reperfusion injury, but no clinical agent is yet available. Recently, we established the structural requirements for potent inhibitors of the Na/H exchanger. In the present work, we designed *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine 3a as a new lead compound for potent inhibitors with good water-solubility, based on the previous information. During the structural optimization, care was taken to keep the hydrophobicity (clogP) in the range of about 1.5—2.0, which is considered optimum for good bioavailability. Various derivatives of 3a were synthesized and the quantitative structure–activity relationship (QSAR) was studied. The QSAR result indicated that the lengths of the substituents at the 2- and the 4-positions of the 2*H*-benzo[1,4]oxazine ring are parabolically related to activity. The most potent compounds were (*R*) and/or (*S*)-*N*-(2-ethyl-4-isopropyl(or ethyl)-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidines 3q—t with IC_{50} values of 0.036—0.073 μM. The water-solubility of the hydrochlorides and methanesulfonates is 3—5 mg/ml, which is sufficient for therapeutic use.

Key words Na/H exchange inhibitor; quantitative structure–activity relationship; water-solubility; *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine

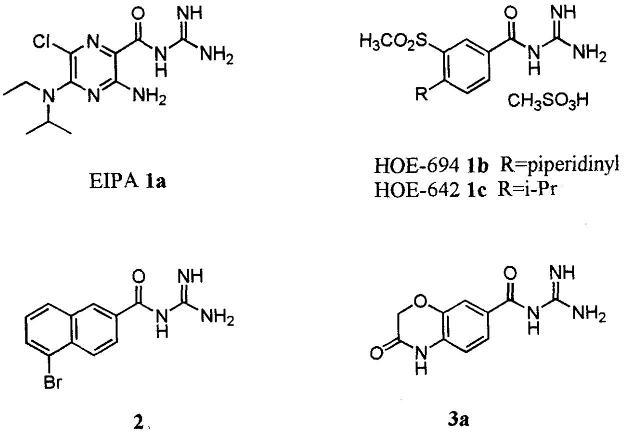
The Na/H exchanger, a plasma membrane ion transporter, plays an important role in the regulation of Na^+ and H^+ concentration and is involved in many physiological processes, for example control of cellular volume and cellular growth. Recently, interest has been focused on the role of Na/H exchange in ischemia-reperfusion injury. Activation of the Na/H exchanger is triggered by post ischemia reperfusion, and results in Na^+ overload, which leads to Ca^{2+} overload in cardiac cells. Ca^{2+} overload causes cellular dysfunction, damage, and necrosis. Therefore, inhibition of the Na/H exchanger is a promising approach for treating ischemia-reperfusion injury, such as arrythmia and cardiac dysfunction.[1)]

In our previous report, we investigated the structural requirements for a potent Na/H exchange inhibitor using the quantitative structure–activity relationship (QSAR) analysis of ethylisopropylamiloride (EIPA) **1a**[2)] and we found that 1) bicyclic aroylguanidines strongly inhibit the Na/H exchanger, 2) the activity was proportionally related to the hydrophobicity of the (pseudo)bicyclic ring structures, and 3) a substituent having appropriate length at the position corresponding to the 5-position of 2-naphthoylguanidine enhanced the activity.[3)] Among 5-substituted-2-naphthoylguanidines, the 5-bromo derivative **2** exhibited strong activity ($IC_{50}=0.010\,\mu$M), but its water-solubility is very low (<0.1 mg/ml). Since low water-solubility is a disadvantage for clinical use, we decided to search for a potent and highly water-soluble Na/H exchange inhibitor. Recently, a Hoechst group reported a benzoylguanidine derivative, HOE-694 **1b** as an Na/H exchange inhibitor, though its water-solubility is limited.[4)] After our project was started, a derivative of HOE-694, HOE-642 **1c**, with good water-solubility was reported by the Hoechst group.[5)]

In this paper, we describe the design and synthesis of *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine **3a** as a new lead compound for potent Na/H exchange inhibitors with good water-solubility.

Design of Lead Compound

Previously reported 5-bromo-2-naphthoylguanidine **2** showed a potent activity[3)], however, it has a clogP value of 2.69, which might be too high for good water-solubility. Yalkowky and Valvani reported that the water-solubility of organic crystals is negatively related to their hydrophobicity (log P) and to their melting point (mp) in general.[6)] Therefore, in order to obtain a highly water-soluble compound, we require a low hydrophobicity and/or low melting point compound. The hydrophobicity (clogP) is easily estimated by computer programs such as PC models,[7)] although it is difficult to predict the melting point of a compound. Thus, we decided to focus on the hydrophobicity to find a compound with high water-solubility. However, Na/H exchange inhibitory activity is proportionally related to the hydrophobicity of the ring



EIPA **1a**

HOE-694 **1b** R=piperidinyl
HOE-642 **1c** R=i-Pr

2

3a

Chart 1. Na/H Exchange Inhibitors

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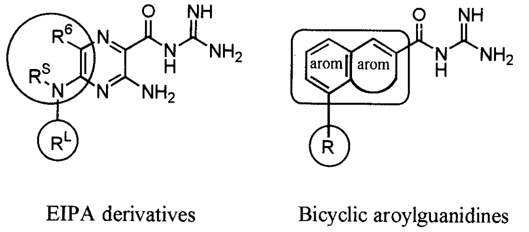
moiety of bicyclic aroylguanidines.[3] Hence, there is an inverse relation between the water-solubility and the inhibitory activity with respect to hydrophobicity. We therefore decided to consider the hydrophobicity from the viewpoint of the bioavailability. The optimum log P value of drugs for good absorption from the intestine is reported to be about 1.5—2.0.[8] Thus, we tried to design a lead compound such that the clogP of the optimized compound would be about 1.5—2.0.

The introduction of a substituent of appropriate length at the position corresponding to the 5-position of 2-naphthoylguanidine enhances the activity (Chart 2).[3] In the EIPA derivatives, the larger substituent (R^L) at the 5-amino group of the pyrazine ring corresponds to the substituent at the 5-position of naphthalene, and QSAR analysis showed that the length (L) of R^L parabolically related to the activity. The parameter L is Verloop's STERIMOL parameter,[9] based on the assumption that R^L is in an extended conformation, and the calculated optimum length $L(R^L)$ was 6.30, which corresponds to that of a butyl group ($L=6.17$).

The hydrophobicity (π) value of the butyl group is 2.05.[10] As the introduction of a butyl group into a lead compound during optimization will increase the clogP of the optimized compound by a clogP of 2.05, the clogP of a lead compound must be about -0.5 in order to achieve the optimum log P value of 1.5—2.0 for good bioavailability. We reported that 2-naphthoylguanidine has strong inhibitory activity in our previous paper,[3] but its clogP is rather high (clogP$=1.83$). Substitution of two carbon atoms in the naphthalene ring by two hetero atoms, such as a nitrogen or an oxygen, decreases the clogP by about 2. A benzene ring was selected as the A ring for ease of synthesis (Chart 3). A nitrogen atom was introduced at the 5-position of 2-naphthoylguanidine, allowing easy synthesis of various derivatives modified at the 5-position. Based on these considerations, we first designed *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine **3a** as a new lead compound.

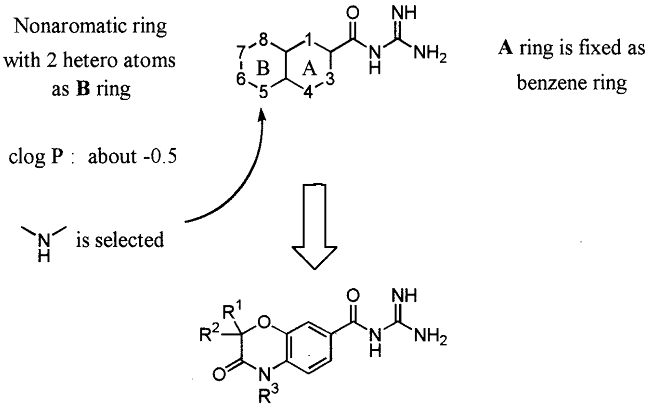
The clogP of the lead compound **3a** was calculated to be -0.36, and its IC_{50} value was predicted to be around 13 μM from the QSAR equation of bicyclic aroylguani-



Ring: A more hydrophobic (pseudo)bicyclic ring is preferred

Substituent: Appropriate length enhances the activity

Chart 2. Structural Requirements for Potent Activity



Lead compound **3** (**3a**: $R^1=R^2=R^3=H$)

Chart 3. Design of the Lead Compound

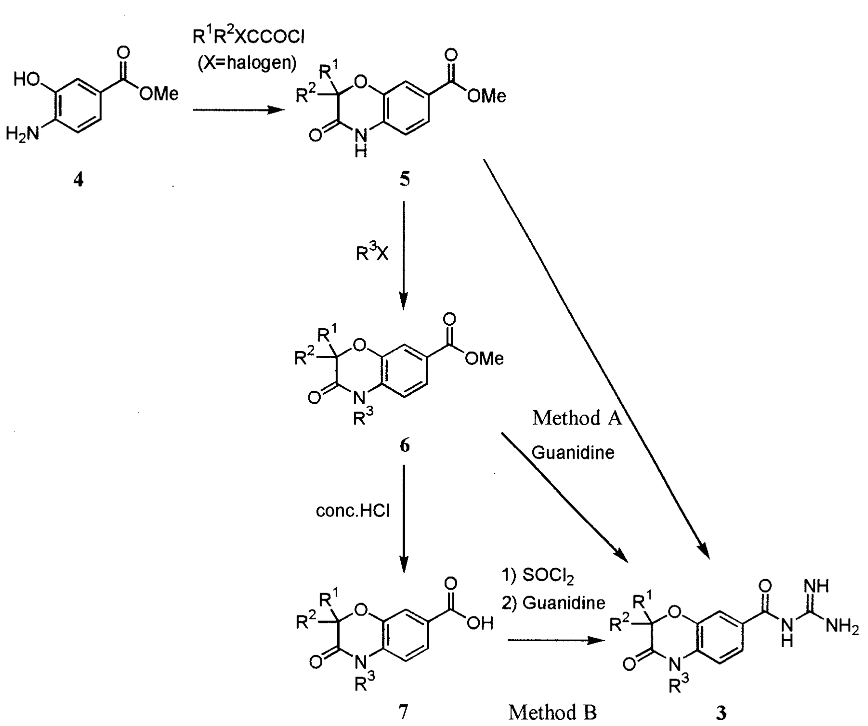


Chart 4. Syntheses of *N*-(2*H*-Benzo[1,4]oxazine-7-carbonyl)guanidines **3**

dines.[11] Although the expected activity of the lead compound **3a** was not high, we anticipated that it would be improved by the introduction of an appropriate substituent.

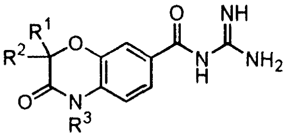
Chemistry

N-(3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidines **3** (Table 1) were synthesized by method A or B in Chart 4.

In method A, *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidines **3a**—**p** were synthesized from methyl 3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carboxylates **5a** and **6a**—**o** by refluxing with excess guanidine in methanol (MeOH) for several hours. The methyl esters **5** were obtained by condensation of methyl 4-amino-3-hydroxybenzoate **4** with 2-haloacid halide. The methyl esters **6a**—**o** were prepared by *N*-alkylation of **5**. Method A was not applicable to the synthesis of optically active derivatives **3q**—**t** at the 2-position of the 2*H*-benzo[1,4]oxazine ring, because the strong basicity of guanidine caused racemization.

Optically active *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]-oxazine-7-carbonyl)guanidines **3q**—**t** were synthesized by method B. The condensation of compound **4** and optically active 2-haloacid chlorides gave 2*H*-benzo[1,4]oxazines **5i** and **5j**, which were alkylated to afford compounds **6p**—**s**. After acid hydrolysis of **6p**—**s**, the obtained acids **7** were converted to the acid chlorides, which were then allowed

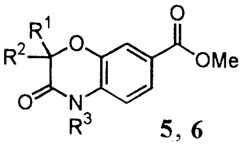
Table 1. Physical Data for *N*-(2*H*-Benzo[1,4]oxazine-7-carbonyl)guanidines **3**



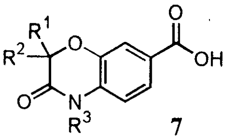
No.	R^1	R^2	R^3	Note[a]	Method	Yield (%)	mp (°C) (recryst. solvent)	Formula	Analysis (%) Calcd (Found)		
									C	H	N
3a	H	H	H		A	7	>300 (MeOH)	$C_{10}H_{10}N_4O_3$ $1/10H_2O$	50.89 (50.86	4.36 4.33	23.74 23.70)
3b	H	H	Me		A	8	254—256 (EtOH)	$C_{11}H_{12}N_4O_3$ $1/10H_2O$	52.87 (52.70	4.86 4.94	22.42 22.34)
3c	H	H	Et		A	66	242—245 (MeOH)	$C_{12}H_{14}N_4O_3$	54.96 (54.77	5.38 5.41	21.36 21.56)
3d	H	H	iso-Pr		A	60	235—237 (MeOH)	$C_{13}H_{16}N_4O_3$	56.51 (56.32	5.84 5.84	20.28 20.34)
3e	H	H	Pr	HCl	A	5	262—263 (EtOH)	$C_{13}H_{16}N_4O_3$ HCl	49.92 (49.86	5.48 5.46	17.91 17.88)
3f	H	H	iso-Butyl	HCl	A	29	256—257 (EtOH)	$C_{14}H_{18}N_4O_3$ HCl	51.46 (51.33	5.86 5.93	17.15 17.03)
3g	H	H	$(CH_2)_2OEt$		A	14	187—189 (MeCN)	$C_{14}H_{18}N_4O_4$ $1/10H_2O$	51.59 (51.32	6.18 5.82	17.19 16.93)
3h	H	H	Pentyl		A	30	176—178 (MeCN)	$C_{15}H_{20}N_4O_3$	59.20 (59.10	6.62 6.60	18.41 18.34)
3i	H	H	Bzl		A	9	240—242 (MeCN)	$C_{17}H_{16}N_4O_3$	62.95 (62.79	4.97 5.06	17.27 17.46)
3j	H	Me	iso-Pr		A	16	228—230 (IPA)	$C_{14}H_{18}N_4O_3$	57.92 (57.94	6.25 6.30	19.30 19.25)
3k	Me	Me	iso-Pr		A	14	230—231 (MeCN)	$C_{15}H_{20}N_4O_3$	59.20 (59.00	6.62 6.60	18.41 18.64)
3l	H	Et	iso-Pr		A	30	192—195 (MeCN)	$C_{15}H_{20}N_4O_3$	59.20 (58.96	6.62 6.60	18.41 18.61)
3m	H	iso-Pr	iso-Pr	HCl	A	43	191—193 (MeCN)	$C_{16}H_{22}N_4O_3$	54.16 (54.20	6.53 6.38	15.79 15.74)
3n	H	Butyl	iso-Pr	HCl	A	24	Amorphous	$C_{17}H_{24}N_4O_3$ HCl $1/2H_2O$	54.03 (54.20	6.93 6.74	14.83 15.04)
3o	H	Ph	iso-Pr	HCl	A	33	Amorphous	$C_{19}H_{20}N_4O_3$ HCl $1/2H_2O$	57.36 (57.34	5.57 5.59	14.08 14.03)
3p	H	Hexyl	iso-Pr	HCl	A	51	Amorphous	$C_{19}H_{28}N_4O_3$ HCl $1/2H_2O$	56.22 (56.48	7.45 7.33	13.80 14.10)
3q	H	Et	iso-Pr	(*R*) 94% e.e. $[\alpha]_D^{25}$ −19	B	33	199—200 (MeCN)	$C_{15}H_{20}N_4O_3$	59.20 (59.20	6.62 6.59	18.41 18.59)
3r	H	Et	iso-Pr	(*S*) 97% e.e $[\alpha]_D^{25}$ +17	B	15	198—199 (MeCN)	$C_{15}H_{20}N_4O_3$	59.20 (58.92	6.62 6.57	18.41 18.49)
3s	H	Et	Et	(*R*)>98% e.e. $[\alpha]_D^{25}$ −19	B	60	181—182 (MeCN)	$C_{14}H_{18}N_4O_3$	57.92 (57.78	6.25 6.21	19.30 19.34)
3t	H	Et	Et	(*S*) 98% e.e. $[\alpha]_D^{25}$ +18	B	45	181—182 (MeCN)	$C_{14}H_{18}N_4O_3$	57.92 (57.95	6.25 6.22	19.30 19.33)

a) Configuration at C2 carbon. Optical rotation: $c=1$, MeOH. Enantiomeric excess (e.e.) was determined by HPLC (see Experimental section).

Table 2. Physical Data for 2*H*-Benzo[1,4]oxazine-7-carboxylic Acids **7** and Methyl Esters **5** and **6**



5, 6



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No.	R^1	R^2	R^3	Note[a]	Yield (%)	mp (°C) (recryst. solvent)	Formula	Analysis(%) Calcd (Found)		
								C	H	N
5a	H	H	H		73	258—260 (MeCN)	$C_{10}H_9NO_4$	57.97 (58.14	4.38 4.45	6.76 6.72)
5b	H	Me	H		71	168—170 (MeCN)	$C_{11}H_{11}NO_4$	59.73 (59.66	6.33 6.36	5.01 5.07)
5c	Me	Me	H		78	183—185 (AcOEt)	$C_{12}H_{13}NO_4$	61.27 (61.38	5.57 5.55	5.95 5.93)
5d	H	Et	H		78	132—134 (MeCN)	$C_{12}H_{13}NO_4$	61.27 (60.95	5.57 5.55	5.95 5.92)
5e	H	iso-Pr	H		69	142—144 (MeCN)	$C_{13}H_{15}NO_4$	62.64 (62.66	6.07 6.07	5.62 5.46)
5f	H	Butyl	H		83	139—140 (Et_2O)	$C_{14}H_{17}NO_4$	63.87 (63.76	6.51 6.48	5.32 5.06)
5g	H	Hexyl	H		75	Oil	$C_{16}H_{21}NO_4$	65.96 (65.86	7.27 7.14	4.81 4.61)
5h	H	Ph	H		68	213—214 (MeCN)	$C_{16}H_{13}NO_4$	67.84 (67.95	4.63 4.66	4.94 4.89)
5i	H	Et	H	(*R*) 95% e.e. $[\alpha]_D^{25}$ −15	79	133—135 (MeCN)	$C_{12}H_{13}NO_4$	61.27 (61.30	5.57 5.56	5.95 5.91)
5j	H	Et	H	(*S*) 96% e.e. $[\alpha]_D^{25}$ +13	89	133—135 (MeCN)	$C_{12}H_{13}NO_4$	61.27 (61.30	5.57 5.58	5.95 6.01)
6a	H	H	Me		77	150—151 (IPA–AcOEt)	$C_{11}H_{11}NO_4$	59.73 (59.68	6.33 6.34	5.01 5.02)
6b	H	H	Et		27	115—116 (AcOEt–hexane)	$C_{12}H_{13}NO_4$	61.27 (61.36	5.57 5.61	5.95 5.98)
6c	H	H	iso-Pr		25	64—66 (IPE)	$C_{13}H_{15}NO_4$	62.64 (62.81	6.07 5.91	5.62 5.68)
6d	H	H	Pr		89	64—66 (IPE)	$C_{13}H_{15}NO_4$	62.64 (62.48	6.07 6.04	5.62 5.57)
6e	H	H	iso-Butyl		73	97—99 (IPE)	$C_{14}H_{17}NO_4$	63.87 (63.78	6.51 6.46	5.32 5.32)
6f	H	H	$(CH_2)_2OEt$		54	Oil	$C_{14}H_{17}NO_5$	60.21 (60.19	6.14 6.20	5.02 4.92)
6g	H	H	Pentyl		65	85—87 (IPE)	$C_{15}H_{19}NO_4$	64.97 (65.05	6.91 6.88	5.05 4.98)
6h	H	H	Bzl		48	113—115 (IPE)	$C_{17}H_{15}NO_4$	68.68 (68.98	5.09 5.20	4.71 4.59)
6i	H	Me	iso-Pr		43	Oil	$C_{14}H_{17}NO_4$	63.87 (63.79	6.51 6.50	5.32 5.35)
6j	Me	Me	iso-Pr		49	62—63 (IPE)	$C_{15}H_{19}NO_4$	64.97 (64.95	6.91 6.91	5.05 5.04)
6k	H	Et	iso-Pr		38	Oil	$C_{15}H_{19}NO_4$	64.97 (64.88	6.91 6.98	5.05 5.00)
6l	H	iso-Pr	iso-Pr		43	Oil	$C_{16}H_{21}NO_4$	65.96 (65.98	7.27 7.36	4.81 4.65)
6m	H	Butyl	iso-Pr		21	Oil	$C_{17}H_{23}NO_4$	66.86 (66.65	7.59 7.51	4.59 4.57)
6n	H	Ph	iso-Pr		36	95—96 (IPE)	$C_{19}H_{19}NO_4$	70.14 (69.98	5.89 5.87	4.30 4.30)
6o	H	Hexyl	iso-Pr		42	Oil	$C_{19}H_{27}NO_4$	68.44 (68.43	8.16 8.17	4.20 3.98)
6p	H	Et	iso-Pr	(*R*) 96% e.e. $[\alpha]_D^{22}$ −8	39	Oil	$C_{15}H_{19}NO_4$	64.97 (64.88	6.91 6.99	5.05 5.14)
6q	H	Et	iso-Pr	(*S*) 88% e.e. $[\alpha]_D^{22}$ +8	48	Oil	$C_{15}H_{19}NO_4$	64.97 (65.12	6.91 7.02	5.05 5.04)
6r	H	Et	Et	(*R*) 96% e.e. $[\alpha]_D^{24}$ −8	89	51—53 (MeCN)	$C_{14}H_{17}NO_4$	63.87 (63.64	6.51 6.43	5.32 5.08)
6s	H	Et	Et	(*S*) 96% e.e. $[\alpha]_D^{24}$ +7	96	51—53 (MeCN)	$C_{14}H_{17}NO_4$	63.87 (63.64	6.51 6.48	5.32 5.28)
7a[b]	H	Et	iso-Pr	(*R*) 85% e.e. $[\alpha]_D^{22}$ −5	99	176—178 (MeCN)	$C_{14}H_{17}NO_4$	63.87 (63.93	6.51 6.57	5.32 5.40)
7b[b]	H	Et	iso-Pr	(*S*) 96% e.e. $[\alpha]_D^{22}$ +5	87	176—178 (MeCN)	$C_{14}H_{17}NO_4$	63.87 (63.73	6.51 6.53	5.32 5.40)
7c[b]	H	Et	Et	(*R*) 97% e.e. $[\alpha]_D^{24}$ −8	70	190—192 (MeCN)	$C_{13}H_{15}NO_4$	62.64 (62.49	6.07 6.05	5.62 5.48)
7d[b]	H	Et	Et	(*S*) 97% e.e. $[\alpha]_D^{25}$ +7	82	190—192 (MeCN)	$C_{13}H_{15}NO_4$	62.64 (62.59	6.07 6.01	5.62 5.59)

a) Configuration at C2 carbon. Optical rotation: $c=1$, MeOH. Enantiomeric excess (e.e.) was determined by HPLC (see Experimental section). *b*) Synthesized by Method B.

Table 3. ^{1}H-NMR Spectral Data for *N*-(2*H*-Benzo[1,4]oxazine-7-carbonyl)guanidines **3**

No.	Spectral data (in DMSO-d_6)
3a	(300 MHz) δ 4.58 (2H, s), 6.71 (2H, br), 6.86 (1H, d, $J=8$ Hz), 7.60 (1H, d, $J=2$ Hz), 7.67 (1H, dd, $J=8$, 2 Hz), 7.75 (2H, br), 10.86 (1H, br)
3b	(300 MHz) δ 3.29 (3H, s), 4.66 (2H, s), 6.75 (2H, br), 7.15 (1H, d, $J=8$ Hz), 7.64 (1H, d, $J=2$ Hz), 7.78 (1H, dd, $J=8$, 2 Hz), 7.91 (2H, br)
3c	(60 MHz) δ 1.20 (3H, t, $J=7$ Hz), 4.00 (2H, q, $J=7$ Hz), 4.59 (2H, s), 7.05 (1H, d, $J=9$ Hz), 7.69 (1H, d, $J=1$ Hz), 7.80 (1H, dd, $J=9$, 1 Hz), 8.10 (4H, br)
3d	(60 MHz) δ 1.58 (6H, d, $J=7$ Hz), 4.42 (2H, s), 4.40—4.90 (1H, m), 7.12 (1H, d, $J=7$ Hz), 7.70 (1H, d, $J=1$ Hz), 7.80 (1H, dd, $J=7$, 1 Hz), 8.00 (4H, br)
3e	(60 MHz) δ 0.90 (3H, t, $J=7$ Hz), 1.48 (2H, tq, $J=7$, 7 Hz), 3.93 (2H, t, $J=7$ Hz), 4.59 (2H, s), 7.30 (1H, d, $J=9$ Hz), 7.79 (1H, d, $J=2$ Hz), 7.99 (1H, dd, $J=9$, 2 Hz), 8.52 (4H, br)
3f	(60 MHz) δ 0.92 (6H, d, $J=7$ Hz), 1.14—1.30 (1H, m), 3.85 (2H, d, $J=7$ Hz), 4.72 (2H, s), 7.35 (1H, d, $J=8$ Hz), 7.79 (1H, d, $J=2$ Hz), 8.06 (1H, dd, $J=8$, 2 Hz), 8.23 (4H, br)
3g	(250 MHz) δ 1.05 (3H, t, $J=7$ Hz), 3.43 (2H, q, $J=7$ Hz), 3.57 (2H, t, $J=5$ Hz), 4.09 (2H, t, $J=5$ Hz), 4.66 (2H, s), 6.75 (2H, br), 7.28 (1H, d, $J=8$ Hz), 7.64 (1H, d, $J=1$ Hz), 7.75 (1H, dd, $J=8$, 1 Hz), 7.85 (2H, br)
3h	(250 MHz) δ 0.86 (3H, t, $J=7$ Hz), 1.22—1.41 (4H, m), 1.43—1.67 (2H, m), 3.91 (2H, t, $J=7$ Hz), 4.64 (2H, s), 6.75 (2H, br), 7.17 (1H, d, $J=8$ Hz), 7.66 (1H, d, $J=1$ Hz), 7.75 (2H, br), 7.78 (1H, dd, $J=8$, 1 Hz), 7.85 (2H, br)
3i	(300 MHz) δ 4.82 (2H, s), 5.18 (2H, s), 6.74 (2H, br), 7.03 (1H, d, $J=8$ Hz), 7.22—7.36 (5H, m), 7.63 (1H, dd, $J=8$, 2 Hz), 7.84 (1H, d, $J=2$ Hz), 7.88 (2H, br)
3j	(60 MHz) δ 1.52 (3H, d, $J=6$ Hz), 1.58 (6H, d, $J=6$ Hz), 4.65 (1H, q, $J=6$ Hz), 4.65—5.10 (1H, m), 7.20 (1H, d, $J=8$ Hz), 7.25 (4H, br), 7.95 (1H, d, $J=1$ Hz), 7.97 (1H, dd, $J=8$, 1 Hz)
3k	(60 MHz) δ 1.33 (6H, s), 1.49 (6H, d, $J=6$ Hz), 4.50—4.90 (1H, m), 7.30 (1H, d, $J=7$ Hz), 7.60—7.95 (6H, br)
3l	(60 MHz) δ 1.09 (3H, t, $J=6$ Hz), 1.59 (6H, d, $J=6$ Hz), 1.60—2.10 (2H, m), 4.30 (1H, t, $J=6$ Hz), 4.50—5.10 (1H, m), 6.50 (4H, br), 7.19 (1H, d, $J=7$ Hz), 7.90 (1H, s), 7.95 (1H, d, $J=7$ Hz)
3m	(250 MHz) δ 0.96 (3H, d, $J=7$ Hz), 1.00 (3H, d, $J=7$ Hz), 1.47 (3H, d, $J=7$ Hz), 1.49 (3H, d, $J=7$ Hz), 1.99—2.19 (1H, m), 4.34 (1H, d, $J=6$ Hz), 4.64—4.84 (1H, m), 7.48 (1H, d, $J=9$ Hz), 7.83 (1H, d, $J=2$ Hz), 7.91 (1H, dd, $J=9$, 2 Hz), 8.55 (2H, br), 8.77 (2H, br), 11.98 (1H, br)
3n	(60 MHz) δ 0.70—1.10 (3H, m), 1.51 (6H, d, $J=7$ Hz), 1.11—2.10 (6H, m), 4.20—5.00 (2H, m), 6.95 (4H, br), 7.10 (1H, d, $J=9$ Hz), 7.76 (1H, d, $J=1$ Hz), 7.86 (1H, dd, $J=9$, 1 Hz)
3o	(60 MHz) δ 1.47 (3H, d, $J=7$ Hz), 1.52 (3H, d, $J=7$ Hz), 4.75—4.64 (1H, m), 5.65 (1H, s), 7.33 (5H, s), 7.47 (1H, d, $J=9$ Hz), 7.76 (1H, d, $J=2$ Hz), 7.80 (1H, dd, $J=9$, 2 Hz), 8.48 (4H, br), 11.92 (1H, br)
3p	(250 MHz) δ 0.85 (3H, t, $J=7$ Hz), 1.47 (6H, d, $J=7$ Hz), 1.00—1.87 (8H, m), 4.57 (1H, dd, $J=5$, 8 Hz), 4.62—4.82 (1H, m), 7.49 (1H, d, $J=9$ Hz), 7.80 (1H, d, $J=2$ Hz), 7.94 (1H, dd, $J=9$, 2 Hz), 8.57 (2H, br), 8.78 (2H, br), 12.01 (1H, br)
3q	(300 MHz) δ 1.06 (3H, t, $J=7$ Hz), 1.52 (3H, d, $J=7$ Hz), 1.54 (3H, d, $J=7$ Hz), 1.70—2.00 (2H, m), 4.34 (1H, dd, $J=9$, 4 Hz), 4.70—4.85 (1H, m), 6.25 (4H, br), 7.10—7.13 (1H, m), 7.82—7.85 (2H, m)
3r	(300 MHz) δ 1.05 (3H, t, $J=7$ Hz), 1.52 (3H, d, $J=7$ Hz), 1.53 (3H, d, $J=7$ Hz), 1.70—2.10 (2H, m), 4.34 (1H, dd, $J=8$, 4 Hz), 4.70—4.90 (1H, m), 6.50 (4H, br), 7.11—7.14 (1H, m), 7.82—7.85 (2H, m)
3s	(250 MHz, $\mathrm{CDCl_3}$) δ 1.07 (3H, t, $J=8$ Hz), 1.27 (3H, t, $J=8$ Hz), 1.70—2.00 (2H, m), 3.99 (2H, q, $J=8$ Hz), 4.47 (1H, dd, $J=9$, 5 Hz), 6.45 (4H, br), 6.98 (1H, d, $J=9$ Hz), 7.82 (1H, d, $J=2$ Hz), 7.86 (1H, dd, $J=9$, 2 Hz)
3t	(250 MHz, $\mathrm{CDCl_3}$) δ 1.07 (3H, t, $J=8$ Hz), 1.27 (3H, t, $J=7$ Hz), 1.75—1.94 (2H, m), 3.99 (2H, q, $J=7$ Hz), 4.47 (1H, dd, $J=9$, 5 Hz), 6.45 (4H, br), 6.98 (1H, d, $J=9$ Hz), 7.82 (1H, d, $J=2$ Hz), 7.86 (1H, dd, $J=9$, 2 Hz)

to react with guanidine to give optically active acylguanidines **3q**—**t**. The physical data are listed in Tables 1—4.

Results and Discussion

N-(3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidines **3** were evaluated for Na/H exchange inhibitory activity based on their inhibition of the platelet swelling induced by sodium propionate in accordance with the method of Rosskoph *et al.*[12] The results are listed in Table 5.

As expected, the new lead compound **3a** showed Na/H exchange inhibitory activity with the IC_{50} value of 2.70 μM. Although this IC_{50} value is smaller than the extrapolatively predicted value of around 13 μM, it is within 95% confidential interval of the prediction.

At first, *N*-substituted derivatives **3b**—**f** and **3h** were synthesized in order to optimize the substituent R^3 at the 4-position, which corresponded to the 5-position of 2-naphthoylguanidine. With respect to the alkyl derivatives **3b**—**f** and **3h**, the activity seemed to be parabolically related to the length of R^3 (Table 5). This SAR was observed in the case of the 4-ethoxyethyl derivative **3g** and the 4-benzyl derivative **3i**. These results are in agreement with the reported QSAR of the EIPA derivatives.[3] Among compounds **3a**—**i**, the *N*-isopropyl derivative **3d** with the IC_{50} value of 0.17 μM is the most potent derivative. The c log P value (0.61) of *N*-isopropyl derivative **3d** was lower than the reported optimum value (about 1.5—2.0). This indicated that the introduction of an alkyl substituent at the 2-position was possible from the viewpoint of c log P. Therefore, the SAR of the substituents R^1 and R^2 at the 2-position was investigated.

Replacement of the hydrogen atom of **3d** with a methyl group (**3j**) caused a two fold enhancement in the potency. However, additional methyl substitution of **3j** to afford **3k** reduced the activity by a factor of two. This may be due to the steric bulkiness of the dimethyl moiety. Therefore, various mono 2-substituted derivatives **3l**—**p** were synthesized. Among compounds **3d** and **3j**—**p**, the ethyl derivative **3l** showed the most potent activity. The derivatives **3m**—**p** which have a longer group, such as butyl, phenyl and hexyl, showed less potent activity. The effect of the substituent R^1 or R^2 on the activity was similar to that of R^3.

In order to confirm the SAR of the substituents R^1, R^2

Table 4. ^{1}H-NMR Spectral Data for 2*H*-Benzo[1,4]oxazine-7-carboxylic Acids **7** and Methyl Esters **5** and **6**

No.	Spectral data (in CDCl$_3$)
5a	(60 MHz, DMSO-d_6) δ 3.85 (3H, s), 4.60 (2H, s), 6.98 (1H, d, $J=8$ Hz), 7.52 (1H, d, $J=1$ Hz), 7.60 (1H, dd, $J=8$, 1 Hz), 10.80 (1H, br)
5b	(60 MHz) δ 1.62 (3H, d, $J=7$ Hz), 3.92 (3H, s), 4.72 (1H, q, $J=7$ Hz), 6.90 (1H, d, $J=7$ Hz), 7.63 (1H, d, $J=1$ Hz), 7.71 (1H, dd, $J=7$, 1 Hz), 9.51 (1H, br)
5c	(60 MHz) δ 1.58 (6H, s), 3.88 (3H, s), 6.88 (1H, d, $J=9$ Hz), 7.60 (1H, d, $J=1$ Hz), 7.70 (1H, dd, $J=9$, 1 Hz), 9.40 (1H, br)
5d	(60 MHz) δ 1.10 (3H, t, $J=7$ Hz), 1.70—2.30 (2H, m), 3.90 (3H, s), 4.50 (1H, t, $J=6$ Hz), 6.86 (1H, d, $J=7$ Hz), 7.60 (1H, d, $J=1$ Hz), 7.66 (1H, dd, $J=7$, 1 Hz)
5e	(250 MHz) δ 1.05 (3H, d, $J=7$ Hz), 1.12 (3H, d, $J=7$ Hz), 2.21—2.41 (1H, m), 3.90 (3H, s), 4.41 (1H, d, $J=6$ Hz), 6.86 (1H, d, $J=9$ Hz), 7.64 (1H, d, $J=2$ Hz), 7.66 (1H, dd, $J=9$, 2 Hz), 9.33 (1H, br)
5f	(60 MHz) δ 0.70—1.10 (3H, m), 1.20—2.10 (6H, m), 3.88 (3H, s), 4.65 (1H, t, $J=7$ Hz), 6.90 (1H, d, $J=9$ Hz), 7.60 (1H, dd, $J=9$, 1 Hz), 7.80 (1H, d, $J=1$ Hz), 9.60 (1H, br)
5g	(250 MHz) δ 0.88 (3H, t, $J=7$ Hz), 1.38—1.92 (10H, m), 3.90 (3H, s), 4.60 (1H, dd, $J=5$, 8 Hz), 6.86 (1H, d, $J=9$ Hz), 7.66 (1H, d, $J=1$ Hz), 7.67 (1H, dd, $J=9$, 1 Hz), 9.70 (1H, br)
5h	(60 MHz) δ 3.81 (3H, s), 5.64 (1H, s), 6.97 (1H, d, $J=8$ Hz), 7.31 (5H, s), 7.60 (1H, d, $J=2$ Hz), 7.61 (1H, dd, $J=8$, 2 Hz), 10.83 (1H, br)
5i	(60 MHz) δ 1.10 (3H, t, $J=7$ Hz), 1.60—2.30 (2H, m), 3.90 (3H, s), 4.50 (1H, dd, $J=7$, 4 Hz), 6.86 (1H, d, $J=7$ Hz), 7.60 (1H, d, $J=1$ Hz), 7.66 (1H, dd, $J=7$, 1 Hz), 9.55 (1H, br)
5j	(60 MHz) δ 1.10 (3H, t, $J=7$ Hz), 1.70—2.30 (2H, m), 3.90 (3H, s), 4.50 (1H, t, $J=7$ Hz), 6.86 (1H, d, $J=7$ Hz), 7.60 (1H, d, $J=1$ Hz), 7.66 (1H, dd, $J=7$, 1 Hz), 9.65 (1H, br)
6a	(300 MHz) δ 3.39 (3H, s), 3.91 (3H, s), 4.65 (2H, s), 7.01 (1H, d, $J=8$ Hz), 7.64 (1H, d, $J=2$ Hz), 7.75 (1H, dd, $J=8$, 2 Hz)
6b	(60 MHz) δ 1.30 (3H, t, $J=6$ Hz), 3.90 (3H, s), 4.05 (2H, q, $J=6$ Hz), 4.65 (2H, s), 7.02 (1H, d, $J=7$ Hz), 7.60 (1H, d, $J=1$ Hz), 7.74 (1H, dd, $J=7$, 1 Hz)
6c	(60 MHz) δ 1.60 (6H, d, $J=7$ Hz), 3.90 (3H, s), 4.53 (2H, s), 4.52—5.00 (1H, m), 7.18 (1H, d, $J=8$ Hz), 7.66 (1H, d, $J=1$ Hz), 7.70 (1H, dd, $J=8$, 1 Hz)
6d	(60 MHz) δ 0.99 (3H, t, $J=7$ Hz), 1.70 (2H, tq, $J=7$, 7 Hz), 3.88 (2H, t, $J=7$ Hz), 3.92 (3H, s), 4.67 (2H, s) 7.07 (1H, d, $J=8$ Hz), 7.71 (1H, d, $J=1$ Hz), 7.83 (1H, dd, $J=8$, 1 Hz)
6e	(60 MHz) δ 0.95 (6H, d, $J=7$ Hz), 2.02—2.22 (1H, m), 3.90 (1H, d, $J=7$ Hz), 3.94 (3H, s), 4.70 (2H, s) 7.13 (1H, d, $J=9$ Hz), 7.79 (1H, d, $J=2$ Hz), 7.87 (1H, dd, $J=9$, 2 Hz)
6f	(300 MHz) δ 0.91 (3H, t, $J=7$ Hz), 1.34—1.40 (4H, m), 1.62—1.72 (2H, m), 3.93 (3H, s), 3.94 (2H, t, $J=8$ Hz), 4.63 (2H, s), 7.01 (1H, d, $J=8$ Hz), 7.64 (1H, d, $J=2$ Hz), 7.73 (1H, dd, $J=8$, 2 Hz)
6g	(250 MHz) δ 1.16 (3H, t, $J=7$ Hz), 1.25—2.15 (6H, m), 3.90 (3H, s), 4.13 (2H, t, $J=5$ Hz), 4.64 (2H, s), 7.30 (1H, d, $J=9$ Hz), 7.63 (1H, d, $J=2$ Hz), 7.73 (1H, dd, $J=9$, 2 Hz)
6h	(250 MHz) δ 3.87 (3H, s), 4.77 (2H, s), 5.19 (2H, s), 6.92 (1H, d, $J=8$ Hz), 7.22—7.33 (5H, m), 7.60 (1H, dd, $J=8$, 2 Hz), 7.64 (1H, d, $J=2$ Hz)
6i	(60 MHz) δ 1.52 (9H, d, $J=7$ Hz), 3.82 (3H, s), 4.30—5.00 (2H, m), 7.10 (1H, d, $J=9$ Hz), 7.60 (1H, d, $J=1$ Hz), 7.65 (1H, dd, $J=9$, 1 Hz)
6j	(60 MHz) δ 1.45 (6H, s), 1.60 (6H, d, $J=6$ Hz), 3.90 (3H, s), 4.50—5.00 (1H, m), 7.15 (1H, d, $J=8$ Hz), 7.65 (1H, d, $J=1$ Hz), 7.75 (1H, dd, $J=8$, 1 Hz)
6k	(60 MHz) δ 1.02 (3H, t, $J=7$ Hz), 1.52 (6H, d, $J=6$ Hz), 1.60—2.20 (2H, m), 3.90 (3H, s), 4.37 (1H, dd, $J=8$, 5 Hz), 4.50—5.00 (1H, m), 7.18 (1H, d, $J=9$ Hz), 7.65 (1H, d, $J=1$ Hz), 7.78 (1H, dd, $J=9$, 1 Hz)
6l	(250 MHz) δ 0.99 (3H, d, $J=7$ Hz), 1.06 (3H, d, $J=7$ Hz), 1.53 (3H, d, $J=7$ Hz), 1.56 (3H, d, $J=7$ Hz), 2.07—2.27 (1H, m), 3.91 (3H, s), 4.21 (1H, d, $J=7$ Hz), 4.68—4.88 (1H, m), 7.14 (1H, d, $J=9$ Hz), 7.66 (1H, d, $J=2$ Hz), 7.70 (1H, dd, $J=9$, 2 Hz)
6m	(60 MHz) δ 0.70—1.10 (3H, m), 1.20—2.10 (6H, m), 1.50 (6H, d, $J=6$ Hz), 3.90 (3H, s), 4.30—5.10 (2H, m), 7.13 (1H, d, $J=8$ Hz), 7.70 (1H, dd, $J=8$, 1 Hz), 7.75 (1H, d, $J=1$ Hz)
6n	(60 MHz) δ 1.61 (3H, d, $J=7$ Hz), 1.78 (3H, d, $J=7$ Hz), 3.91 (3H, s), 4.83—5.03 (1H, m), 5.77 (1H, s), 7.31 (1H, d, $J=8$ Hz), 7.49 (5H, s), 7.88 (1H, d, $J=2$ Hz), 7.89 (1H, dd, $J=8$, 2 Hz)
6o	(250 MHz) δ 0.88 (3H, t, $J=7$ Hz), 1.23—1.40 (6H, m), 1.43—1.93 (4H, m), 1.52 (3H, d, $J=7$ Hz), 1.55 (3H, d, $J=7$ Hz), 3.90 (3H, s), 4.43 (1H, dd, $J=4$, 9 Hz), 4.66—4.86 (1H, m), 7.15 (1H, d, $J=9$ Hz), 7.65 (1H, d, $J=2$ Hz), 7.71 (1H, dd, $J=9$, 2 Hz)
6p	(60 MHz) δ 1.02 (3H, t, $J=7$ Hz), 1.52 (6H, d, $J=6$ Hz), 1.40—2.20 (2H, m), 3.89 (3H, s), 4.20—5.10 (2H, m), 7.18 (1H, d, $J=7$ Hz), 7.65 (1H, s), 7.78 (1H, d, $J=7$ Hz)
6q	(60 MHz) δ 1.02 (3H, t, $J=7$ Hz), 1.52 (6H, d, $J=6$ Hz), 1.30—2.20 (2H, m), 3.90 (3H, s), 4.20—5.10 (2H, m), 7.18 (1H, d, $J=9$ Hz), 7.65 (1H, d, $J=1$ Hz), 7.78 (1H, dd, $J=9$, 1 Hz)
6r	(250 MHz) δ 1.07 (3H, t, $J=8$ Hz), 1.27 (3H, t, $J=7$ Hz), 1.70—2.10 (2H, m), 3.91 (3H, s), 4.00 (2H, q, $J=7$ Hz), 4.59 (1H, dd, $J=9$, 5 Hz), 7.00 (1H, d, $J=9$ Hz), 7.66 (1H, d, $J=2$ Hz), 7.73 (1H, dd, $J=9$, 2 Hz)
6s	(250 MHz) δ 1.08 (3H, t, $J=7$ Hz), 1.28 (3H, t, $J=7$ Hz), 1.76—1.99 (2H, m), 3.91 (3H, s), 4.00 (2H, q, $J=7$ Hz), 4.50 (1H, dd, $J=9$, 5 Hz), 7.00 (1H, d, $J=9$ Hz), 7.66 (1H, d, $J=2$ Hz), 7.73 (1H, dd, $J=9$, 2 Hz)
7a	(250 MHz) δ 1.07 (3H, t, $J=8$ Hz), 1.54 (3H, d, $J=7$ Hz), 1.56 (3H, d, $J=7$ Hz), 1.70—2.10 (2H, m), 4.40 (1H, dd, $J=8$, 5 Hz), 4.60—4.90 (1H, m), 7.18 (1H, d, $J=9$ Hz), 7.73 (1H, d, $J=2$ Hz), 7.79 (1H, dd, $J=9$, 2 Hz)
7b	(60 MHz) δ 1.10 (3H, t, $J=6$ Hz), 1.60 (6H, d, $J=6$ Hz), 1.70—2.10 (2H, m), 4.20—5.10 (2H, m), 7.16 (1H, d, $J=7$ Hz), 7.70 (1H, d, $J=1$ Hz), 7.78 (1H, dd, $J=7$, 1 Hz), 9.45 (1H, br)
7c	(250 MHz) δ 1.09 (3H, t, $J=8$ Hz), 1.29 (3H, t, $J=7$ Hz), 1.70—2.10 (2H, m), 4.02 (2H, q, $J=7$ Hz), 4.52 (1H, dd, $J=9$, 5 Hz), 7.04 (1H, d, $J=9$ Hz), 7.73 (1H, d, $J=2$ Hz), 7.81 (1H, dd, $J=9$, 2 Hz), 9.05 (1H, br)
7d	(250 MHz) δ 1.09 (3H, t, $J=8$ Hz), 1.29 (3H, t, $J=7$ Hz), 1.88—1.99 (2H, m), 4.02 (2H, q, $J=7$ Hz), 4.53 (1H, dd, $J=9$, 5 Hz), 7.04 (1H, d, $J=9$ Hz), 7.73 (1H, d, $J=2$ Hz), 7.82 (1H, dd, $J=9$, 2 Hz), 9.70 (1H, br)

and R^3, QSAR analysis of compounds **3a**—**j** and **3l**—**o** was performed using the Hansch-Fujita method. In the analysis, **3k** was excluded, because it was the only disubstituted compound at the 2-position and disubstitution might be unfavorable for the activity. The best equation was Eq. 1.

$$\mathrm{p(IC_{50})} = 1.38(\pm 0.79)L(\mathrm{R}^{1,2}) - 0.17(\pm 0.10)L(\mathrm{R}^{1,2})^2 + 1.13(\pm 0.69)L(\mathrm{R}^3) - 0.12(\pm 0.07)L(\mathrm{R}^3)^2 - 4.28(\pm 1.78) \quad (1)$$

$$n=14,\ r=0.919,\ s=0.244,\ F=12.24,\ L(\mathrm{R}^{1,2})_{\mathrm{opt}}=4.19,\ L(\mathrm{R}^3)_{\mathrm{opt}}=4.68$$

In Eq. 1, $L(\mathrm{R}^{1,2})$ is the larger value of $L(\mathrm{R}^1)$ and $L(\mathrm{R}^2)$, the number in parentheses is the 95% confidential 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. $L(\mathrm{R}^{1,2})_{\mathrm{opt}}$ and $L(\mathrm{R}^3)_{\mathrm{opt}}$ are the calculated optimum values of $L(\mathrm{R}^{1,2})$ and $L(\mathrm{R}^3)$, respectively. Eq. 1 indicates that the activity is parabolically related to the length of the substituents R^1 or R^2, and R^3, independently. Using the hydrophobic parameter for R^1 or R^2, and R^3 did not give a good correlation. The calculated optimum $L(\mathrm{R}^3)$ is 4.68, which is somewhat lower than the calculated optimum value (6.30) for R^{L} of EIPA derivatives. One reason for this may be that the direction of the L axis is different between the 2*H*-benzo[1,4]oxazine derivatives **3** and EIPA derivatives, since the hybridization at the 4-nitrogen atom of compounds **3** is sp^2 and that of the 5-position of EIPA derivatives is sp^3. The optimum substituents for R^1 or R^2, and R^3 are an ethyl group and isopropyl group. The optimum compounds are the 2-ethyl-4-isopropyl derivative **3l** and the 2,4-diethyl derivative.

The compounds **3j** and **3l**—**p** have an asymmetric carbon at the 2-position of the 2*H*-benzo[1,4]oxazine ring. Both optical isomers **3q** and **3r** of the 2-ethyl-4-isoproyl derivative, and both optical isomers **3s** and **3t** of the 2,4-diethyl derivative were synthesized to investigate the effect of the chirality. It was found that the compounds **3q**—**t** have almost equally potent activities with IC_{50} values of 0.036—0.071 μM, and therefore the chirality of the 2-position did not affect the activity. These results are consistent with Eq. 1, which did not include the optical parameter. The optical active isomers **3q**—**t** were added to **3a**—**j** and **3l**—**o** for QSAR analysis, and the statistically significant Eq. 2 was obtained.

$$\mathrm{p(IC_{50})} = 1.45(\pm 0.49)L(\mathrm{R}^{1,2}) - 0.17(\pm 0.06)L(\mathrm{R}^{1,2})^2 + 1.12(\pm 0.58L(\mathrm{R}^3) - 0.12(\pm 0.06)L(\mathrm{R}^3)^2 - 4.38(\pm 1.35) \quad (2)$$

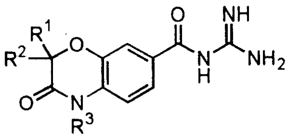
$$n=18,\ r=0.945,\ s=0.216,\ F=26.87,\ L(\mathrm{R}^{1,2})_{\mathrm{opt}}=4.19,\ L(\mathrm{R}^3)_{\mathrm{opt}}=4.68$$

Eq. 2 is statistically equivalent to Eq. 1. Eq. 2 indicates that compounds **3q**—**t** are among the optimized derivatives.

The clogP of **3d**, obtained by the optimization of the *N*-substituent R^3 of the lead compound **3a**, is 0.61, and is lower than the optimum value of 1.5—2.0 for good bioavailability. The further optimization of the substituents R^1 and R^2 was achieved by the incorporation of an ethyl group to give compounds **3q**—**t**. The clogP values of **3q** and **3r** are 1.66, and those of **3s** and **3t** are 1.35. These values are nearly equal to the optimum clogP for good bioavailability.

The hydrochlorides and methanesulfonates of **3q**—**t** were prepared in order to increase the water-solubility. The water-solubility of these salts was in the range of

Table 5. Observed and Calculated Na/H Exchange Inhibitory Activity of *N*-(2*H*-Benzo[1,4]oxazine-7-carbonyl)guanidines **3**



No.	R^1	R^2	R^3	Note	IC_{50} (μM)	$L(\mathrm{R}^{1,2})$	$L(\mathrm{R}^3)$	clogP	pIC_{50}		
									Obsd.	Eq. 1	Eq. 2
3a	H	H	H		2.70	2.06	2.06	−0.36	−0.43	−0.32	−0.32
3b	H	H	Me		0.56	2.06	3.00	−0.23	0.25	0.16	0.16
3c	H	H	Et		0.36	2.06	4.11	0.30	0.44	0.46	0.46
3d	H	H	iso-Pr		0.17	2.06	4.11	0.61	0.77	0.46	0.46
3e	H	H	Pr	HCl[a]	0.41	2.06	5.05	0.83	0.39	0.49	0.49
3f	H	H	iso-Butyl	HCl[a]	0.65	2.06	5.05	1.23	0.19	0.49	0.49
3g	H	H	$(\mathrm{CH_2})_2\mathrm{OEt}$		1.30	2.06	6.99	0.37	−0.11	−0.14	−0.14
3h	H	H	Pentyl		1.40	2.06	7.11	1.89	−0.15	−0.21	−0.21
3i	H	H	Bzl		0.38	2.06	3.63	1.48	0.42	0.37	0.37
3j	H	Me	iso-Pr		0.094	3.00	4.11	1.13	1.03	0.98	1.00
3k	Me	Me	iso-Pr		0.20	3.00	4.11	1.65	0.70	NR[c]	NR[c]
3l	H	Et	iso-Pr		0.034	4.11	4.11	1.66	1.47	1.21	1.25
3m	H	iso-Pr	iso-Pr	HCl[a]	0.13	4.11	4.11	1.99	0.89	1.21	1.25
3n	H	Butyl	iso-Pr	HCl[a]	0.14	6.17	4.11	2.72	0.85	0.57	0.57
3o	H	Ph	iso-Pr	HCl[a]	0.57	6.28	4.11	2.44	0.24	0.49	0.49
3p	H	Hexyl	iso-Pr	HCl[a]	>10	8.24	4.11	3.78	<−1	NR[c]	NR[c]
3q	H	Et	iso-Pr	(*R*)[b]	0.036	4.11	4.11	1.66	1.44	NR[c]	1.25
3r	H	Et	iso-Pr	(*S*)[b]	0.073	4.11	4.11	1.66	1.14	NR[c]	1.25
3s	H	Et	Et	(*R*)[b]	0.071	4.11	4.11	1.35	1.15	NR[c]	1.25
3t	H	Et	Et	(*S*)[b]	0.045	4.11	4.11	1.35	1.35	NR[c]	1.25

[a] HCl salt. [b] Configuration at C2 carbon. [c] NR: Not included in regression.

3—5 mg/ml, as we expected.

Conclusion

Based on the QSAR of (pseudo)bicyclic aroylguanidines as Na/H exchange inhibitors,[3] we designed *N*-(3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine **3a** as a new lead compound. The hydrophobicity was chosen so that the optimized compounds would have high water-solubility and good bioavailability. The QSAR result for the derivatives **3** indicates that the lengths of the substituents at the 2- and 4-position of the 2*H*-benzo[1,4]oxazine ring are parabolically related to the activity, independently. As we had expected, the optimization of **3a** afforded potent inhibitors, (*R*) or (*S*)-*N*-(2-ethyl-4-isopropyl (or ethyl)-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine **3q**—**t** with IC_{50} values of 0.036—0.073 μM. The water-solubility of the hydrochlorides and methanesulfonates of **3q**—**t** is in the range of 3—5 mg/ml, which is sufficient for therapeutic use.

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). Optical rotations were measured with a JASCO DIP-140 polarimeter. Elemental analysis was performed with Yanagimoto CHN-CORDER MT-5. HPLC was performed under the following conditions: column for determination of enantiomeric excess (Chiralpak AS, 4.6 mm i.d. × 250 mm, Daicell Inc.), eluent (hexane–EtOH (10 : 1, v/v) for **3q**—**3r**, hexane–EtOH (100 : 1, v/v) for **5i** and **5j**, hexane–EtOH (1000 : 1, v/v) for **6p** and **6q**, hexane–EtOH (500 : 1, v/v) for **6r** and **6s**, hexane–EtOH (30 : 1, v/v) +0.1% CF_3CO_2H for **7a** and **7b**, hexane–EtOH (10 : 1, v/v) +0.1% CF_3CO_2H for **7c** and **7d**), UV detector (254 nm).

General Procedure for the Preparation of Methyl 3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carboxylates 5 A 2-haloacid halide (10 mmol) was added to a mixture of methyl 4-amino-3-hydroxybenzoate **4** (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, and dried over anhydrous magnesium sulfate ($MgSO_4$). The solvent was removed *in vacuo*. Then 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. 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* to give **5**. 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-7-carboxylates 6 A (substituted)alkyl halide (15 mmol) was added to a mixture of methyl 3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carboxylate **5** (10 mmol) and 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 **6**. Physical data are listed in Tables 2 and 4.

General Procedure for the Preparation of 2-Ethyl-4-isopropyl(or ethyl)-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carboxylic Acids 7 A mixture of methyl 2-ethyl-4-isopropyl(or ethyl)-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carboxylate **6p**—**s** (10 mmol) and concentrated HCl (10 ml) was refluxed overnight. The precipitates were collected by filtration, washed with hexane and 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]oxazine-7-carbonyl)guanidines 3 Method A: Guanidine hydrochloride (55 mmol) was added to a sodium methoxide (NaOMe) solution, prepared from sodium (Na) (50 mmol) and MeOH (25 ml). The mixture was refluxed for 30 min, and filtered, then a methyl 3-oxo(-4-substituted)-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carboxylate **5** (**6**) (5 mmol) was added to the filtrate. The mixture was refluxed for several hours, diluted with water, and extracted with ethyl acetate (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 the corresponding *N*-(3,4-dihydro(-4-substituted)-3-oxo-2*H*-benzo[1,4]oxazine-7-carbonyl)guanidine **3a**—**p**. Physical data are listed in Tables 1 and 3.

Method B: Guanidine hydrochloride (55 mmol) was added to a NaOMe solution, prepared from Na (50 mmol) and MeOH (25 ml). The mixture was refluxed for 30 min, and filtered. The filtrate was concentrated *in vacuo*. The residue was taken up in 1,2-dimethoxyethane (DME) (10 ml), and a suspension of 2-ethyl-4-isopropyl(or ethyl)-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carbonyl chloride, prepared from 2-ethyl-4-isopropyl(or ethyl)-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carboxylic acid **7** (10 mmol) and thionyl chloride, in DME (20 ml) was added. The mixture was stirred at room temperature for 0.5 h, water was added, and the whole was extracted with AcOEt. The extract was washed with water, dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The residue was chromatographed on silica gel (chloroform–MeOH) and recrystallized to give **3p**—**t** as colorless crystals. 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 Rosskoph *et al.*[12] Platelet-rich plasma was prepared as described by Mammen *et al.*[13] 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 and the treated blood was centrifuged at 90 × *g* for 10 min. The supernatant was separated to prepare platelet-rich plasma. Then, a solution of a test compound in dimethylsulfoxide (DMSO) was 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 the 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.

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

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

References and Notes

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