Synthesis and Biological Evaluation of 4-Heteroaryl-2-amino-5-methylimidazole Analogs as NHE-1 Inhibitors

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To identify a non-acylguanidine NHE-1 inhibitor, an acylguanidne group was replaced with an imidazole group in the potent NHE-1 inhibitors with furan or benzothiphene core template, found from our previous studies. We synthesized and biologically evaluated 4-heteroaryl-2-amino-5-methylimidazole derivatives. All those imidazole compounds (**16-18**) represented the potent NHE-1 inhibitory activities, similar to the corresponding acylguanidine compounds.

Key Words: Sodium hydrogen exchanger type 1 (NHE-1) inhibitor, Non-acylguanidine, 4-Heteroaryl-2-amino-5-methylimidazole, Ischemia/reperfusion injury, Ischemic disease

Introduction

Because myocardial ischemia is a leading cause of death in the western world, clinical application to treat ischemic diseases such as myocardial infarction, arrhythmia, and angina, has attracted considerable attention.[1] Administration of sodium hydrogen exchanger type 1 (NHE-1) inhibitors reduced infarct size, myocardial stunning, arrhythmia, and endothelial dysfunction caused by ischemia/reperfusion injury in various pre-clinical works, which indicates the potential of NHE-1 inhibitors to treat ischemic diseases.[2-4]

Most known NHE-1 inhibitors have an acylguanidine functionality as key pharmacophore.[5] However, there are concerns on the acylguanidine group which has the possibility to release guanidine under metabolic conditions, a known cause of toxicity. Then efforts to discover non-acylguanidine NHE-1 inhibitors have been made,[6] and several compounds containing imidazole as an acylguanidine surrogate group have been reported.[7,8]

We previously found the potent acylguanidine NHE-1 inhibitors with furan[9] or benzothiophene[10] core template, and attempted to replace the acylguanidine group with imidazole (Figure 1). This paper describes the synthesis, and biological evaluation of 4-heteroaryl-2-amino-5-methylimidazole derivatives, and compared with the corresponding acylguanidine compounds.

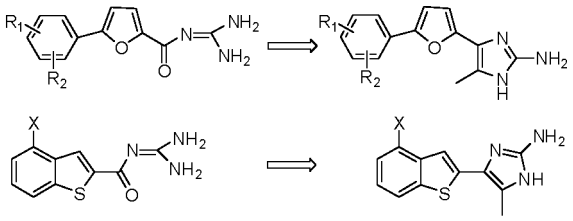
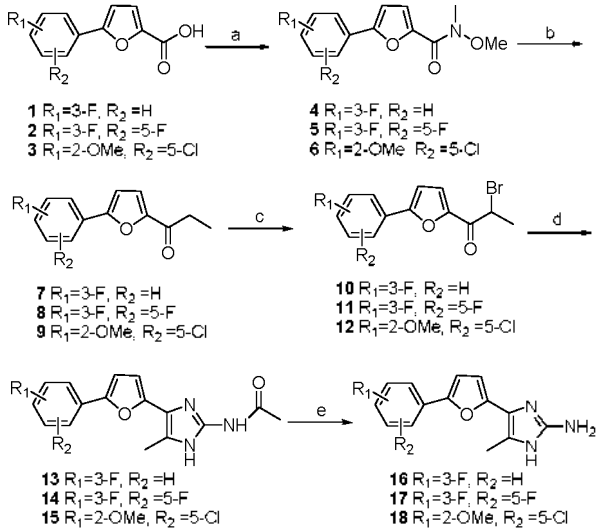


Figure 1. Replacement of Acylguanidine.

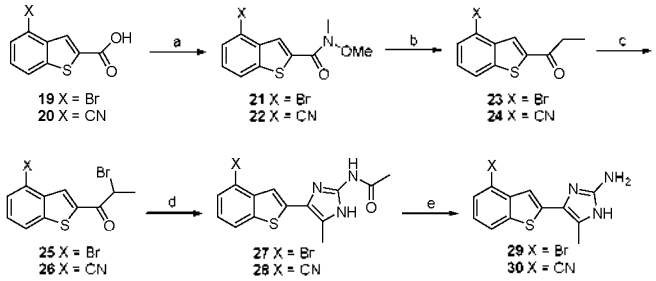
Chemistry

Synthesis of the 4-(5-arylfuran-2-yl)-2-amino-5-methylimidazoles (**16-18**) started with the 5-arylfuran-2-carboxylic acids (**1-3**)[9] as shown in Scheme 1. The activation of carboxylic acids with 1,1-carbonyldiimidazole (CDI) and subsequent treatment with *N,O*-dimethylhydroxylamine hydrochloride in CH_2Cl_2 gave the *N*-methoxy-*N*-methyl amides, known as Weinreb amides (**4-6**).[11] The nucleophilic displacement of Weinreb amides with Grignard reagent (ethylmagnesium bromide) proceeded smoothly to yield ketones (**7-9**) in reasonable yields



Scheme 1. Reagents; (a) 1,1-carbonyldiimidazole, *N,O*-dimethylhydroxylamine hydrochloride, THF; (b) EtMgBr, THF; (c) Br_2, CH_2Cl_2; (d) 1-acetylguanidine, DMF; (e) HCl, MeOH.

(65 - 78%).[12] The α-bromination of ketones with bromine in CH_2Cl_2 provided the α-bromoketones (**10-12**),[13] which reacted with *N*-acetylguanidine and cyclized to form the 2-(acetylamino)imidazoles (**13-15**).[14] Finally, the deacetylation reaction was carried out under HCl in methanol to yield the 4-(5-arylfuran-2-yl)-2-amino-5-methylimidazoles (**16-18**).



Scheme 2. Reagents: (a) 1,1-carbonyldiimidazole, *N,O*-dimethylhydroxylamine hydrochloride, THF; (b) EtMgBr, THF; (c) Br_2, CH_2Cl_2; (d) 1-acetylguanidine, DMF; (e) HCl, MeOH.

Starting from the benzothiophene-2-carboxylic acids (**19** and **20**), the 4-(benzothiophen-2-yl)-2-amino-5-methylimidazoles (**21** and **22**) were prepared according to the same procedure with the furan derivatives (Scheme 2).

Biological Evaluation

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.[15] Using this method the IC_{50} value for cariporide, known NHE-1 inhibitor, was measured as 1.0 μM.

Results and Discussion

The inhibitory effects of synthesized compounds on NHE-1 were determined as IC_{50}. Previously studied acylguanidines were comparable to or much more potent than cariporide. Especially the 2-methoxy-5-chlorophenyl furan compound **33** (IC_{50} = 0.06 μM) was 10 times more potent than cariporide on NHE-1, and also showed excellent cardioprotective efficacy in both in vitro function assay and in vivo ischemia animal model.[9] 2-Amino-4-methylimidazole derivatives (**16-18**, **29** and **30**) were as active as the corresponding acylguanidines, which indicates that the 2-amino-4-methylimidazole moiety can be an acylguanidine surrogate. We will continue the study on this series of compounds including function assay, in vivo animal model, pharmacokinetic and toxicological properties to identify an effective and safe anti-ischemic agent.

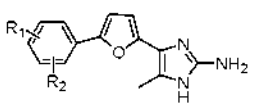
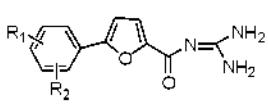
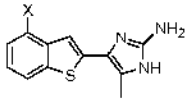
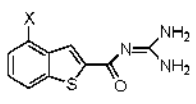
Experimental Section

Chemistry. Melting points were determined on a capillary melting point apparatus and are uncorrected. Anhydrous solvents were dried by conventional methods. Reagents of commercial quality were used from freshly opened containers unless otherwise stated. ^{1}H NMR spectra were recorded on a Varian Gemini 200 or a Bruker DRX-300 spectrometer. ^{13}C NMR were obtained on a Bruker AMX-300 spectormeter. Mass spectra were obtained with a JEOL JMS-DM 303 instrument by using electron impact or chemical ionization techniques.

5-(3-Fluorophenyl)-*N*-methoxy-*N*-methylfuran-2-carboxamide (4): To a solution of 5-(3-fluorophenyl)-2-carboxylic acid (1.71 g, 8.3 mmol) **1** in THF (25 mL) was added 1,1-carbonyldiimidazole (1.62 g, 9.95 mmol), and the reaction mixture was stirred at rt for an hr following the addition of HCl salt of *N,O*-dimethylhydroxylamine (1.21 g, 12.4 mmol), and continuously stirring at rt for 12 hr. Water (20 mL) was added, and the mixture was extracted with ethyl acetate (60 mL) twice. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1) to yield **4** as a pale yellow solid (1.8 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H), 3.82 (s, 3H), 6.77(d, 1H, J = 3.6 Hz), 7.02 (m, 1H), 7.23(d, 1H, J = 9.9 Hz), 7.56 (dd, 1H, J = 0.9, 7.5 Hz); MS(M^+) 249.

5-(3,5-Difluorophenyl)-*N*-methoxy-*N*-methylfuran-2-carboxamide (5): Starting from 5-(3,5-difluorophenyl)-2-carboxylic acid (775.7 mg, 3.46 mmol) **2**, the compound **5** was obtained as a white solid (693.2 mg, 75%) by the same procedure to pre-

Table 1. NHE-1 inhibitory activities of 2-Aminoimidazoles *vs* Acylguanidines.

cariporide			IC_{50} (μM)		
			1.0		
R_1	R_2	Compd		Compd	
3-F	H	**16**	0.2	**31**[9]	1.0
3-F	5-F	**17**	0.5	**32**[9]	0.6
2-OMe	5-Cl	**18**	0.07	**33**[9]	0.06
X					
Br		**29**	0.9	**34**[10]	0.3
CN		**30**	1.0	**35**[10]	3.0

pare **4**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H), 3.82 (s, 3H), 6.77(m, 1H), 6.79 (d, 1H, J = 3.7 Hz), 7.23(d, 1H, J = 3.7 Hz), 7.26-7.32 (m, 2H); MS(M^-) 267.

5-(2-Methoxy-5-chlorophenyl)-*N*-methoxy-*N*-methylfuran-2-carboxamide (6): Starting from 5-(2-methoxy-5-fluorophenyl)- 2-carboxylic acid (2.4 g, 9.5 mmol) **3**, the compound **6** was obtained as a white solid (2.5 g, 89%) by the same procedure to prepare **4**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H), 3.82 (s, 3H), 3.94 (s, 3H), 6.89(d, 1H, J = 8.7 Hz), 7.05 (d, 1H, J = 3.6 Hz), 7.22-7.26 (m, 2H), 7.98 (d, 1H, J = 2.7 Hz); MS (M^-) 295.

4-Bromo-*N*-methoxy-*N*-methylbenzo[*b*]thiophene-2-carboxamide (21): Starting from 4-bromobenzo[*b*]thiophene-2-carboxylic acid (847 mg, 3.29 mmol) **19**, the compound **21** was obtained as a pale yellow solid (296 mg, 28%) by the same procedure to prepare **4**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.45 (s, 3H), 3.85 (s, 3H), 7.30 (m, 1H), 7.59 (d, 1H, J = 7.8 Hz), 7.78 (d, 1H, J = 8.0 Hz), 8.34 (s, 1H); MS(M^-) 301, 299.

4-Cyano-*N*-methoxy-*N*-methylbenzo[*b*]thiophene-2-carboxamide (22): Starting from 4-cyanobenzo[*b*]thiophene-2-carboxylic acid (460 mg, 2.26 mmol) **20**, the compound **22** was obtained as a white solid (327 mg, 59%) by the same procedure to prepare **4**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.47 (s, 3H), 3.87 (s, 3H), 7.53 (dd, 1H, J = 7.6, 8.0 Hz), 7.81 (d, 1H, J = 7.6 Hz), 8.01 (d, 1H, J = 8.0 Hz), 8.43 (s, 1H); MS(M^+) 246.

1-[5-(3-Fluorophenyl)furan-2-yl]propan-1-one (7): A solution of **4** (1.7 g, 6.9 mmol) in THF (18 mL) was cooled to −78 °C, and ethylmagnesium bromide (2 M in THF, 17.3 mL) was slowly added during 1 h with stirring. After the completion of reaction, water (20 mL) was added, and the mixture was extracted with ethyl acetate (60 mL) twice. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 10:1) to yield **7** as an oil (1.03 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 3H, J = 7.4 Hz), 2.92 (q, 2H, J = 7.4 Hz), 6.79 (d, 1H, J = 3.7 Hz), 7.06 (m, 1H), 7.25(d, 1H, J = 3.7 Hz), 7.39-7.49 (m, 2H), 7.55 (m, 1H); MS(M^-) 218.

1-[5-(3,5-Difluorophenyl)furan-2-yl]propan-1-one (8): Starting from **5** (667.2 mg, 2.5 mmol), the compound **8** was obtained as a pale yellow solid (500.7 mg, 85%) by the same procedure to prepare **7**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 3H, J = 7.2 Hz), 2.92 (q, 2H, J = 7.2 Hz), 6.77-6.85 (m, 2H), 7.24-7.32 (m, 3H); MS(M^-) 236.

1-[5-(2-Methoxy-5-chlorophenyl)furan-2-yl]propan-1-one (9): Starting from **6** (2.5 g, 8.5 mmol), the compound **9** was obtained as a pale green solid (1.75 g, 78%) by the same procedure to prepare **7**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 3H, J = 7.3 Hz), 3.94 (s, 3H), 6.90 (d, 1H, J = 5.6 Hz), 7.07 (d, 1H, J = 3.7 Hz), 7.23-7.33 (m, 2H), 7.94 (d, 1H, J = 2.6 Hz); MS(M^-) 264.

1-(4-Bromobenzo[*b*]thiophen-2-yl)propan-1-one (23): Starting from **21** (539 mg, 1.80 mmol), the compound **23** was obtained as a white solid (361 mg, 75%) by the same procedure to prepare **7**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, 3H, J = 7.2 Hz), 3.09 (q, 2H, J = 7.2 Hz), 7.33 (d, 1H, J = 8.2 Hz), 7.58 (dd, 1H, J = 7.6, 8.2 Hz), 7.79 (d, 1H, J = 7.6 Hz), 8.07 (s, 1H); MS (M^-) 270, 268.

1-(4-Cyanobenzo[*b*]thiophen-2-yl)propan-1-one (24): Starting from **22** (325 mg, 1.32 mmol), the compound **24** was obtained as a white solid (185 mg, 65%) by the same procedure to prepare **7**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (*t*, 3H, J = 7.4 Hz), 3.13 (q, 2H, J = 7.4 Hz), 7.35 (dd, 1H, J = 7.2, 8.4 Hz), 7.99 (d, 1H, J = 7.2 Hz), 8.11 (d, 1H, J = 8.4 Hz), 8.14 (s, 1H); MS (M^-) 215.

2-Bromo-1-[5-(3-fluorophenyl)furan-2-yl]propan-1-one (10): To a solution of **7** (1.03 g, 4.7 mmol) in CH_2Cl_2 (15 mL) was added Br_2 at 0 °C, and the reaction mixture was stirred for an hr at rt. After the completion of reaction, water (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (50 mL) twice. The organic layer was washed with saturated $NaHCO_3$ solution and then brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 10:1) to yield **10** as a pale yellow solid (904 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.91 (d, 3H, J = 6.9 Hz), 5.20 (q, 1H, J = 6.9 Hz), 6.84 (d, 1H, J = 3.7 Hz), 7.09 (m, 1H), 7.41-7.51(m, 3H), 7.58 (dd, 1H, J = 6.6 Hz); MS(M^+) 298, 296.

2-Bromo-1-[5-(3,5-difluorophenyl)furan-2-yl]propan-1-one (11): Starting from **8** (450 mg, 1.9 mmol), the compound **11** was obtained as a pale yellow solid (480 mg, 80%) by the same procedure to prepare **10**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.91 (d, 3H, J = 6.9 Hz), 5.18 (q, 2H, J = 6.9 Hz), 6.80-6.88 (m, 2H), 7.30 (dd, 2H, J = 2.1, 8.1 Hz), 7.41 (d, 1H, J = 3.9 Hz); MS(M^+) 316, 314.

2-Bromo-1-[5-(2-methoxy-5-chlorophenyl)furan-2-yl]propan-1-one (12): Starting from **9** (1.03 g, 3.9 mmol), the compound **12** was obtained as a pale yellow solid (803 mg, 60%) by the same procedure to prepare **10**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.91 (d, 3H, J = 6.8 Hz), 5.22 (q, 1H, J = 6.8 Hz), 6.93 (d, 1H, J = 8.9 Hz), 7.12 (d, 1H, J = 3.8 Hz), 7.30 (dd, 2H, J = 2.6, 8.9 Hz), 7.94 (d, 1H, J = 2.6 Hz); MS(M^+) 344, 342.

2-Bromo-1-(4-bromobenzo[*b*]thiophen-2-yl)propan-1-one (25): Starting from **23** (275 mg, 1.02 mmol), the compound **25** was obtained as a pale yellow solid (286 mg, 86%) by the same procedure to prepare **10**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.97 (d, 3H, J = 6.8 Hz), 5.33 (q, 1H, J = 6.8 Hz), 7.35 (m, 1H), 7.62 (d, 1H, J = 7.2 Hz), 7.82 (d, 1H, J = 8.4 Hz), 8.21 (s, 1H); MS (M^-) 348.

2-Bromo-1-(4-cyanobenzo[*b*]thiophen-2-yl)propan-1-one (26): Starting from **24** (166 mg, 0.77 mmol), the compound **26** was obtained as a pale yellow solid (223 mg, 98%) by the same procedure to prepare **10**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.98 (*d*, 3H, J = 6.9 Hz), 3.28 (q, 1H, J = 6.9 Hz), 7.56 (dd, 1H, J = 7.8, 8.0 Hz), 7.82 (d, 1H, J = 8.0 Hz), 8.12 (d, 1H, J = 8.0 Hz), 8.26 (s, 1H); MS(M^-) 295, 293.

4-[5-(3-Fluorophenyl)furan-2-yl]-2-acetylamino-5-methylimidazole (13): To a solution of **10** (500 mg, 1.68 mmol) in DMF (7 mL) was added 1-acetylguanidine (510.4 mg, 5.05 mmol), and the reaction mixture was stirred at 60 °C for 12 hr. After the completion of reaction, water (5 mL) was added, and the mixture was extracted with ethyl acetate (50 mL) twice. The organic layer was washed with saturated $NaHCO_3$ solution and then brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to yield **13** as a pale yellow solid (196.5 mg,

39%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.14 (s, 3H), 2.50 (s, 3H), 6.51 (d, 1H, J = 3.5 Hz), 6.74 (d, 1H, J = 3.5 Hz), 6.95 (m, 1H), 7.30-7.38 (m, 1H), 7.42-7.47 (m, 2H); MS(M^-) 299.

4-[5-(3,5-Difluorophenyl)furan-2-yl]-2-acetylaminoamino-5-methylimidazole (14): Starting from **11** (460 mg, 1.46 mmol), the compound **14** was obtained as a pale yellow solid (181.2 mg, 39%) by the same procedure to prepare **13**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.13 (s, 3H), 2.51 (s, 3H), 6.50 (d, 1H, J = 3.6 Hz), 6.68 (m, 1H), 6.76 (d, 1H, J = 3.6 Hz), 7.17 (dd, 2H, J = 2.1, 8.7 Hz); MS(M^+) 317.

4-[5-(2-Methoxy-5-chlorophenyl)furan-2-yl]-2-acetylaminoamino-5-methylimidazole (15): Starting from **12** (150 mg, 0.44 mmol), the compound **15** was obtained as a pale yellow solid (61.5 mg, 41%) by the same procedure to prepare **13**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.24 (s, 3H), 2.48 (3, 3H), 3.94 (s, 3H), 6.50 (d, 1H, J = 3.5 Hz), 6.87 (d, 1H, J = 8.8 Hz), 7.04 (d, 1H, J = 3.5 Hz), 7.16 (dd, 2H, J = 2.6, 8.8 Hz), 7.95 (brs, 1H); MS(M^-) 345.

4-(4-Bromobenzo[*b*]thiophen-2-yl)-2-acetylaminoamino-5-methylimidazole (27): Starting from **25** (260 mg, 0.804 mmol), the compound **27** was obtained as a brown solid (100 mg, 36%) by the same procedure to prepare **13**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.14 (s, 3H), 2.55 (s, 3H), 7.15 (m, 1H), 7.46 (s, 1H), 7.52 (d, 1H, J = 8.0 Hz), 7.75 (d, 1H, J = 8.4 Hz); MS(M^-) 349, 351.

4-(4-Cyanobenzo[*b*]thiophen-2-yl)-2-acetylaminoamino-5-methylimidazole (28): Starting from **26** (220 mg, 0.748 mmol), the compound **28** was obtained as a pale yellow solid (64 mg, 29%) by the same procedure to prepare **13**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.14 (*s*, 3H), 2.57 (s, 3H), 7.34 (dd, 1H, J = 7.6, 8.2 Hz), 7.55 (s, 1H), 7.68 (d, 1H, J = 7.6 Hz), 7.99 (d, 1H, J = 8.2 Hz), 8.04 (s, 1H), 10.76 (brs, 1H); MS(M^+) 296.

4-[5-(3-Fluorophenyl)furan-2-yl]-2-amino-5-methylimidazole methanesulfonate (16): The compound **13** (160 mg, 0.53 mmol) was dissolved in methanol (3 mL), and 8 N HCl (1.5 mL) was added. The reaction mixture was heated at reflux with stirring for 4 hr, and cooled to rt, following the basification with 6 N NaOH to pH 10, and extraction with ethyl acetate (30 mL) twice. The organic layer was washed brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20% methanol in CH_2Cl_2), which was dissolved in acetone (4 mL), treated with methanesulfonic acid with stirring, and cooled to 0 °C. The resulting precipitates were filtered to yield **16** as a pale brown solid. (38.5 mg, 21%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44 (s, 3H), 2.73 (s, 3H), 6.71 (d, 1H, J = 3.6 Hz), 7.00 (d, 1H, J = 3.6 Hz), 6.95 (m, 1H), 7.02-7.08 (m, 1H), 7.41-7.51 (m, 2H), 7.58 (d, 1H, J = 7.9 Hz); MS(M^+) 257.

4-[5-(3,5-Difluorophenyl)furan-2-yl]-2-amino-5-methylimidazole (17): Starting from **14** (139.6 mg, 0.44 mmol), the compound **17** was obtained as a pale yellow solid (63.9 mg, 53%) by the same procedure to prepare **16**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.33 (s, 3H), 4.68-4.74 (brm, 3H), 6.36 (d, 1H, J = 3.3 Hz), 6.58 (m, 1H), 6.62 (d, 1H, J = 3.3 Hz), 6.99 (d, 2H, J = 6.6 Hz); MS(M^+) 275.

4-[5-(2-Methoxy-5-chlorophenyl)furan-2-yl]-2-amino-5-methylimidazole methanesulfonate (18): Starting from **15** (146 mg, 0.42 mmol), the compound **18** was obtained as a white solid (26 mg, 16%) by the same procedure to prepare **16**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H), 2.73 (3, 3H), 3.99 (s, 3H), 6.70 (d, 1H, J = 3.6 Hz), 7.07-7.12 (m, 2H), 7.28 (dd, 1H, J = 2.6, 8.8 Hz), 7.86 (d, 2H, J = 2.6); MS(M^+) 303.

4-(4-Bromobenzo[*b*]thiophen-2-yl)-2-amino-5-methylimidazole (29): Starting from **27** (90 mg, 0.257 mmol), the compound **29** was obtained as a pale brown solid (63 mg, 80%) by the same procedure to prepare **16**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H), 7.18 (dd, 1H, J = 7.8, 7.8 Hz), 7.40 (s, 1H), 7.55 (d, 1H, J = 7.8 Hz), 7.83 (d, 1H, J = 7.8 Hz); MS(M^-) 309, 307.

4-(4-Cyanobenzo[*b*]thiophen-2-yl)-2-amino-5-methylimidazole methanesulfonate (30): Starting from **28** (60 mg, 0.202 mmol), the compound **30** was obtained as a pale yellow solid (30 mg, 42%) by the same procedure to prepare **18**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.33 (*s*, 3H), 7.58 (dd, 1H, J = 6.0, 9.0 Hz), 7.72 (s, 2H), 7.85 (s, 1H), 7.96 (d, 1H, J = 6.0 Hz), 8.39 (d, 1H, J = 9.0 Hz), 12.64 (brs, 1H); MS(M^+) 254.

Biology. Inhibitory effect on NHE-1: Na^+/H^+-exchanger (NHE-1) inhibitory activity was used as our primary screen and measured by the rate of NHE-1 mediated recovery of intracellular pH (pH*i*) in a 96-well microplate using a pH sensitive fluorescent dye, 2',7'-bis-2-carboxyethyl-5-(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM, Sigma- Aldrich Co., MO, USA).[23,24] PS120 Fibroblast cells expressing human NHE-1 were obtained from Professor J. Pouyssegur (Nice, France),[20] and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% penicillin/ streptomycin (100X solution), 1% L-glutamine (200 mM solution), and 10% fetal bovine serum at 37 °C, in humidified atmosphere of 5% CO_2 and 95% air. Cells were grown to confluency and then harvested from 100 mm Culture dishes using Trypsin-EDTA solution. Cells were washed twice with Na-free buffer (138.2 mM choline chloride, 4.9 mM KCl, 1.5 mM $CaCl_2.2H_2O$, 1.2 mM $MgSO_4{\cdot}7H_2O$, 1.2 mM KH_2PO_4, 15 mM D-glucose, 20 mM HEPES, at pH 7.4) and incubated with 10 μM of BCECF-AM and 20 mM NH_4Cl at 37 °C for 30 min. The BCECF- and NH_4Cl-loaded cells were then washed, resuspended in Na-free buffer, and kept on ice.

The 10 μL of the BCECF- and acid-loaded cells (2.5×10^4 cells) was added to 180 μL of HBS buffer containing NaCl (137 mM NaCl, 4.9 mM KCl, 1.5 mM $CaCl_2.2H_2O$, 1.2 mM $MgSO_4{\cdot}7H_2O$, 1.2 mM KH_2PO_4, 15 mM D-glucose, 20 mM HEPES, pH 7.4) with 10 μL of DMSO or compounds in 96-well microplate. NHE activity was initiated by NaCl, and fluorescence readings were taken 4 min after the addition of acidified cell to each microplate containing NaCl, at 444 nm excitation/ 535 nm emission and also at 485 nm excitation/535 nm emission using a spectrofluorometer (GEMINI-XS, Molecular Device, CA, USA) at room temperature. Activity in the absence of NaCl was subtracted from the activity in the presence of NaCl. The initial increase in pH*i* in response to NaCl was taken as an estimate of Na^+/H^+-exchange activity, and the inhibitory effect of the compounds was evaluated as IC_{50} value (concentration required to inhibit pH*i* recovery by 50%). Calibration of pH*i* was accomplished using the high K^+- nigericin method.[25] The BCECF fluorescence ratio (485/444) was plotted against pH*i* and fitted by linear regression.

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