Synthesis and Na^+/H^+ Exchanger-1 Inhibitory Activity of Substituted (Quinolinecarbonyl)guanidine Derivatives

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The Na^+/H^+ exchanger (NHE) is a protein expressed in many mammalian cell types. It is involved in intracellular pH (pH_i) homeostasis by exchanging extracellular Na^+ for intracellular H^+. To date, nine NHE isoforms (NHE1–NHE9) have been identified. NHE1 is the most predominant isoform expressed in mammalian cardiac muscle. A novel series of substituted (quinolinecarbonyl)guanidine derivatives were designed and synthesized as NHE inhibitors. Most compounds can inhibit NHE1-mediated platelet swelling in a concentration-dependent manner, among which compound **7f** was the most active and more potent than cariporide. Furthermore, compound **7f** has also been demonstrated to exhibit the *in vivo* cardioprotective effects against SD rat myocardial ischemic-reperfusion injury superior to those of cariporide.

Introduction. – The mammalian Na^+/H^+ exchangers (NHEs) are ubiquitously expressed integral membrane proteins that regulate the intracellular pH by removing a H-atom in exchange for an extracellular Na^+ ion. NHEs consist of nine known isoforms (NHE1–NHE9). The NHE1 isoform was the first discovered and best characterized, and it exists on the plasma membrane of all mammalian cells [1][2]. NHE1 is involved in numerous physiological processes in mammals, including regulation of the intracellular pH, cell-volume control, cytoskeletal organization, heart disease, and cancer [3–5]. The activity of NHE1 is elevated in animal models of myocardial infarcts and in left ventricular hypertrophy. During ischemia and reperfusion of the myocardium, NHE activity catalyzes increased uptake of intracellular Na^+. This, in turn, is exchanged for extracellular Ca^{2+} by the Na^+/Ca^{2+} exchanger, resulting in Ca^{2+} overload and damage to the myocardium, such as myocardial infarction activation, stunning, and tissue necrosis [6][7].

NHE1 inhibitors, in their cation forms, combine with NHE1 at the extracellular Na^+-binding site, to competitively inhibit the NHE1 function and reduce Na^+ and Ca^{2+} influx, and hence abolish the post-ischemia Ca^{2+} overload in myocardial cells and lower the risk of cell dysfunction and injury.

Aroylguanidine, a subunit typically presented in most of the known NHE1 inhibitors, such as cariporide, sabiporide, and MS-31038 (*Fig.*) [7][8], is well-considered as the possible pharmacophore among the reported NHE1 inhibitors. Upon analyzing sabiporide and MS-31038 structures, we reasoned that (quinolinecarbonyl)guanidine should serve as our core structure. We also introduced a (trimethoxy-

benzyl)piperazinyl moiety to the (quinolinecarbonyl)guanidine core, and thus synthesized our target compounds **6** and **7a**–**7l** (*Table 1*).

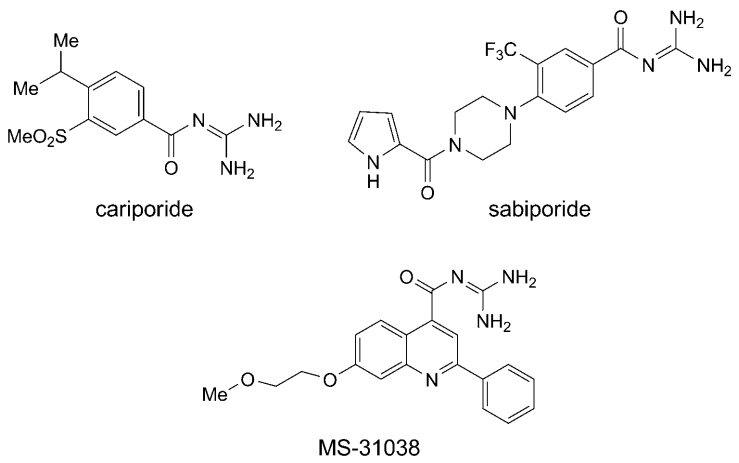
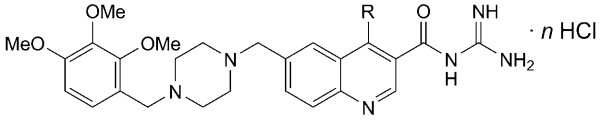


Figure. *Structures of cariporide, sabiporide, and MS-31038*

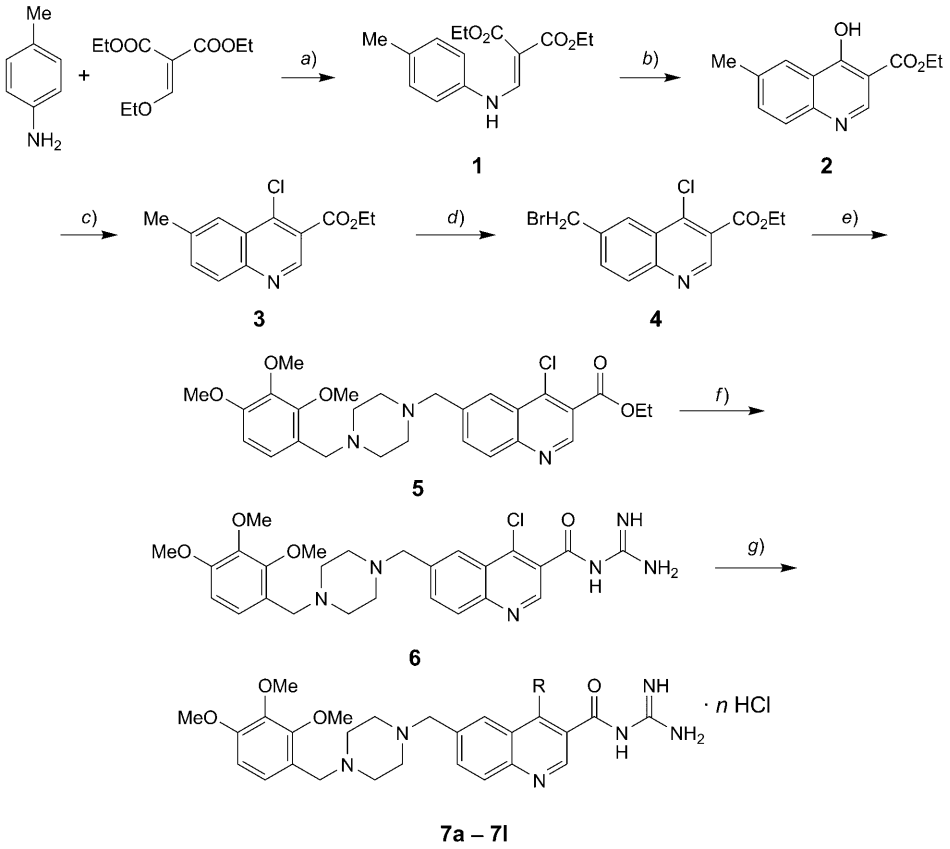
Table 1. *Chemical Structure of Compounds* **6** *and* **7a**–**7l**



	R	*n*		R	*n*
6	Cl	0	**7g**	4-Me–C_6H_4–NH–	4
7a	C_3H_7–NH–	4	**7h**	3-Me–C_6H_4–NH–	0
7b	C_4H_9–NH–	4	**7i**	3,4-Me_2–C_6H_4–NH–	4
7c	Ph–NH–	4	**7j**	3,5-$(MeO)_2$–C_6H_4–NH–	4
7d	4-MeO–C_6H_4–NH–	0	**7k**	4-Cl–C_6H_4–NH–	4
7e	3-MeO–C_6H_4–NH–	4	**7l**	4-Br–C_6H_4–NH–	4
7f	4-EtO–C_6H_4–NH–	4			

Results and Discussion. – *Synthesis.* The synthetic route for the target compounds **6** and **7a**–**7l** is depicted in *Scheme 1*. Diethyl 2-[(*p*-tolylamino)methylidene]malonate (**1**), obtained by condensation of *p*-tolylamine and diethyl 2-(ethoxymethylidene)malonate, was cyclized at 250° to afford ethyl 4-hydroxy-6-methylquinoline-3-carboxylate (**2**), which was treated with $POCl_3$ to afford ethyl 4-chloro-6-methylquinoline-3-carboxylate (**3**) and subsequently brominated with NBS to form ethyl 6-(bromomethyl)-4-chloroquinoline-3-carboxylate (**4**). Compound **4** was coupled with 1-(2,3,4-trimethoxybenzyl)piperazine (trimetazidine) to give intermediate **5**, which was treated with excess guanidine in anhydrous i-PrOH to give the target compound **6** [9]. Target compounds **7a**–**7l** were obtained by nucleophilic replacement of Cl of **6** with an

Scheme 1



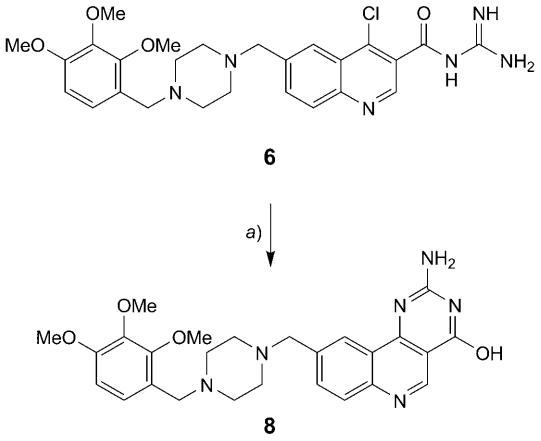
a) 110°, N_2. *b*) Ph_2O, 250°, N_2. *c*) $POCl_3$, reflux. *d*) *N*-Bromosuccinimide (NBS), CCl_4, benzoyl peroxide, reflux. *e*) Trimetazidine, MeCN, K_2CO_3, r.t. *f*) Guanidine, i-PrOH, r.t. *g*) 1. Alkylamine or substituted phenylamine, i-PrOH, K_2CO_3, reflux, 2. sat. HCl (g) in anh. AcOEt (except for **7d** and **7h**).

alkylamine or a substituted phenylamine. Crude products were purified by silica-gel column chromatography (AcOEt/MeOH 8–12 : 1), followed by formation of the HCl salt (except for **7d** and **7h**) with saturated HCl (g) in anhydrous AcOEt.

A by-product **8** (*Scheme 2*) has been isolated during the preparation of the target compounds **7a**–**7l**. The structure was characterized by ^{1}H-NMR spectroscopy and mass spectrometry. In some cases, this by-product **8** was the major product isolated from the reaction. Due to double activation by both the N(1)-atom and 3-acyl group, Cl of the quinoline served as a good leaving group, and the intramolecular nucleophilic attack of the guanidine led to **8**. Furthermore, treatment of compound **6** with anhydrous K_2CO_3 in acetone or EtOH afforded compound **8** as the major product.

Biological Activity. Thirteen target compounds, along with the reference compound cariporide, were evaluated in rat platelet-swelling assay (PSA) for NHE1 inhibitory activity screening. The experimental procedure was similar to that described in

Scheme 2



a) Alkylamine or substituted phenylamine, i-PrOH, K_2CO_3, reflux, or acetone or EtOH, K_2CO_3, reflux.

[10][11], with only minor modifications. The IC_{50} values of the tested compounds were obtained from the linear part of the relationship between the log concentration and NHE activity using linear-regression analysis.

The PSA results revealed that most of the tested compounds did inhibit rat platelet NHE-1 in a concentration-dependent manner. Compounds **7a**, **7c**, **7d**, **7f**, and **7j** were more active than cariporide in NHE1 inhibition (*Table 2*). Compound **7f** (IC_{50} = 2.91 nM) was 23 times more potent than cariporide (IC_{50} = 68.2 nM).

Table 2. IC_{50} *Values of Cariporide, and Compounds* **6** *and* **7a**–**7l** *for Inhibition of NHE1*

	IC_{50}[a]) [nmol/l]		IC_{50}[a]) [nmol/l]
Cariporide	68.2 ± 9.1	**7f**	2.91 ± 0.32
6	245 ± 19.7	**7g**	201 ± 33.7
7a	30.9 ± 4.8	**7h**	1406 ± 286
7b	107 ± 9.4	**7i**	880 ± 107
7c	50.9 ± 6.6	**7j**	43.8 ± 5.3
7d	39.1 ± 4.5	**7k**	282 ± 38
7e	570 ± 72.4	**7l**	287 ± 42

[a]) IC_{50} was expressed as drug concentration to achieve half-maximal inhibition of acid-induced swelling in rat platelets. Values are means ± SD, $n = 3$.

Target compounds **7a**, **7f**, **7j**, and cariporide were tested for the protection effects against myocardial ischemic-reperfusion injury in SD rat hearts [12]. The *in vivo* testing results revealed that the infarct size, and the CK level of **7a**, **7f**, and **7j** at the same dose were significantly lower than those of the ischemia-reperfusion group ($p < 0.01$) and were comparable with those of cariporide (*Table 3*). Among them, both parameters of **7f** were lower than those of cariporide and implied a more favorable activity than cariporide.

Table 3. *Cardioprotective Activity of Compounds* **7a**, **7f**, *and* **7j** *against Ischemic-Reperfusion Injury in SD Rat Hearts*[a])

	Dosage [mg/kg]	CK[b]) [U/ml]	Infarct size[c]) [%]
Ischemia/reperfusion	–	5.69 ± 1.18	69 ± 4
Cariporide	1.0	3.53 ± 0.97**[d])	46 ± 3**
7a	1.0	4.06 ± 1.32*	48 ± 3**
7f	1.0	3.04 ± 1.02**	35 ± 3**
	0.5	3.58 ± 0.86**	43 ± 2**
7j	1.0	3.63 ± 0.93**	53 ± 7*

[a]) Cariporide and the tested compounds were injected intravenously 5 min before LAD occlusion. [b]) The amount of creatine kinase (CK) was determined using a CK-NAC kit (*Nanjing Jiancheng Bioengineering Institute*, Nanjing, P. R. China) and a *722* grating photospectrometer (*Shanghai Precision & Scientific Instrument Co., Ltd.*, Shanghai, P. R. China). Serum CK activity was expressed as U/ml. Values are means ± SD, $n = 6$ or higher. [c]) Infarct size was expressed as the ratio of myocardial infarct weight to weight of ventricle at risk. Values are means ± SD, $n = 6$ or higher. [d]) *: $p < 0.05$, **: $p < 0.01$ compared with ischemia-reperfusion group.

The preliminary results of the pharmacological tests showed that 4-(*para*-substituted phenylamino)-substituted (quinolinecarbonyl)guanidines exhibit stronger inhibitory effects on NHE1 than those of 4-(*meta*-substituted phenylamino)-substituted (quinolinecarbonyl)guanidines. The 4-halogeno-substituted or 4-[(*para*-halogenophenyl)amino]-substituted (quinolinecarbonyl)guanidines (*i.e.*, **6**, or **7l** and **7k**, resp.) showed low inhibitory potency for NHE1. The above encouraging results gave us a useful lead for further design of novel NHE1 inhibitors.

Conclusions. – In conclusion, a series of novel (quinolinecarbonyl)guanidine derivatives were synthesized. Some of them showed better NHE1 inhibitory activity than cariporide, and the most active one was compound **7f**. *In vivo* testing showed that the infarct size and the CK level of **7f** was significantly lower than those of the ischemia-reperfusion group ($p < 0.01$) and was lower than those of cariporide. Structure–activity relationships were briefly investigated and discussed. One by-product was isolated, and its structure was characterized.

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Experimental Part

1. *General.* M.p.: *RDCSY-I* Apparatus; uncorrected. IR Spectra: *Bruker Tensor 27* spectrophotometer, in KBr; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker AM-300* spectrometer; δ in ppm rel. to Me_4Si, J in Hz. ESI-MS: *HP1100* mass spectrometer; in m/z (rel. %). Elemental analyses: *Elementar Vario EL III* instrument.

2. *Synthesis. Diethyl 2-[(*p-*Tolylamino)methylidene]malonate* (**1**). *para*-Toluidine (21.4 g, 0.2 mol) and diethyl 2-(ethoxymethylidene)malonate (EMME) (48.0 g, 0.22 mol) were dissolved in a 250-ml three-neck flask. The mixture was stirred for 20 h at 110° while vaporizing. The mixture became sticky brownish red at the end of the reaction. After cooling, the product was directly used without further purification. Yield: 80%. M.p. 45–46°.

Ethyl 4-Hydroxy-6-methylquinoline-3-carboxylate (**2**). Under mechanical stirring, 50 ml of Ph_2O was heated to 250° under N_2, and a soln. of **1** was added quickly. The mixture was stirred for 1 h at 250°, to become a dark brown-to-black soln. After concentration, this soln. was allowed to cool to r.t. and yielded a dark brown solid. To the resulting solid was added 100 ml of petroleum ether (PE), and the mixture was then filtered. The filter cake was washed with PE, followed by drying under IR lamp, to give **2** (32.8 g, 59.1%). A brown yellow solid product. M.p. 211–213°.

Ethyl 4-Chloro-6-methylquinoline-3-carboxylate (**3**). The soln. of **2** (30.0 g, 0.13 mol) in 40 ml of $POCl_3$ was refluxed for 4 h. Upon concentration, the black sticky residue was dissolved in 50 ml of $CHCl_3$, and then poured into ice-water while stirring. The mixture was neutralized by sat. Na_2CO_3 soln., then extracted with $CHCl_3$ three times. The combined org. layers were washed with H_2O, dried (Na_2SO_4), filtered and concentrated to give **3** (22.4 g, 69.3%) after column chromatography (CC) (PE/AcOEt 10:1). Yellow solid. M.p. 62–63°. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.47 (*t*, *J* = 6.9, Me); 2.61 (*s*, Me); 4.51 (*q*, *J* = 7.2, CH_2); 7.68 (*dd*, *J* = 1.5, 8.4, 1 arom. H); 8.04 (*d*, *J* = 8.4, 1 arom. H); 8.18 (*s*, 1 arom. H); 9.14 (*s*, 1 arom. H).

Ethyl 6-(Bromomethyl)-4-chloroquinoline-3-carboxylate (**4**). To a soln. of **3** (20.0 g, 0.08 mol) in 200 ml of anh. CCl_4 was added NBS (14.3 g, 0.08 mol) and benzoyl peroxide in cat. amount. The mixture was heated smoothly to reflux for 18 h under light, and then filtered and concentrated to give **4** (19.5 g, 74.4%) after CC (PE/AcOEt 8:1). White flocculent solid. M.p. 124–126°. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.48 (*t*, *J* = 7.2, Me); 4.52 (*q*, *J* = 7.2, CH_2); 4.70 (*s*, CH_2); 7.90 (*dd*, *J* = 2, 8.7, 1 arom. H); 8.20 (*d*, *J* = 8.7, 1 arom. H); 8.41 (*d*, *J* = 2, 1 arom. H); 9.22 (*s*, 1 arom. H).

Ethyl 4-Chloro-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carboxylate (**5**). *Preparation of the Free Trimetazidine* (= *1-(2,3,4-Trimethoxybenzyl)piperazine*). A soln. of trimetazidine hydrochloride (15.5 g, 0.046 mol) in 20 ml of H_2O was basified to pH 11 using 40% KOH soln. and extracted with AcOEt (50 ml, 3 ×). The combined org. soln. was washed with sat. aq. NaCl soln. to pH 7, and dried (Na_2SO_4) overnight. Then, the org. layer was filtered and concentrated under reduced pressure for later use. To a soln. of **4** (15.0 g, 0.046 mol) in 500 ml of anh. MeCN was added anh. K_2CO_3 (19 g, 0.138 mol). The free trimetazidine produced from above was added dropwise. TLC showed the completion of the reaction in seconds, and then the mixture was filtered, concentrated, and submitted to CC (PE/AcOEt 4:1). After recovering unreacted bromo-compounds, **5** (20.3 g, 86.1%) was obtained after CC ($CHCl_2$/MeOH 20:1). Oil ^{1}H-NMR (500 MHz, $CDCl_3$): 1.47 (*t*, *J* = 7.1, Me); 2.5–2.75 (*m*, 8 piperazine H); 3.71–3.76 (*m*, 2 CH_2); 3.85 (*s*, MeO); 3.86 (*s*, MeO); 3.90 (*s*, MeO); 4.50 (*q*, *J* = 7.1, CH_2); 6.66 (*d*, *J* = 8.5, 1 arom. H); 7.13 (br. *s*, 1 arom. H); 7.86 (*dd*, *J* = 1.6, 8.6, 1 arom. H); 8.08 (*d*, *J* = 8.6, 1 arom. H); 8.27 (*s*, 1 arom. H); 9.16 (*s*, 1 arom. H).

1-(4-Chloro-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine (**6**). A suspension of **5** (20.0 g, 0.038 mol) in 45 ml of abs. i-PrOH was added dropwise to the mixture of 25 ml of abs. i-PrOH and free guanidine (46.0 g, 0.78 mol), and then reacted for 1 h at r.t. The mixture was poured into a mixture of 250 ml of AcOEt/H_2O 1.5:1, while cooling in an ice bath, and extracted with 150 ml of AcOEt for three times. The combined org. soln. was then washed with sat. aq. NaCl soln., dried (Na_2SO_4), filtered, and concentrated. The residue gave **6** (10.6 g, 51.7%) after CC (AcOEt/MeOH 10:1). White solid. M.p. 228–229°. IR (KBr): 3404, 2990, 2955, 2802, 1657, 1603, 1580, 1532, 1494, 1372, 1092 (MeO), 840, 805. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.30–2.48 (*m*, 8 piperazine H); 3.40 (*s*, CH_2); 3.71 (*s*, CH_2); 3.73 (*s*, MeO); 3.77 (*s*, 2 MeO); 6.75 (*d*, *J* = 8.6, 1 arom. H); 6.96 (*d*, *J* = 8.6, 1 arom. H); 7.80 (*dd*, *J* = 8.6, 1.7, 1 arom. H); 8.03 (*d*, *J* = 8.6, 1 arom. H); 8.16 (*s*, 1 arom. H); 8.96 (*s*, 1 arom. H); 6.90 (br. *s*, 2 NH); 8.00 (br. *s*, 2 NH). ^{13}C-NMR (300 MHz, (D_6)DMSO): 52.1 (piperazine C); 52.3 (piperazine C); 55.3; 55.4; 59.8; 60.4; 61.2; 107.0; 122.7; 123.2; 124.2; 124.8; 128.7; 131.0; 132.4; 136.6; 138.4; 141.4; 146.6; 149.2; 151.5; 152.0; 162.3; 174.1 (CO). ESI-MS: 527.2 ($[M+H]^+$), 529.1 ($[M+2H]^+$), 549.1 ($[M+Na]^+$). Anal. calc. for $C_{26}H_{31}N_6O_4 \cdot 0.5\ H_2O$: C 58.26, H 6.02, N 15.68; found: C 58.62, H 5.93, N 15.84.

General Procedure for the Synthesis of **7b**, **7c**, **7e**–**7g**, *and* **7i**–**7l**. $BuNH_2$ (0.30 g, 0.0038 mol) (or aniline, *m*-methoxyaniline, *p*-ethoxyaniline, *p*-toluidine, 3,4-dimethylaniline, 3,5-dimethylaniline, 4-chloroaniline, 4-bromoaniline) and 5 ml of abs. i-PrOH and anh. K_2CO_3 (0.5 g, 0.0038 mol) were mixed, and the mixture was then heated to 60° while stirring. The soln. of **6** in anh. i-PrOH was added (0.2 g, 0.00038 mol) and refluxed for 3 h. The mixture was filtered, concentrated, and the remaining $BuNH_2$ was removed by CC (PE/AcOEt 4:1). The product was purified by CC (AcOEt/MeOH 8:1). The resulting

yellow oil was dissolved in AcOEt and then treated with HCl (g) in AcOEt, The resulting HCl salt was then filtered as a brownish yellow solid.

1-(6-{[4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl]methyl}-4-(butylamino)quinoline-3-carbonyl)guanidine Hydrochloride (**7b**). Yield: 0.08 g (36.9%). Brownish yellow solid. M.p. 194–196°. IR (KBr): 3385, 2958–2560, 1703, 1626, 1601, 1585, 1497, 1286, 1098, 935, 792. ^{1}H-NMR (300 MHz, (D_6)DMSO + D_2O): 0.88 (*t*, *J* = 7.5, Me); 1.24–1.37 (*m*, CH_2); 1.46–1.56 (*m*, CH_2); 2.75 (*t*, *J* = 7.5, NCH_2); 3.35–3.70 (*m*, 8 H, piperazine); 3.74 (*s*, MeO); 3.80 (*s*, MeO); 3.85 (*s*, MeO); 4.26 (*s*, CH_2); 4.50 (*s*, CH_2); 6.87 (*d*, *J* = 8.7, 1 arom. H); 7.30 (*d*, *J* = 8.7, 1 arom. H); 8.16 (*d*, *J* = 8.4, 1 arom. H); 8.28 (*d*, *J* = 8.7, 1 arom. H); 8.74 (*s*, 1 arom. H); 9.29 (*s*, 1 arom. H). ESI-MS: 564.3 ($[M+H]^+$). Anal. calc. for $C_{30}H_{45}Cl_4N_7O_4 \cdot H_2O$: C 49.53, H 6.51, N 13.48; found: C 49.72, H 6.56, N 13.24.

1-(4-(Phenylamino)-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine Hydrochloride (**7c**). Yield: 38.1%. Yellow solid. M.p. 262–263°. IR (KBr): 3385, 3001–2719, 1701, 1624, 1578, 1531, 1494, 1285, 1099, 934. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.05–3.60 (*m*, 8 piperazine H); 3.76 (*s*, MeO); 3.82 (*s*, MeO); 3.87 (*s*, MeO); 4.23 (*s*, CH_2); 4.50 (br. *s*, CH_2); 6.88 (*d*, *J* = 8.7, 1 arom. H); 7.32–7.37 (*m*, 2 arom. H); 7.42 (*d*, *J* = 7.2, 1 arom. H); 7.93 (*d*, *J* = 8.7, 1 arom. H); 8.14–8.17 (*m*, 1 arom. H); 8.48 (*s*, 1 arom. H); 8.57 (br. *s*, 1 NH); 8.91 (*d*, *J* = 6.3, 1 arom. H); 8.99 (*s*, 1 arom. H); 9.09 (br. *s*, 1 NH); 13.27 (*s*, 1 N^+H); 13.89 (*s*, 1 N^+H). ESI-MS: 584.4 ($[M+H]^+$). Anal. calc. for $C_{32}H_{41}Cl_4N_7O_4 \cdot H_2O$: C 51.41, H 5.80, N 13.12; found: C 51.24, H 5.92, N 13.24.

1-(4-[(3-Methoxyphenyl)amino]-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine Hydrochloride (**7e**). Yield: 43.2%. Yellow solid. M.p. 279–281°. IR (KBr): 3377, 3077–2657, 2987, 1701, 1623, 1572, 1530, 1493, 1286, 1096, 806. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.35–3.65 (*m*, 8 piperazine H); 3.75 (*s*, MeO); 3.77 (*s*, MeO); 3.81 (*s*, MeO); 3.86 (*s*, MeO); 4.25 (*s*, CH_2); 4.28 (br. *s*, 1 NH); 4.59 (*s*, CH_2); 6.87 (*d*, *J* = 8.7, 1 arom. H); 6.93 (*s*, 1 arom. H); 7.35 (*d*, *J* = 7.8, 1 arom. H); 7.95 (*d*, *J* = 8.4, 1 arom. H); 8.15 (*d*, *J* = 6.6, 1 arom. H); 8.51 (*s*, 1 arom. H); 8.65 (br. *s*, 1 NH); 8.88 (*d*, *J* = 6.6, 1 arom. H); 8.98 (*s*, 1 arom. H); 9.00 (br. *s*, 1 NH); 9.19 (*s*, 1 arom. H); 13.24 (*s*, 1 NH^+); 14.07 (*s*, 1 NH^+). ESI-MS: 614.5 ($[M+H]^+$). Anal. calc. for $C_{33}H_{43}Cl_4N_7O_5 \cdot 2\,H_2O$: C 49.82, H 5.95, N 12.32; found: C 50.04, H 5.82, N 12.33.

1-(4-[(4-Ethoxyphenyl)amino]-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine Hydrochloride (**7f**). Yield: 52.7%. Brownish yellow solid. M.p. 257–260°. IR (KBr): 3382, 2982–2560, 1700, 1623, 1581, 1532, 1451, 1286, 1100, 926, 802. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.34 (*t*, *J* = 6.6, Me); 3.22–3.63(*m*, 8 piperazine H); 3.75 (*s*, MeO); 3.81 (*s*, MeO); 3.86 (*s*, MeO); 4.05 (*q*, *J* = 6.9, CH_2O); 4.24 (*s*, CH_2); 4.34 (br. *s*, CH_2); 6.87 (*d*, *J* = 8.7, 1 arom. H); 6.95 (*d*, *J* = 8.7, 1 arom. H); 7.29 (*d*, *J* = 8.7, 1 arom. H); 7.34 (*d*, *J* = 8.7, 1 arom. H); 8.00 (*s*, 1 arom. H); 8.15 (*d*, *J* = 8.7, 1 arom. H); 8.28 (*d*, *J* = 9, 1 arom. H); 8.45 (br. *s*, 1 NH); 8.49 (*s*, 1 arom. H); 8.91 (*s*, 1 arom. H); 9.19 (br. *s*, 1 NH); 11.58 (*s*, 1 NH); 12.71 (*s*, 1 NH); 13.25 (*s*, 1 NH^+); 13.96 (*s*, 1 NH^+). ESI-MS: 628.3 ($[M+H]^+$). Anal. calc. for $C_{34}H_{45}Cl_4N_7O_5 \cdot H_2O$: C 51.59, H 5.98, N 12.39; found: C 51.24, H 6.07, N 12.24.

*1-(4-(*p*-Toluidino)-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine Hydrochloride* (**7g**). Yield: 36.4%. Yellow solid. M.p. 260–261°. IR (KBr): 3384, 3075–2659, 1701, 1623, 1570, 1530, 1494, 1286, 1204, 1097, 930, 805. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.50 (*s*, Me); 3.35–3.65 (*m*, 8 piperazine H); 3.75 (*s*, MeO); 3.81 (*s*, MeO); 3.86 (*s*, MeO), 4.22 (br. *s*, CH_2); 4.30 (br. *s*, 1 NH); 4.58 (*s*, CH_2); 6.87 (*d*, *J* = 8.7, 1 arom. H); 7.35 (*d*, *J* = 8.4, 1 arom. H); 7.94 (*d*, *J* = 8.4, 1 arom. H); 8.15 (*d*, *J* = 7.8, 1 arom. H); 8.50 (*s*, 1 arom. H); 8.59 (br. *s*, 1 NH); 8.90 (*d*, *J* = 6.6, 2 arom. H); 9.09 (br. *s*, 1 NH); 9.19 (*s*, 1 arom. H); 13.25 (*s*, 1 NH^+); 13.96 (*s*, 1 NH^+). ESI-MS: 598.3 ($[M+H]^+$). Anal. calc. for $C_{33}H_{43}Cl_4N_7O_4 \cdot H_2O$: C 52.04, H 5.96, N 12.87; found: C 51.84, H 6.07, N 12.74.

1-(4-[(3,4-Dimethylphenyl)amino]-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine Hydrochloride (**7i**). Yield: 46.1%. Light-yellow solid. M.p. 210–213°. IR (KBr): 3445, 2935, 2813, 1621, 1585, 1518, 1498, 1287, 1095, 874, 806. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.12 (*s*, Me); 2.15 (*s*, Me); 2.17–2.52 (*m*, 8 piperazine H); 3.34 (*s*, CH_2); 3.44 (*s*, CH_2); 3.85 (*s*, MeO); 3.87 (*s*, 2 MeO); 6.63 (*d*, *J* = 8.4, 2 arom. H); 6.74 (*d*, *J* = 7.8, 1 arom. H); 6.81 (*s*, 1 arom. H); 6.96 (*dd*, *J* = 3.6, 8.4, 1 arom. H); 7.56 (*s*, 1 arom. H); 7.90 (*d*, *J* = 9, 1 arom. H); 8.04 (br. *s*, 1 NH); 8.21 (br. *s*, 1 NH); 9.49 (*s*, 1 arom. H). ^{13}C-NMR (300 MHz, (D_6)DMSO): 19.14; 19.78; 52.87; 52.94; 56.06; 56.56; 60.82; 61.22; 62.72; 76.62; 77.04; 77.25; 77.47; 107.06; 114.31; 119.71; 119.78; 123.66; 124.17; 125.17; 127.12; 127.61; 130.32; 132.32; 132.38; 132.72; 133.88; 137.52; 141.18; 142.36; 147.79; 151.28; 152.64; 152.91; 161.91; 178.05. ESI-MS:

612.3 ([M+H]$^+$). Anal. calc. for $C_{34}H_{45}Cl_4N_7O_4 \cdot 1.5\ H_2O$: C 52.05, H 6.17, N 12.50; found: C 52.54, H 6.07, N 12.74.

1-(4-[(3,5-Dimethylphenyl)amino]-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine Hydrochloride (**7j**). Yield: 42.3%. Yellow solid. M.p. 242–243°. IR (KBr): 3379, 3001–2554, 1700, 1622, 1579, 1530, 1495, 1285, 1098, 933, 804. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.26 (*s*, Me); 2.50 (*s*, Me); 3.32–3.71 (*m*, 8 piperazine H); 3.75 (*s*, MeO); 3.81 (*s*, MeO); 3.86 (*s*, MeO); 4.21 (*s*, CH_2); 4.47 (br. *s*, CH_2); 6.87 (*d*, J = 8.7, 1 arom. H); 6.94 (*s*, 1 arom. H); 7.32 (*d*, J = 8.7, 1 arom. H); 7.92 (*d*, J = 8.4, 1 arom. H); 8.13 (*d*, J = 9, 1 arom. H); 8.46 (*s*, 1 arom. H); 8.52 (br. *s*, 1 NH); 8.91 (*d*, J = 6.6, 1 arom. H); 8.94 (*s*, 1 arom. H); 9.08 (br. *s*, 1 NH); 11.46 (br. *s*, 1 NH); 12.83 (br. *s*, 1 NH); 13.26 (*s*, 1 NH$^+$); 13.85 (*s*, 1 NH$^+$). ESI-MS: 612.5 ([M+H]$^+$). Anal. calc. for $C_{34}H_{45}Cl_4N_7O_4 \cdot H_2O$: C 52.65, H 6.11, N 12.64; found: C 52.49, H 6.18, N 12.85.

1-(4-[(4-Chlorophenyl)amino]-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine Hydrochloride (**7k**). Yield: 42.1%. Yellow solid. M.p. 275–278°. IR (KBr): 3373, 3078–2656, 2996, 1701, 1623, 1570, 1530, 1493, 1285, 1097, 903, 804. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.33–3.72 (*m*, 8 piperazine H); 3.75 (*s*, MeO); 3.81 (*s*, MeO); 3.86 (*s*, MeO); 4.03 (br. *s*, 1 NH); 4.25 (*s*, CH_2); 4.59 (*s*, CH_2); 6.87 (*d*, J = 8.7, 1 arom. H); 7.34–7.41 (*m*, 1 arom. H); 7.48 (*d*, J = 8.7, 1 arom. H); 7.95 (*d*, J = 8.4, 1 arom. H); 8.15 (*d*, J = 7.8, 1 arom. H); 8.51 (*s*, 1 arom. H); 8.63 (br. *s*, 1 NH); 8.89 (*d*, J = 6.6, 1 arom. H); 9.02 (*s*, 1 arom. H); 9.08 (br. *s*, 1 NH); 13.24 (*s*, 1 NH$^+$); 14.02 (*s*, 1 NH$^+$). ESI-MS: 618.2 ([M+H]$^+$). Anal. calc. for $C_{32}H_{40}Cl_5N_7O_4 \cdot H_2O$: C 49.15, H 5.41, N 12.54; found: C 49.35, H 5.48, N 12.51.

1-(4-[(4-Bromophenyl)amino]-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine Hydrochloride (**7l**). Yield: 32.7%. Yellow solid. M.p. 241–244°. IR (KBr): 3376, 3080–2713, 3001, 1701, 1623, 1568, 1529, 1492, 1285, 1098 (MeO), 930, 804. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.35–3.70 (*m*, 8 piperazine H); 3.75 (*s*, MeO); 3.81 (*s*, MeO); 3.86 (*s*, MeO); 4.24 (*s*, CH_2); 4.30 (br. *s*, 1 NH); 4.57 (*s*, CH_2); 6.87 (*d*, J = 8.7, 1 arom. H); 7.30–7.36 (*m*, 3 arom. H); 7.60 (*d*, J = 8.4, 2 arom. H); 7.94 (*d*, J = 8.4, 1 arom. H); 8.14 (*d*, J = 8.4, 1 arom. H); 8.50 (*s*, 1 arom. H); 8.61 (br. *s*, 3 NH); 8.89 (*s*, 1 arom. H); 9.08 (br. *s*, 3 NH); 13.25 (*s*, 1 NH$^+$); 13.99 (*s*, 1 NH$^+$). ESI-MS: 662.4 ([M+H]$^+$), 664.4 ([M+H]$^+$, isotopic peak). Anal. calc. for $C_{32}H_{40}BrCl_4N_7O_4 \cdot H_2O$: C 46.51, H 5.12, N 11.86; found: C 46.39, H 5.08, N 12.15.

General Procedure for the Synthesis of **7d** *and* **7h**. To a hot (60°) soln. of **6** (0.2 g, 0.00038 mol) in 10 ml of abs. EtOH, anh. K_2CO_3 (0.5 g, 0.0038 mol) and 4-methoxyaniline (0.47 g, 0.0038 mol) were added. The resulting mixture was refluxed for 12 h. After filtration and concentration under reduced pressure, the residue was purified by large panel CC (AcOEt/MeOH 32 : 1, 16 : 1).

1-(4-[(4-Methoxyphenyl)amino]-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine (**7d**). Yield: 0.10 g (45.9%). Yellow solid. M.p. 268–270°. IR (KBr): 3386, 2934, 2817, 1637, 1584, 1508, 1461, 1286, 1095, 898, 827. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.19–2.40 (*m*, 8 piperazine H); 3.31–3.37 (*m*, 2 CH_2); 3.61 (*s*, MeO); 3.73 (*s*, MeO); 3.76 (*s*, 2 MeO); 6.77 (*dd*, J = 2.4, 8.1, 2 arom. H); 6.92 (*dd*, J = 3.3, 8.0, 2 arom. H); 7.14 (br. *s*, 2 NH); 7.49 (*s*, 1 arom. H); 7.78 (*d*, J = 8.7, 1 arom. H); 7.88 (*d*, J = 8.4, 1 arom. H); 8.48 (*s*, 1 arom. H); 9.04 (*s*, 1 arom. H); 9.27 (*s*, 1 arom. H); 11.36 (br. *s*, 1 NH); 11.76 (*s*, 1 NH). ESI-MS: 614.5 ([M+H]$^+$). Anal. calc. for $C_{33}H_{39}N_7O_5 \cdot H_2O$: C 62.74, H 6.54, N 15.52; found: C 62.97, H 6.58, N 15.35.

*1-(4-(*m*-Toluidino)-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine* (**7h**). Yield: 39.9%. Yellow solid. M.p. 275–277°. IR (KBr): 3405, 2990, 2956, 2802, 1657, 1602, 1579, 1533, 1495, 1327, 1092, 840, 806. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.15 (*s*, Me); 2.19–2.31 (*m*, 8 piperazine H); 3.24 (*s*, CH_2); 3.39 (*s*, CH_2); 3.77 (*s*, MeO); 3.78 (*s*, MeO); 3.79 (*s*, MeO); 6.55 (*d*, J = 8.7, 1 arom. H); 6.77 (*s*, 1 arom. H); 6.82 (*d*, J = 7.2, 2 arom. H); 6.90 (*d*, J = 8.4, 1 arom. H); 7.05 (*t*, J = 7.5, 1 arom. H); 7.43 (*s*, 1 arom. H); 7.50 (*d*, J = 9, 1 arom. H); 7.85 (*d*, J = 8.7, 1 arom. H); 9.40 (*s*, 1 arom. H); 11.99 (*s*, 2 NH). ESI-MS: 598.3 ([M+H]$^+$). Anal. calc. for $C_{33}H_{39}N_7O_4 \cdot 2\ H_2O$: C 62.54, H 6.84, N 15.47; found: C 62.57, H 7.17, N 15.44.

1-(4-(Propylamino)-6-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]methyl}quinoline-3-carbonyl)guanidine Hydrochloride (**7a**). To a soln. of **6** (0.2 g, 0.00038 mol) in 10 ml of abs. i-PrOH, $PrNH_2$ (0.23 g, 0.0038 mol) and anh. K_2CO_3 (0.52 g, 0.0038 mol) were added. The resulting mixture was stirred and refluxed for 3 h. After filtration and concentration, the residue was purified by large panel (AcOEt/

MeOH 32:1) to afford a yellow oil (0.07 g). The oil was dissolved in AcOEt and cooled in ice bath, and sat. HCl (g) in anh. AcOEt was added. The resulting precipitate was filtered to give **7a** (32.8%). Light-yellow solid. M.p. 183–186°. IR (KBr): 3407, 966, 1703, 1628, 1603, 1536, 1496, 1285, 1199, 1097, 933. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.17 (*t*, *J* = 6.9, Me); 1.77–1.86 (*m*, CH_2); 3.49–3.73 (*m*, 8 piperazine H); 3.75 (*s*, MeO); 3.82 (*s*, MeO); 3.86 (*s*, MeO); 3.99–4.06 (*m*, CH_2); 4.23 (*s*, CH_2); 4.48 (*s*, CH_2); 6.87 (*d*, *J* = 8.7, 1 arom. H); 7.35 (*d*, *J* = 8.4, 1 arom. H); 8.06 (*d*, *J* = 8.4, 1 arom. H); 8.24 (*d*, *J* = 9, 1 arom. H); 8.49 (*s*, 1 arom. H); 8.77 (br. *s*, 2 NH); 9.15 (br. *s*, 1 NH); 9.27(*s*, 1 arom. H); 9.83 (br. *s*, 1 NH); 13.08 (br. *s*, 1 N^+H); 15.13 (br. *s*, 1 NH^+). ESI-MS: 550.3($[M+H]^+$). Anal. calc. for $C_{34}H_{45}Cl_4N_7O_4 \cdot H_2O$: C 48.82, H 6.36, N 13.74; found: C 50.01, H 6.28, N 13.85.

3. *Bioassay.* Our study conformed with the *Guide for the Care and Use of Laboratory Animals*, published by the U.S. National Institute of Health.

Rat Platelet-Swelling Assay. Sprague–Dawley rats (380–420 g) were anesthetized with Et_2O, and blood was collected from their eyeholes with 25% (*v*/*v*) acid-citrate-dextrose (ACD; sodium citrate, 2.23 g; citric acid, 0.86 g; and glucose, 2.47 g in 100 ml of dist. H_2O). Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 1300*g*/min for 10 min at r.t. The upper 2/3 of the supernatants were used for the further measurements and stored at r.t. until used. All measurements were performed within 4–5 h.

All compounds were dissolved in DMSO and diluted with propionate medium (pH 7.4). A soln. of the tested compound (25 µl) was added to 175 µl of propionate buffer (sodium propionate, 140 mmol/l; HEPES, 20 qs; glucose, 10 mmol/l; KCl, 5 mmol/l; $MgCl_2$, 1 mmol/l; $CaCl_2$, 1 mmol/l; pH 6.7) contained in a spectrophotometer cuvette. Then, 50 µl of PRP prewarmed to 37° was added. The suspension was stirred, and the change in optical density (*OD*) was recorded each 7.5 s for 2 min at 550 nm (*Thermo Multiscan Spectrum*). The decrease of *OD* corresponded to a monoexponential curve following the equation $OD_{(t)} = OD_{t=0}\, e^{-kt}$, where *t* is the time (in s) corresponding to the recorded *OD*, and *k* is the decrease rate constant. For each compound, the concentrations were plotted against their corresponding *k* values. The maximum platelet swelling was measured in absence of any drug. The minimum swelling was observed in presence of cariporide (10^{-5} mol/l) and is the result of a completely inhibited NHE1. Sigmoidal curves were drawn by non-linear regression analysis (*Graphpad Prism* software). They led to calculation of the drug concentration (IC_{50}) which decreases 50% of the platelet swelling. For each molecule, the measurements were performed in triplicate.

Cardioprotective Effects in Rat Model of Ischemic Heart. Sprague–Dawley rats (380–420 g) were anesthetized with sodium pentobarbital (60 mg/kg; ip). Coronary artery occlusion was produced by ligating the left anterior descending coronary artery (LAD) for 1 h. Then, the coronary artery was reperfused by loosing the ligature. After 2 h of reperfusion, the coronary artery was re-occluded, and 2 ml of a 1% *Evans* blue was injected *via* tail vein. Then, the heart was removed and blood sample was collected.

The left ventricle of the removed heart was dissected free from other structures and sliced transversely into 1-mm-thick sections. The sections were then incubated in 1% triphenyltetrazolium chloride for 15 min at 37° and then fixed for 20–24 h in a 10% formalin soln. to determine the infarct size. The infarct size was calculated by the following formula:

$$\text{Myocardial infarct size [\%]} = (\text{the weight of undyed myocardium/the weight of left ventricle}) \times 100$$

The blood samples were centrifuged at 3000*g*/min for 10 min. The supernatant serum was removed and stored in liq. N_2, until the biochemical analysis was performed. Creatine kinase (CK) in serum was measured by TU-1800 (*Purkinje General Instrument Co. Ltd*, P. R. China) using commercial kits (*Jiancheng Bioengineering Institute*, P. R. China).

Target compounds **7a**, **7f**, **7j**, and cariporide were intravenously given 5 min before reperfusion.

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