Structural Requirements for Potent Na/H Exchange Inhibitors Obtained from Quantitative Structure–Activity Relationships of Monocyclic and Bicyclic Aroylguanidines

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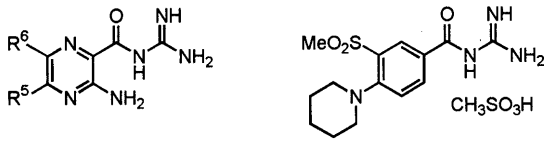
The quantitative structure–activity relationship (QSAR) of *N*-(3-amino-6-chloro-5-ethylisopropylaminopyrazine-4-carbonyl)guanidine (EIPA) 1ac and its derivatives as Na/H exchange inhibitors was analyzed using the steric parameters and an indicator variable. The results indicated that bicyclic aroylguanidines might have Na/H exchange inhibitory activity. Therefore, various bicyclic aroylguanidines were synthesized and tested for Na/H exchange inhibitory activity. The QSAR study of the bicyclic aroylguanidines showed that hydrophobic bicyclic rings seemed to be preferable for potent activity. The hydrophobicity of the aroyl ring moiety was thought to be paticularly important. Thus, the QSAR of EIPA and its derivatives was re-analyzed using hydrophobicity and steric parameters. The results indicated that high hydrophobicity of the pseudo-ring moiety and a substituent of appropriate length at the position corresponding to the 5-position of the naphthalene ring enhance the activity. As expected from the results, 5-bromo-2-naphthoylguanidine 3b and 5-methoxy-2-naphthoylguanidine 3c exhibited strong activity. These findings will be helpful to design new, potent Na/H exchange inhibitors.

Key words Na/H exchange inhibitor; quantitative structure-activity relationship; aroylguanidine; structural requirement

The Na/H exchanger is a plasma membrane transporter and is the major pathway for regulation of intracellular pH by extrusion of H^+ and influx of Na^+ into the cell. The Na/H exchanger is stimulated by a decrease of intracellular pH caused by myocardial ischemia. Activation of the Na/H exchanger on reperfusion is the major mechanism of increase of intracellular pH after ischemia-induced acidosis. Although activation of the Na/H exchanger is essential for the restoration of normal pH, it results in a deleterious Na^+ overload. This in turn may activate the Na/Ca exchanger, resulting in intracellular Ca^{2+} overload and leading to myocardial dysfunction and arrhythmia. Thus, the Na/H exchange inhibitors are expected to be useful drugs for arrhythmia and for reducing myocardial ischemic cell injury by preventing Na^+ overload.[1)]

N-(3,5-Diamino-6-chloropyrazine-4-carbonyl)guanidine (amiloride) **1a**, a potent diuretic, is known to have a weak Na/H exchange inhibitory activity (Chart 1). Extensive studies on amiloride derivatives by the Merck group led to the discovery of potent inhibitors,[2)] such as ethylisopropylamiloride (EIPA) **1ac**. The Na/H exchange inhibitory activity of EIPA is potent ($IC_{50}=0.070\ \mu M$).[3)] Recently, Hoe-694 **2**, a benzoylguanidine derivative, was reported.[4)] Although the aromatic rings of these compounds are different, both compounds have a characteristic monocyclic aroylguanidine structure. Therefore, such a structure might be essential for the Na/H exchange inhibitory activity. However, other structural requirements for potent inhibitors have not been established. The inhibitory activities of EIPA **1ac** and its derivatives **1** have been studied,[2)] but that of Hoe-694 has not been reported. Therefore in this study, we analyzed the structure–activity relationships (SARs) of EIPA **1ac** and its derivatives **1** and investigated the structural requirements for potent activity.

Synthesis As shown in Chart 2, 2-naphthoylguanidine **3a** was synthesized from 2-naphthoyl chloride and excess guanidine by stirring at room temperature in 1,2-dimethoxyethane (method A). Other bicyclic aroylguanidines **3b—d** and **4—7** were synthesized from the corresponding methyl carboxylates and excess guanidine by refluxing in methanol (method B). The physical data are listed in Tables 1 and 2.



$R^5=NH_2$, $R^6=Cl$: amiloride **1**

$R^5=NEt^iPr$, $R^6=Cl$: EIPA **1ac** Hoe-694 **2**

Chart 1. Structures of Known Na/H Exchange Inhibitors

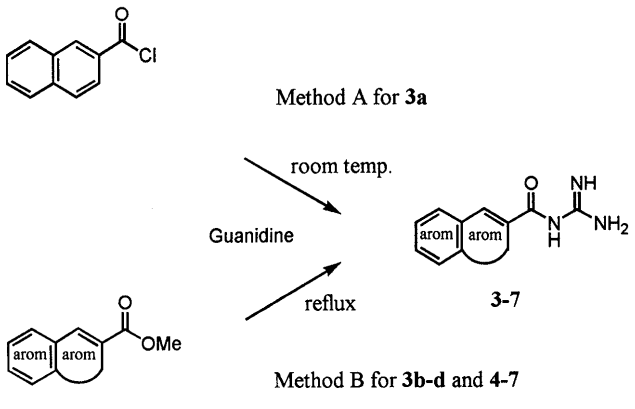


Chart 2. Synthesis of Bicyclic Aroylguanidines

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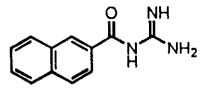
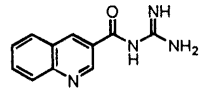
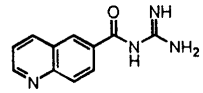
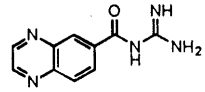
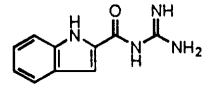
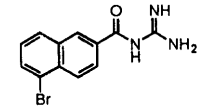
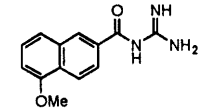
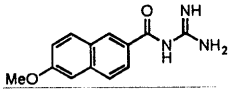
Table 1. Na/H Exchange Inhibitory Activity and Physical Data for Bicyclic Aroylguanidines **3**—**7**

No.	Structure	Na/H exchange inhibitory activity IC_{50} (μM)	Synth. method[a]	Yield (%)	mp (°C)	Recryst. solvent	Formula	Analysis (%) Calcd (Found) C	H	N
3a		0.091	A	68	151—153	$CHCl_3$	$C_{12}H_{11}N_3O$	67.59 (67.72	5.20 5.20	19.71 19.81)
4		0.41	B	32	221—223	EtOH	$C_{11}H_{10}N_4O \cdot 1/10H_2O$	61.16 (61.17	4.76 4.71	25.94 25.98)
5		1.0	B	58	252—253	EtOH–H_2O	$C_{11}H_{10}N_4O$	61.67 (61.86	4.70 4.74	26.15 26.06)
6		5.9	B	40	231—233	IPA–AcOEt	$C_{10}H_9N_5O \cdot 1/5H_2O$	54.89 (54.84	4.33 4.37	32.01 32.27)
7		0.23	B	92	204—206	AcOEt	$C_{10}H_{10}N_4O$	59.40 (59.46	4.98 5.02	27.71 27.62)
3b		0.010	B	26	188—190	MeCN	$C_{12}H_{10}BrN_3O$	49.34 (49.40	3.45 3.53	14.38 14.34)
3c		0.023	B	8	162—165	MeCN	$C_{13}H_{13}N_3O_2 \cdot 1/3HCl$	61.13 (61.13	5.26 5.19	16.45 16.54)
3d		0.1	B	37	203—206	MeCN	$C_{13}H_{13}N_3O_2$	64.19 (64.20	5.39 5.37	17.27 17.33)

a) See Chart 2.

Table 2. ^{1}H-NMR Spectral Data for Bicyclic Aroylguanidines **3**—**7**

No.	Spectral data (DMSO-d_6)
3a	(300 MHz) δ 7.00 (4H, br), 7.50—7.56 (2H, m), 7.60—8.05 (3H, m), 8.20 (1H, dd, J=9, 2 Hz), 8.66 (1H, s)
4	(300 MHz) δ 7.50 (4H, br), 7.65 (1H, ddd, J=9, 9, 2 Hz), 7.82 (1H, ddd, J=9, 9, 2 Hz), 8.08 (1H, dd, J=9, 2 Hz), 8.11 (1H, dd, J=9, 2 Hz), 8.92 (1H, d, J=2 Hz), 9.50 (1H, d, J=2 Hz)
5	(300 MHz) δ 6.62 (2H, br), 7.57 (1H, dd, J=9, 2 Hz), 8.02 (1H, d, J=9 Hz), 8.10 (2H, br), 8.44 (1H, dd, J=9, 2 Hz), 8.47 (1H, dd, J=9, 2 Hz), 8.70 (1H, d, J=2 Hz), 8.94 (1H, dd, J=2, 2 Hz)
6	(250 MHz) δ 6.87 (2H, br), 8.09 (1H, d, J=9 Hz), 8.15 (2H, br), 8.50 (1H, dd, J=9, 2 Hz), 8.77 (1H, d, J=2 Hz), 8.77—9.97 (2H, m)
7	(300 MHz) δ 6.91 (1H, s), 6.98 (1H, dd, J=7, 8 Hz), 7.13 (1H, dd, J=7, 8 Hz), 7.30 (4H, br), 7.41 (1H, d, J=8 Hz), 7.56 (1H, d, J=8 Hz), 11.18 (1H, br)
3b	(250 MHz) δ 6.85 (2H, br), 7.42 (1H, t, J=8 Hz), 7.91 (1H, dd, J=8, 1 Hz), 8.04 (1H, d, J=8 Hz), 8.10 (2H, br), 8.11 (1H, d, J=9 Hz), 8.36 (1H, dd, J=9, 1 Hz), 8.65 (1H, d, J=1 Hz)
3c	(60 MHz) δ 4.00 (3H, s), 7.01 (1H, dd, J=3, 6 Hz), 7.49 (1H, d, J=3 Hz), 7.50 (1H, d, J=6 Hz), 7.66 (4H, br), 8.20 (2H, d, J=1 Hz), 8.67 (1H, d, J=1 Hz)
3d	(60 MHz) δ 3.90 (3H, s), 7.25 (1H, dd, J=8, 2 Hz), 7.30 (1H, s), 7.35 (1H, s), 7.35 (4H, br), 7.75 (1H, d, J=8 Hz), 7.91 (1H, d, J=8 Hz), 8.25 (1H, dd, J=8, 2 Hz), 8.68 (1H, s)

Results and Discussion

Analysis of Known Inhibitors First, we analyzed the quantitative SAR (QSAR) of the known Na/H exchange inhibitors, EIPA **1ac** and its derivatives **1**, for which the Na/H exchange inhibitory activities have been reported (Table 3).[2] The QSAR was analyzed using the Hansch–Fujita method and Eq. 1 was obtained as one of the best equations.

$$\begin{aligned} pIC_{50} = {} & 1.068(\pm 0.331)MR(R^5) - 0.127(\pm 0.046)MR(R^5)^2 + \\ & 1.195(\pm 0.360)B_4(R^6) + 1.210(\pm 0.247)D + 0.721(\pm 0.852) \quad (1) \\ & n=45,\ r=0.939,\ s=0.364,\ F=74.11,\ MR(R^5)_{opt}=4.20 \end{aligned}$$

In Eq. 1, IC_{50}(M) is the value reported in reference 2, the number in parentheses is the 95% confidential interval, $MR(R^5)$ is the molecular refractivity value of the R^5 group calculated by means of the CLOGP program,[5] $B_4(R^6)$ is Verloop's STERIMOL parameter,[6] which represents the maximum width of the R^6 group, D is an indicator variable, which takes the value one when R^5 is a tertiary amino group and otherwise takes the value zero, 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. $MR(R^5)_{opt}$ is the calculated optimum value of $MR(R^5)$.

All compounds in the paper[2] were included in the QSAR analysis. Although in Eq. 1, we use the STERMOL

Table 3. Na/H Exchange Inhibitory Activity of EIPA **1ac** and Its Derivatives **1**

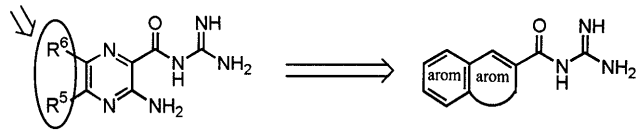
No.	R^6	R^5	$MR(R^5)^{a)}$	$B_4(R^6)^{b)}$	$L(R^L)^{b)}$	$\pi(R^6)+\pi(NHR^5)^{a)}$	$D^{c)}$	$pIC_{50}{}^{d)}$	Eq. 1	Eq. 3
1a	Cl	NH_2	1.40	1.80	2.06	−1.23	0	4.08	4.12	4.08
1b	H	NH_2	1.40	1.00	2.06	−1.23	0	2.98	3.16	3.11
1c	F	NH_2	1.40	1.35	2.06	−1.23	0	3.16	3.58	3.54
1d	Br	NH_2	1.40	1.95	2.06	−1.23	0	4.36	4.30	4.26
1e	I	NH_2	1.40	2.15	2.06	−1.23	0	4.74	4.54	4.51
1f	Cl	H	1.03	1.80	0.00	0.00	0	3.78	3.84	3.91
1g	Cl	NHEt	2.33	1.80	4.11	−1.23	0	4.95	4.67	4.93
1h	Cl	NH^cPr	2.66	1.80	4.14	−1.23	0	4.88	4.82	4.94
1i	Cl	NHallyl	2.77	1.80	5.11	−1.23	0	4.95	4.86	5.15
1j	Cl	NH^tBu	3.26	1.80	4.11	−1.23	0	5.49	5.01	4.93
1k	Cl	NHHexyl	4.18	1.80	8.22	−1.23	0	5.49	5.12	5.00
1l	Cl	NH-1-Adamantyl	5.34	1.80	5.05	−1.23	0	4.78	4.96	5.14
1m	Cl	$NHCH_2(CHOH)_4CH_2OH$	4.49	1.80	5.97	−1.23	0	5.19	5.11	5.23
1n	Cl	$NH(CH_2)_2NMe_2$	3.62	1.80	5.58	−1.23	0	3.85	5.08	5.21
1o	Cl	$NHCH_2C(CH_3)_2NH_2$	4.09	1.80	6.07	−1.23	0	4.69	5.12	5.24
1p	Cl	$NHC(=NH)NH_2$	2.42	1.80	4.00	−1.23	0	4.63	4.72	4.90
1q	Cl	$NHCH_2Ph$	4.38	1.80	3.63	−1.23	0	4.90	5.12	4.78
1r	Cl	$NHCH_2$–C_6H_4-2-F	4.39	1.80	2.73	−1.23	0	5.03	5.12	4.42
1s	Cl	$NHCH_2$–C_6H_4-4-Cl	4.87	1.80	4.42	−1.23	0	5.08	5.07	5.01
1t	Cl	$NHCH_2$–C_6H_4-4-Me	4.84	1.80	4.29	−1.23	0	5.06	5.07	4.98
1u	Cl	$NHCH_2$–C_6H_2-2,4,6-Me_3	5.77	1.80	4.29	−1.23	0	4.60	4.82	4.98
1v	Cl	NHPh	3.91	1.80	6.28	−1.23	0	5.42	5.11	5.24
1w	Cl	NMe_2	2.33	1.80	3.00	−0.47	1	5.16	5.89	5.30
1x	Cl	NMeEt	2.79	1.80	4.11	−0.47	1	6.20	6.08	5.69
1y	Cl	NMe^iPr	3.26	1.80	4.11	−0.47	1	6.62	6.22	5.69
1z	Cl	N–$(CH_2)_4$–	3.08	1.80	4.11	0.08	1	6.17	6.17	6.24
1aa	Cl	NMeBu	3.72	1.80	6.17	−0.47	1	6.62	6.30	6.00
1ab	Cl	NMe^tBu	3.72	1.80	5.05	−0.47	1	6.36	6.30	5.90
1ac	Cl	NEt^iPr	3.72	1.80	4.11	0.08	1	6.43	6.30	6.24
1ad	Cl	$NMeCH_2C(Me)=CH_2$	3.69	1.80	5.11	−0.47	1	6.09	6.30	5.91
1ae	Cl	N–$(CH_2)_6$–	4.01	1.80	5.05	0.08	1	6.80	6.33	6.45
1af	Cl	NPrBu	5.11	1.80	6.17	0.62	1	6.43	6.23	7.09
1ag	Cl	$NMeCH_2$–C_6H_3-2,4-Cl_2	5.82	1.80	4.42	−0.47	1	5.54	6.01	5.77
1ah	Cl	$NEtCH_2$–C_6H_4-4-Cl	5.80	1.80	4.42	0.08	1	5.86	6.02	6.32
1ai	Cl	$NEtCH_2$–C_6H_3-2-OMe-5-NO_2	6.65	1.80	3.63	0.08	1	6.17	5.58	6.09
1aj	Cl	NMeOMe	2.48	1.80	3.98	−0.47	0	5.19	4.74	5.66
1ak	Cl	$NMeNH_2$	2.23	1.80	3.00	−0.47	0	5.02	4.62	5.30
1al	Cl	$NMeCH_2CONHC(=NH)NH_2$	4.22	1.80	7.11	−0.47	1	5.87	6.34	5.96
1am	H	H	1.03	1.00	0.00	0.00	0	2.98	2.88	2.94
1an	C_6H_4-4-Cl	H	1.03	3.11	0.00	0.00	0	5.40	5.40	5.50
1ao	H	NH^tBu	3.25	1.00	4.11	−1.23	0	4.31	4.05	3.96
1ap	Br	H	1.03	1.95	0.00	0.00	0	4.08	4.02	4.09
1aq	Br	N–$(CH_2)_6$–	4.00	1.95	5.05	0.08	1	6.83	6.51	6.63
1ar	I	NMe_2	2.32	2.15	3.00	−0.47	1	5.91	6.30	5.72
1as	I	NEt^iPr	3.72	2.15	4.11	0.08	1	6.57	6.72	6.67

a) Calculated by the CLOGP program.[5] *b*) From reference 6. *c*) See text. *d*) From reference 2.

Table 4. Correlation Matrix of Variables Used in Eq. 1

	$MR(R^5)$	$MR(R^5)^2$	$B_4(R^6)$	D
$MR(R^5)$	1.000			
$MR(R^5)^2$	0.972	1.000		
$B_4(R^6)$	0.006	0.003	1.000	
D	0.351	0.311	0.129	1.000

Steric factors are important



EIPA 1ac and its derivatives 1 **Bicyclic aroylguanidine**

Chart 3. Design of Bicyclic Aroylguanidines

parameter B_4, other parameters such as B_1, which indicates the minimum width of the substituent R^6, also gave a statistically significant equation. The reason why both B_4 and B_1 are applicable seems that the values of B_4 and B_1 of the R^6 groups are equal, except for the 4-chlorophenyl group (**1an**) (Table 3). The QSAR results indicate that a wider or a larger substituent is preferable for R^6 and that the inhibitory activity is parabolically related to the steric factor of R^5. The meaning of the indicator variable D is not clear, but it appears that a tertiary amino group enhances the activity by a factor of about ten compared with a primary amino or a secondary amino group.

Equation 1 indicates that only the steric factors of the substituents R^5 and R^6 of EIPA **1ac** and its derivatives **1** are related to the Na/H exchange inhibitory activity. Therefore, another new ring formed by the cyclization of the *meta*- and *para*-position of monocyclic aroylguani-

dines is expected to have a similar effect to the substituents R^5 and R^6 of EIPA and its derivatives, and bicyclic aroylguanidines should have Na/H exchanger inhibitory activity (Chart 3). The above consideration prompted us to synthesize bicyclic aroylguanidines.

QSAR of Bicyclic Aroylguanidines We synthesized the simplest bicyclic aroylguanidine, 2-naphthoylguanidine **3a**, first of all, because of the ease of the synthesis. The activity was evaluated in terms of ability to inhibit the platelet swelling induced by sodium propionate, in accordance with the method of Rosskoph *et al.*[3] As expected from our QSAR analysis of EIPA and its derivatives, 2-naphthoylguanidine **3a** showed potent inhibitory activity ($IC_{50}=0.091\ \mu M$) (Table 1). Various other bicyclic aroylguanidines **4**—**7** were therefore synthesized and tested. The activity order is **3a** > **4** > **5** > **6** > **7**. The Hansch–Fujita method was applied to these compounds. Equation 2 was obtained as the best equation, which is statistically highly significant.

$$pIC_{50}=1.043(\pm0.864)\text{clogP}+5.267(\pm0.945) \quad (2)$$
$$n=5,\ r=0.912,\ s=0.327,\ F=14.75$$

In Eq. 2, clogP represents the hydrophobicity of the compound calculated by the CLOGP program.[5] Equation 2 indicates that the more hydrophobic bicyclic rings are preferred for potent Na/H exchange inhibitory activity.

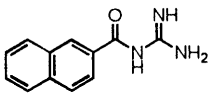
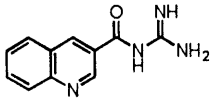
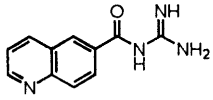
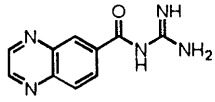
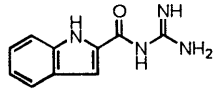
Re-analysis of EIPA and Its Derivatives On the assumption that both monocyclic aroylguanidines and bicyclic aroylguanidines **3a** and **4**—**7** interact with the same site of the Na/H exchanger, Eq. 2 indicates that the QSAR of EIPA **1ac** and its derivatives **1** could be analyzed using a hydrophobicity parameter. Therefore, in order to obtain further infomation from EIPA and its derivatives, we tried to re-analyze the QSAR of EIPA and its derivatives, based on the hypothesis that the substituents R^5 and R^6 of EIPA and its derivatives form a pseudo-ring (Chart 4). Most of the substituents R^5 are amino groups, such as NR^LR^S in which R^L is larger than R^S. The larger group R^L should be oriented far from the R^6 group owing to steric repulsion. The "NR^S" is thought to correspond to the pseudo-ring, and R^L is supposed to function as a substituent of the bicyclic ring. Thus, the π of NHR^S was used as the hydrophobicity parameter of R^5 for the re-analysis. Based on these assumptions, Eq. 3 was obtained as the best equation.

$$pIC_{50}=0.868(\pm0.200)L(R^L)-0.069(\pm0.026)L(R^L)^2 + 1.016(\pm0.169)[\pi(NHR^S)+\pi(R^6)]+3.304(\pm0.395) \quad (3)$$
$$n=45,\ r=0.921,\ s=0.405,\ F=76.68,\ L(R^L)_{opt}=6.30$$

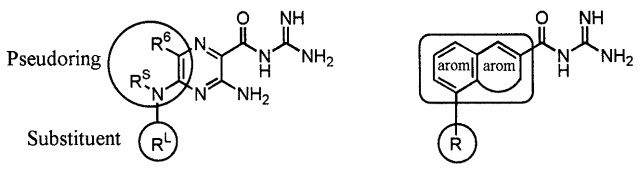
In Eq. 3, L is Verloop's STERIMOL parameter[6] and represents the length of substituent, which is assumed to be in an extended conformation. Although the simple use of the hydrophobicity parameter of the substituent R^5 did not afford a good result, we could obtain the statistically highly significant Eq. 3 by dividing R^5 into two moieties NHR^S and R^L. This supports the hypothetical orientations of R^5 and R^6. It is important that the analysis does not include the indicator variable D. It had been recognized only qualitatively, using the indicator variable, that the tertiary amino group was preferable for the substituent R^5 until Eq. 3 was formulated. Further information was not obtained from the simple analysis of EIPA **1ac** and its derivatives **1** (Eq. 1).

Equation 3 shows that the combined hydrophobicity of R^6 and NHR^S, and the length of R^L considerably affect the potency (Chart 4). The coefficients for the hydrophobic parameter (clogP and π) in Eq. 2 and Eq. 3 are statistically identical (1.043 and 1.016), and are nearly unity, which is a meaningful value.[7] These results support our hypothesis that the monocyclic aroylguanidines and the bicyclic ones interact at the same site of the Na/H exchanger. If Eq. 3 is applicable to bicyclic aroylguanidines, R^L corresponds to the substituent at the 5-position of 2-naphthoylguanidine and the activity of the 5-substituted 2-naphthoylguanidines should be increased.

Table 5. Na/H Exchange Inhibitory Activity and clogP of Bicyclic Aroylguanidines **3a** and **4**—**7**

No.	Structure	clogP[a]	pIC_{50} (M) Obsd.	pIC_{50} (M) Calcd. (Eq. 2)
3a		1.83	7.04	7.18
4		0.95	6.39	6.26
5		0.95	6.00	6.26
6		0.13	5.23	5.40
7		0.90	6.64	5.79

a) Calculated by the CLOGP program.[5]



Ring: The more hydrophobic, the more potent

Substituent: Appropriate length leads to optimum activity

Chart 4. Structural Requirements for Potent Na/H Exchange Inhibitors

Table 6. Correlation Matrix of Variables Used in Eq. 3

	$L(R^L)$	$L(R^L)^2$	$\pi(NHR^S)+\pi(R^6)$
$L(R^L)$	1.000		
$L(R^L)^2$	0.938	1.000	
$\pi(NHR^S)+\pi(R^6)$	0.162	0.097	1.000

SAR of the Substituent of 2-Naphthoylguanidines Based on the above consideration, 5- or 6-substituted 2-naphthoylguanidines **3b—d** were synthesized and evaluated for Na/H exchange inhibitory activity in order to confirm the SAR of the substituents of the bicyclic aroylguanidines. The 5-bromo derivative **3b** is about nine times more potent than 2-naphthoylguanidine **3a** (Table 1). The value of the length parameter L of a bromine atom is 3.83, and that of a hydrogen atom is 2.06. Changing the 5-bromine atom to a 5-methoxy group (**3c**) retains the potent inhibitory activity, while the 6-methoxy derivative **3d** shows almost the same activity as 2-naphthoylguanidine **3a**. The value of the length parameter L of a methoxy group is 3.98. These results indicate that the length of the 5-substitutent is also important for the inhibitory activity of bicyclic aroylguanidines and the SAR of the substituent R^L in EIPA and its derivatives is also applicable to the bicyclic aroylguanidines. From the above analysis, the hydrophobicity and/or electronic factors of the 5-substituent are not related to the Na/H exchange inhibitory activity in a series of (pseudo) bicyclic aroylguanidines with appropriate hydrophobicity.

Conclusion

In order to investigate the structural requirements for potent Na/H exchange inhibitory activity, we analyzed the QSAR of EIPA **1ac** and its derivatives **1**. Based on the result of the QSAR analysis (Eq. 1), various bicyclic aroylguanidines were synthesized. The QSAR of the bicyclic aroylguanidines **3a** and **4—7** was analyzed and we found that the more hydrophobic rings have more potent activity (Eq. 2). On the assumption that these two series of compounds interact with the same site of the Na/H exchanger, we re-analyzed the QSAR of EIPA and its derivatives by dividing R^5 into two moieties NHR^S and R^L. The result of the QSAR (Eq. 3) indicates that the hydrophobicity of the pseudo-ring and the length of the substituent R^L are related to the activity. Finally, the SAR study of substituted 2-naphthoylguanidines confirmed that a substituent with the appropriate length at the 5-position enhances the activity.

In conclusion, the structural requirements for strong activity of the bicyclic aroylguanidines are appropriate hydrophobicity of the ring system and appropriate length of the substituent (Chart 4). These structural requirements should also be applicable to other types of bicyclic aroylguanidines and should be helpful in the design of new, potent Na/H exchange inhibitors.

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.

Procedure for the Preparation of Bicyclic Aroylguanidines 3—7 Physical data are listed in Tables 1 and 2.

Method A for **3a**: Guanidine hydrochloride (1.11 g, 11.6 mmol) was added to a sodium methoxide (NaOMe) solution, which was prepared from sodium hydride (0.27 g, 11.3 mmol) and methanol (MeOH) (10 ml). The mixture was refluxed for 30 min, and filtered. To the filtrate was added a solution of 2-naphthoyl chloride (2.88 mmol) in 1,2-dimethoxyethane (20 ml). The mixture was stirred at room temperature for 2 h, and then concentrated *in vacuo*. The residue was chromatographed on silica gel (chloroform–MeOH) and the product was recrystallized to give 2-naphthoylguanidine **3a**.

Method B for **3b—d** and **4—7**: Guanidine hydrochloride (6.00 g, 63 mmol) was added to a NaOMe solution, which was prepared from sodium (1.50 g, 65 mmol) and MeOH (15 ml). The mixture was refluxed for 30 min, and filtered. To the filtrate was added a bicyclic aroylcarboxylic acid methyl ester (5.7 mmol). The mixture was refluxed for 1 h, then diluted with water, and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel (chloroform–MeOH) and the product was recrystallized to give the corresponding bicyclic aroylguanidine **3b—d** and **4—7**.

Na/H Exchange Inhibitory Activity Na/H exchange inhibitory activity was determined based on the ability to inhibit sodium propionate-induced swelling of platelet in accordance with the method of Rosskoph *et al.*[3] Platelet-rich plasma was prepared in accordance with the method of Mammen *et al.*[8] Wistar male rats (200—300 g) were anesthetized with ether, and blood was taken from the abdominal aorta. To inhibit blood coagulation, acid citrate dextrose 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 $90 \times g$ for 10 min. The supernatant was separated to prepare platelet-rich plasma. Then, a solution of a test compound in dimethyl sulfoxide (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. 4.

swelling inhibitory rate (Na/H exchange inhibitory rate, %)

$$= (1 - D/C) \cdot 100 \quad (4)$$

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

References and Notes

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