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(2-Aryl-5-methylimidazol-4-ylcarbonyl)guanidines and (2-aryl-5-methyloxazol-4-ylcarbonyl)guanidines as NHE-1 inhibitors

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ABSTRACT

A series of (2-aryl-5-methylimidazol-4-ylcarbonyl)guanidines and (2-aryl-5-methyloxazol-4-ylcarbonyl)guanidines were synthesized and evaluated as NHE-1 inhibitors. The structure–activity relationships well matched those of furan derivatives, which were previously investigated. The (2,5-disubstituted)phenyl compounds showed better activities than the other analogues in both imidazole and oxazole compounds. Especially, 2-(2,5-dichlorophenyl)imidazole 52, and 2-(2-methoxy-5-chlorophenyl)imidazole 54 compounds exhibited potent cardioprotective efficacy both in vitro and in vivo as well as high NHE-1 inhibitory activities.

During ischemia-reperfusion injuries, the accumulation of intracellular protons leads to the activation of Na^+/H^+ exchanger type-1 (NHE-1), which exchanges intracellular H^+ for extracellular Na^+ to regulate intracellular pH.[1] The consequent increase of intracellular Na^+ concentration causes intracellular Ca^{2+} overload through the Na^+/Ca^{2+} exchanger, resulting in detrimental effects including myocardial contracture, stunning, necrosis, and arrhythmia.[2] Inhibition of NHE-1 overactivity would be an effective obstruction of this chain of events and would prevent damage to the myocardium in ischemia-reperfusion.[3]

While most NHE-1 inhibitors have acylguanidine structure,[4] the aryl ring templates are quite diverse including benzene, pyrazole, quinoline, and indole, etc.[5] Our previous studies to identify a novel NHE-1 inhibitor, especially based on 5-membered heterocycle, demonstrated that a series of (5-arylfuran-2-ylcarbonyl)guanidines are potent NHE-1 inhibitors showing in vitro and in vivo anti-ischemic efficacies.[6] As the extension of our efforts to examine the SAR and continuously search the NHE-1 inhibitor with better profile, we replaced the furan with imidazole and oxazole (Fig. 1). This paper describes the synthesis, biological evaluation, and structure–activity relationships of those derivatives.

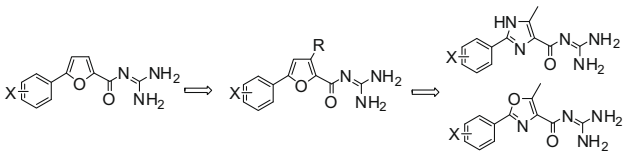
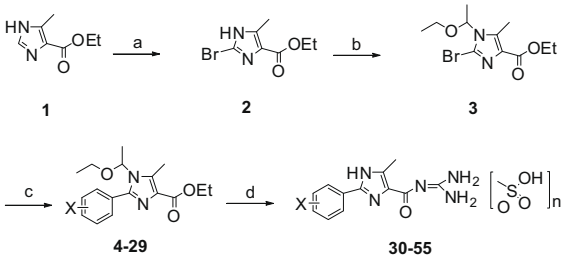


Figure 1. Design of (2-aryl-5-methylimidazol-4-ylcarbonyl)guanidines and (2-aryl-5-methyloxazol-4-ylcarbonyl)guanidines.



Scheme 1. Reagents and conditions: (a) NBS, CH_3CN, rt, 63%; (b) ethyl vinylether, CH_2Cl_2, 0 °C–rt, 93%; (c) arylboronic acid, $Pd(Ph_3)_4$, $Ba(OH)_2.H_2O$ or K_2CO_3, toluene or DME, reflux, 60–80% (d) i—guanidine, DMF, rt, 60–80%; ii—CH_3SO_3H, acetone, rt, 55–80%.

The imidazole analogues with variously substituted 2-aryl group **30**–**55** were synthesized through the sequence of simple steps outlined in Scheme 1. Treatment of the commercially available ethyl 5-methyl-4-imidazolecarboxylate **1** with *N*-bromosuccinimide (NBS) afforded the 2-brominated compound **2**.[7] Initially, we attempted Suzuki coupling reaction[8] of **2** with aryl boronic acid without the prior protection of NH group to introduce the 2-aryl group, but didn't get any reasonable and consistent yield of the product. Even though there are several reports for the successful 2-arylation of imidazole containing the base-sensitive free NH,[9] we decided to protect the amine before Suzuki reaction because

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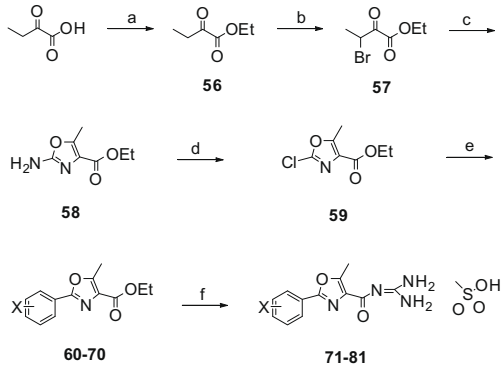
the reaction of *N*-unprotected 1*H*-imidazole-4-carboxylic ester with guanidine was not successful, either. The treatment of **2** with ethyl vinyl ether gave the *N*-(1-ethoxyethyl) product **3**,[10] and subsequent Pd-catalyzed Suzuki coupling reaction proceeded smoothly to yield the variously substituted 2-aryl imidazoles **4**–**29**. The acylguanidines **30**–**55** were prepared from the reaction of corresponding esters **4**–**29** with excess guanidine in DMF. The 1-ethoxyethyl protecting group was removed by treating with methanesulfonic acid in acetone, which simultaneously formed the 1 or 2 equivalents of methanesulfonic acid salts. Final salts **30**–**55** were purified by recrystallization in hot acetone.

Ethyl 2-amino-5-methyloxazole-4-carboxylate **58**, the key intermediate of oxazole derivatives, was prepared starting from 2-ketobutyric acid through the sequence of reactions including acid catalyzed esterification of carboxylic acid, α-bromination using $CuBr_2$, and the condensation of 3-bromo-2-ketobutyrate **57** with urea (Scheme 2).[11] Treatment of the 2-aminooxazole **58** with 1.5 equiv of *tert*-butyl nitrite and $CuCl_2$ produced 2-chlorooxazole **59** in 80% yield.[12] Under standard Suzuki coupling condition, the chlorooxazole **59** was reacted with aryl boronic acid smoothly to yield the series of 2-aryloxazoles **60**–**70**. Final guanidine methanesulfonates **71**–**81** were obtained by the same procedure with the imidazole analogues.

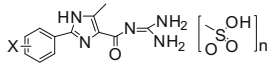
Primarily, the NHE-1 inhibitory activities of the synthesized compounds were determined by measuring their ability to inhibit NHE-1 mediated recovery of intracellular pH following an imposed acidosis in PS120 variant cells selectively expressing the human NHE-1.[13] Using this method the IC_{50} value for cariporide was measured as 1.2 μM. As shown in Table 1, the para substituted phenyl derivatives of imidazole analogues (**33**, **39**, **44**, **50**) were not active, similar to the furan analogues. Generally, addition of 2,5-, or 3,5-disubstitutents improved the activity on NHE-1 over unsubstituted and mono-substituted derivatives. The 2,5-disubstituted compounds represented the most potent inhibition on NHE-1, which is also same tendency with the furan compounds. 2,5-Dichloro **52** (IC_{50} = 0.10 μM) and 2-methoxy-5-chloro **54** (IC_{50} = 0.24 μM) compounds were 12 and 5 times more potent than cariporide, respectively. The activities of corresponding furan analogues (2,5-diCl: IC_{50} = 0.12 μM, 2-OMe-5-Cl: IC_{50} = 0.081 μM) were similar or a little superior to this imidazoles.[6a] Oxazole analogues showed the same tendency of structure activity relationship with the imidazoles (Table 2). 4-Chloro compound **79** was not active (IC_{50} > 30 μM), while 2,5-dichloro compound **80** was very potent on NHE-1 inhibition (IC_{50} = 0.072 μM).

Next, the cardioprotective efficacies against ischemia/reperfusion injury were examined for the compounds showing good NHE-1 inhibitory activity (Table 3). The isolated rat heart model



Scheme 2. (a) 0.2 equiv H_2SO_4, EtOH, reflux, 97%; (b) $CuBr_2$, $CHCl_3$, reflux, 58%; (c) urea, EtOH, reflux, 85%; (d) ^tBuONO, $CuCl_2$, CH_3CN, 80 °C , 80%; (e) aryl boronic acid, $Pd(Ph_3)_4$, $Ba(OH)_2{\cdot}H_2O$ or K_2CO_3, toluene or DME, reflux, 61–93% (d) i—guanidine, DMF, rt; ii—CH_3SO_3H, acetone, rt, 51–77%.

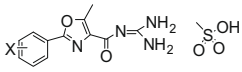
Table 1
Inhibitory effect on NHE-1 of (2-aryl-5-methylimidazol-4-ylcarbonyl)guanidines



	X	*n*	IC_{50} (μM)[a]		X	*n*	IC_{50} (μM)[a]
Cariporide			1.2	**43**	3-F	1	4.5
30	H	1	12.0	**44**	4-F	2	>30
31	2-CH_3	2	2.4	**45**	2,3-diF	2	6.6
32	3-CH_3	1	12.0	**46**	2,5-diF	2	3.0
33	4-CH_3	2	>30	**47**	3,5-diF	1	2.7
34	2,3-diCH_3	2	>30	**48**	2-Cl	2	1.4
35	2,5-diCH_3	2	1.1	**49**	3-Cl	1	4.5
36	3,5-diCH_3	1	5.0	**50**	4-Cl	2	>30
37	2-OCH_3	2	3.1	**51**	2,3-diCl	2	0.50
38	3-OCH_3	2	4.1	**52**	2,5-diCl	2	0.10
39	4-OCH_3	2	>30	**53**	3,5-diCl	1	0.52
40	2,3-diOCH_3	2	>30	**54**	2-OCH_3-5-Cl	2	0.24
41	2,5-diOCH_3	2	2.1	**55**	2-OCH_3-5-F	2	0.80
42	2-F	2	7.2				

[a] Values are means of three experiments and standard deviations for the values are generally within the 15% of IC_{50} value.

Table 2
Inhibitory effect on NHE-1 of (2-aryl-5-methyloxazol-5-ylcarbonyl)guanidines



	X	IC_{50} (μM)[a]		X	IC_{50} (μM)[a]
Cariporide		1.2	**76**	2,5-diF	2.8
71	H	8.2	**77**	3,5-diF	3.3
72	3-CH_3	6.6	**78**	3-Cl	1.7
73	2,5-diCH_3	0.22	**79**	4-Cl	>30
74	3,5-diCH_3	7.1	**80**	2,5-diCl	0.072
75	3-F	4.2	**81**	3,5-diCl	0.89

[a] Values are means of three experiments and standard deviations for the values are generally within the 15% of IC_{50} value.

Table 3
Cardioprotective efficacy against ischemia-reperfusion injury

Compounds	X	IC_{50} (μM)	Langendorff[a]		In vivo[b] IS/AAR (%)
			RPP(%)	LVEDP (mmHg)	
Control			16 ± 1.5	55 ± 2.4	59 ± 1.5
Cariporide		1.2	48 ± 5.0	22 ± 3.0	41 ± 2.1
35	2,5-diCH_3	1.1	33 ± 5.7	32 ± 7.2	45 ± 3.2
48	2-Cl	1.4	69 ± 10	35 ± 4.8	50 ± 3.9
52	2,5-diCl	0.10	59 ± 9.2	25 ± 4.9	40 ± 1.3
53	3,5-diCl	0.52	23 ± 5.4	28 ± 6.7	
54	2-OCH_3-5-Cl	0.24	60 ± 5.4	28 ± 4.7	34 ± 1.1
55	2-OCH_3-5-F	0.80	27 ± 4.3	29 ± 2.9	
73	2,5-diCH_3	0.22	59 ± 4.0	9.0 ± 6.7	47 ± 2.1
80	2,5-diCl	0.072	19 ± 2.4	27 ± 3.8	39 ± 2.3
81	3,5-diCl	0.89	26 ± 3.2	25 ± 2.1	

[a] In vitro cardioprotective effect was evaluated by measuring % RPP (LVDP × HR) to pre-ischemic value, LVEDP (10 μM). n = 3 or higher.

[b] In vivo cardioprotective effect was determined by measuring a ratio of myocardial infarct size to area at risk (IS/AAR) in rat myocardial infarction model (0.1 mg/kg). Values are means, n = 3 or higher.

was employed for the measurement of in vitro cardioprotective effect. Each isolated rat heart treated with 10 μM of the compound for 10 min, subjected to 30 min global ischemia followed by 30 min reperfusion.[14] The cardioprotective effect was measured as an index of cardiac contractile function based on the percent recovery of rate pressure product (RPP, HR × LVDP, heart rate - left ventricular developing pressure) at the end of reperfusion to the pre-ischemic value. Additionally left ventricular end diastolic pressure (LVEDP) was used as an indicator of cardiac contracture.

Additionally cardioprotective in vivo efficacy was determined by measuring a ratio of myocardial infarction size to area at risk (IS/AAR) by using a rat myocardial infarction model[13] that was stabilized for 20 min after a left thoracotomy operation, subjected to 45 min coronary artery occlusion, following 90 min reperfusion. The vehicle or compounds were intravenously administered by bolus injection at 5 min prior to onset of ischemia into the femoral vein.

2,5-Dichloro **52** (59% RPP, 25 mmHg LVEDP) and 2-methoxy-5-chloro **54** (60% RPP, 28 mmHg LVEDP) significantly improved the recovery of cardiac contractility and contracture compared with vehicle (16% RPP, 55 mmHg LVEDP), that is similar to or slightly better than that of cariporide (48% RPP, 22 mmHg LVEDP). Both **52** and **54** represented potent protective effect in rat myocardial infarction model, showing 40% and 34% IS/AAR compared with 59% of the vehicle, which seemed to be similar or superior to cariporide (41% IS/AAR). In the case of furan analogues, functionality adjacent to the acylguanidine moiety improved NHE-1 inhibitory potency, but did not show any significant protective activity against ischemia-reperfusion injury in the Langendorff model.[15] Even none of compounds display protective efficacy in the rat myocardial infarction model. Then, there might be a possibility that the differences are presumably attributable to the 3-substituents next to 2-acylguanidine. But this imidazole analogues with methyl group next to acylguanidine, showed good correlation between NHE-1 inhibitory potency and both in vitro and in vivo cadioprotective efficacies. The oxazole analogues gave the somehow complicated results. 2,5-Dichloro compound **80** significantly reduced infarct size (39% IS/AAR) with the potent NHE-1 inhibitory activity, but didn't show any protective efficacy in Langendorff experiment. In the case of 2,5-dimethyl compound **73** markedly improved cardiac contractile function and contracture (59% RPP, 9 mmHg LVEDP), however was not protective in rat myocardial infarction model. It needs more studies to explain those discrepancies.

In summary, a series of (2-aryl-5-methylimidazol-4-ylcarbonyl)guanidines and (2-aryl-5-methyloxazol-4-ylcarbonyl)guanidines were synthesized and evaluated for their NHE-1 inhibitory activities and in vitro and in vivo cardioprotective efficacies. Oxazole analogues showed poent NHE-1 inhibitory activities comparable to furan and imidazole compounds, but in vitro and in vivo cardioprotective efficacies were not well correlated each other. Among a series of imidazole analogues, the 2,5-disubstituted phenyl derivatives, especially 2,5-dichloro **52** and 2-methoxy-5-chloro **54** compounds showed the potent inhibitory activity on NHE-1, and good in vitro and in vivo cardioprotective efficacy against ischemia/reperfusion injury. Continuing studies including pharmacokinetic and metabolic studies are underway to identify the new cardioprotective agent.

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