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Bioorganic & Medicinal Chemistry Letters 15 (2005) 2998–3001
Bioorganic & Medicinal Chemistry Letters

4-Substituted (benzo[*b*]thiophene-2-carbonyl)guanidines as novel Na^+/H^+ exchanger isoform-1 (NHE-1) inhibitors

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Received 21 February 2005; revised 15 April 2005; accepted 22 April 2005

Abstract—A series of 4-substituted (benzo[*b*]thiophene-2-carbonyl)guanidines was synthesized and evaluated for the NHE-1 inhibitory activity and cardioprotective efficacy both in vitro and in vivo. Several analogs exhibited a strong inhibition on NHE-1, and which was generally well correlated with their cardioprotective efficacy. Especially the 4-nitro **20** and cyano **50** compounds excellently improved the cardiac function and reduced infarct size against ischemia/reperfusion injury.

Since the excessive activation of Na^+/H^+ exchanger isoform-1 (NHE-1) has been known to play an important role in the progression of ischemia/reperfusion injury,[1,2] many efforts have been devoted to develop a potent and selective NHE-1 inhibitor as cardioprotective drug. Through the replacement of a pyrazine ring of amiloride[3] known as a non-selective NHE-1 inhibitor, several monocyclic acylguanidines such as cariporide,[4] eniporide, and zoniporide[5] have been identified as the selective and potent NHE-1 inhibitors. Later, several compounds based on a bicyclic template including a quinoline, indole, benzoxazinone, tetrahydronaphthalene, or tetrahydrocyclohepta-pyridine have been designed with the aim to discover more potent and highly water-soluble inhibitor.[6,7] In our preliminary study to identify a novel NHE-1 inhibitor, a variety of bicyclic acylguanidines were investigated, and (4-bromobenzo[*b*]thiophene-2-carbonyl)guanidine **15** was found as a potent NHE-1 inhibitor. Starting with **15**, a series of (benzo[*b*]thiophene-2-carbonyl)guanidines with various 4-substituents were synthesized, and evaluated for the NHE-1 inhibitory activity and cardioprotective efficacy.

As a stating point, 4-substituted benzo[*b*]thiophene-2-carboxylates **6**–**10** were prepared by a simple 2-step procedure (Scheme 1).[8] A regioselective lithiation[9] of 3-substituted fluorobenzene using lithium diisopropylamide (LDA), followed by formylation with dimethylformamide (DMF) provided 6-substituted 2-fluorobenzaldehydes **1**–**5**. Subsequent treatment of **1**–**5** with thioglycolate and K_2CO_3 in DMF gave **6**–**10** through the nucleophilic displacement of aromatic fluorine and base-induced ring-closure in a single step. Based on the rate of nucleophilic displacement ($F > NO_2 > Cl > Br$)[10] and an enhanced effect of carboxaldehyde function on the displacement lability, the fluorine of **1**–**5** was displaced with thioglycolate in mild condition. The 4-nitro **11** and chloro **12** compounds were similarly prepared from 2,6-dinitro- and 2-chloro-6-nitrobenzaldehyde, respectively. The 4-amino compound **13** was obtained by Raney-nickel catalyzed hydrogenation of the nitro compound **11**. In this case, Pd-catalyzed reduction did not proceed, presumably due to sulfur of benzothiophene. Commercially available 4-trifluoromethyl compound **14** was also utilized.

The 4-bromine of **6** was further converted to the alkyl, cyano, and aryl functions (Scheme 2). The alkyl and aryl substituted compounds were obtained by a Pd-catalyzed cross coupling reaction. While the methyl **24** and aryl compounds **29**–**45** were synthesized via the Suzuki reaction,[11] the vinyl compound **25** was prepared employing the Stille condition.[12] Raney-nickel catalyzed hydrogenation of **25** provided the 4-ethyl compound **26**.

Keywords: Sodium hydrogen exchanger; (Benzo[*b*]thiophen-2-carbonyl)guanidine; Cardioprotective.
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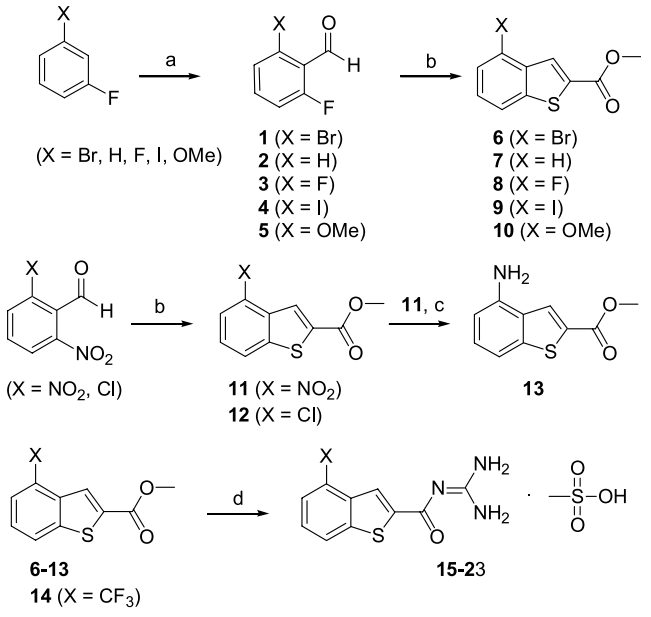
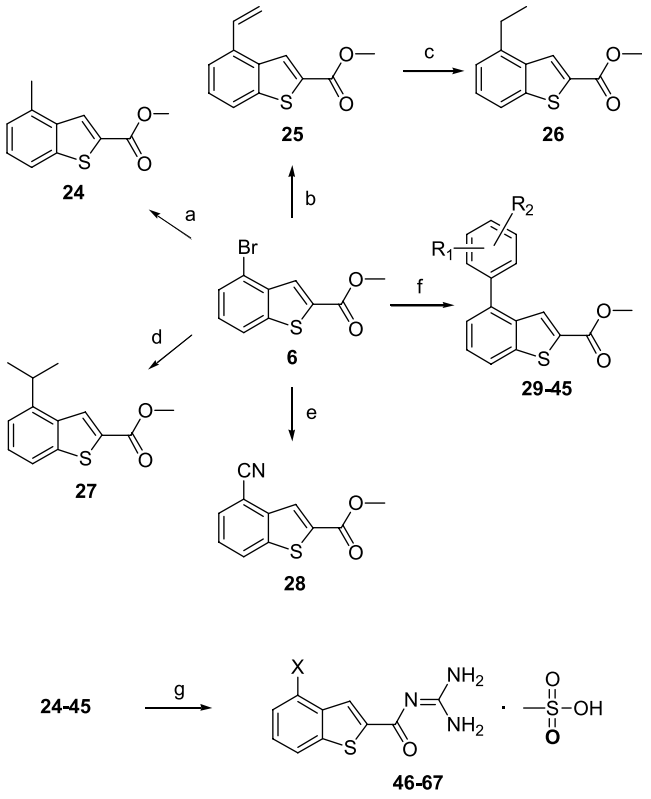
doi:10.1016/j.bmcl.2005.04.046



Scheme 1. Reagents and conditions: (a) (i) LDA, THF, −78 °C, (ii) DMF, 60–95%; (b) methyl thioglycolate, K_2CO_3, DMF, 65–95%; (c) 40 psi H_2, 50% Raney-Ni, CH_3OH, rt, 98%; (d) (i) guanidine, DMF, rt, (ii) CH_3SO_3H, acetone, 50–80%.



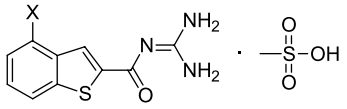
Scheme 2. Reagents and conditions: (a) trimethylboroxine, $Pd(PPh_3)_4$, K_2CO_3, DMF, reflux, 92%; (b) tributyl(vinyl)tin, $Pd(PPh_3)_4$, toluene, reflux, 61%; (c) 40 psi H_2, 50% Raney-Ni, CH_3OH, rt, 72%; (d) (i) isopropylmagnesium chloride, $ZnCl_2$, THF, 50 °C, (ii) **6**, CuI, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, THF, rt, 69%; (e) CuCN, microwave, DMF, 200 °C, 20 min, 80%; (f) arylboronic acid, $Pd(PPh_3)_4$, $Ba(OH)_2{\cdot}H_2O$, or K_2CO_3, toluene or DME, reflux, 60–95%; (g) (i) guanidine, DMF, rt, (ii) CH_3SO_3H, acetone, 50–90%.

The treatment of **6** with isopropylzinc slurry and copper(I) catalyst as well as Pd(0) catalyst afforded the isopropyl compound **27**.[13] The nitrile compound **28** was obtained by microwave-promoted nucleophilic substitution using CuCN in DMF from **6** in 20 min.

(Benzo[*b*]thiophene-2-carbonyl)guanidines (**15**–**23**, **46**–**67**) were prepared from the corresponding carboxylic esters by treating with excess guanidine (free base) in methanol or in DMF. The final acylguanidines were purified by crystallization as a methanesulfonic acid salt.

The NHE-1 inhibitory activity of the synthesized compounds was determined by measuring their ability to inhibit the sodium dependent recovery of pH following an imposed acidosis, in PS120 variant cells in which the human NHE-1 was selectively expressed.[14] Primarily, the IC_{50} values of 4-, 5-, and 6-bromobenzo[*b*]thiophene compounds were determined as 0.20, 12, and 16 μM, respectively. The 4-bromo compound **15** exhibited a higher potency on NHE-1 than cariporide (IC_{50} = 0.68 μM). Then various substituents were introduced at the 4-position to optimize the activity of this series of compounds

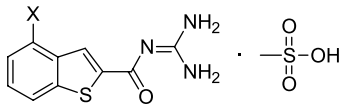
Table 1. Inhibitory effect on NHE-1 of compounds **15**–**23** and **46**–**67**



Compds	X	IC_{50}, μM[a]
Cariporide		0.68
15	Br	0.20
16	H	12
17	F	1.7
18	I	0.17
19	OMe	3.4
20	NO_2	2.3
21	Cl	0.20
22	NH_2	>30
23	CF_3	0.40
46	Me	0.48
47	Vinyl	0.31
48	Et	0.27
49	*i*-Pr	0.85
50	CN	2.0
51	Ph	8.4
52	2-F–Ph	11
53	3-F–Ph	5.6
54	4-F–Ph	3.9
55	2-Cl–Ph	4.2
56	3-Cl–Ph	2.5
57	4-Cl–Ph	>30
58	2-Me–Ph	7.7
59	3-Me–Ph	3.4
60	4-Me–Ph	>30
61	3-OMe–Ph	11
62	3-CF_3	4.4
63	2,3-diF–Ph	8.9
64	3,5-diF–Ph	5.1
65	2,5-diF–Ph	8.6
66	3,5-diCl–Ph	0.68
67	3-Cl-4-F–Ph	2.5

[a] n = 3.

Table 2. Cardioprotective efficacy against ischemia–reperfusion injury



Compds	X	Langendorff[a]			In vivo[b] IS/AAR (%)
		RPP (%)	LVEDP (mmHg)	LDH (IU/g)	
Control		13 ± 1	55 ± 2	28 ± 2	59 ± 2
Cariporide		44 ± 4	44 ± 3	12 ± 2	41 ± 2
15	Br	55 ± 13	34 ± 2	na[c]	46 ± 7
17	F	47 ± 12	38 ± 7	7.0 ± 3	45 ± 2
18	I	66 ± 13	19 ± 3	7.1 ± 3	49 ± 4
20	NO_2	73 ± 8	11 ± 4	11 ± 2	36 ± 5
21	Cl	61 ± 17	25 ± 11	9 ± 1	48 ± 4
23	CF_3	81 ± 17	6 ± 4	5 ± 3	45 ± 3
46	Me	40 ± 4	38 ± 3	17 ± 2	na[c]
47	vinyl	51 ± 23	42 ± 9	13 ± 1	na[c]
48	Et	43 ± 13	38 ± 9	7 ± 3	52 ± 2
50	CN	63 ± 6	19 ± 6	13 ± 2	38 ± 4
66	3,5-diCl–Ph	19 ± 7	64 ± 8	na[c]	na[c]
67	3-Cl–4-F–Ph	6 ± 1	68 ± 9	26 ± 2	na[c]

[a] In vitro cardioprotective effect was evaluated by measuring % RPP (LVDP × HR) to pre-ischemic value, LVEDP, and LDH release in the isolated ischemic rat heart (10 μM). Values are means ± SEM, $n = 3$ 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 ± SEM, $n = 3$ or higher.

[c] na: not assayed.

(Table 1). The unsubstituted analog **16** (IC_{50} = 12 μM) represented far less activity than **15**. The 4-iodo **18** (IC_{50} = 0.17 μM), chloro **21** (IC_{50} = 0.20 μM), and trifluoromethyl **23** (IC_{50} = 0.40 μM) compounds were highly active, similarly to **15**. While the 4-amino compound **22** was inactive, the fluoro **17**, methoxy **19**, nitro **20**, and nitrile **50** compounds maintained an activity. The 4-alkyl substituted compounds **46**–**49** demonstrated a good activity. The potency was increased from methyl **46**, vinyl **47**, to ethyl **48**, but decreased by the substitution with isopropyl group **49**. Presumably there may be an optimum size for the activity. Additionally the 4-aryl substituted compounds were investigated. The 4-phenyl compound **51** exhibited a moderate activity (IC_{50} = 8.4 μM). The meta substituted phenyl analogs of **51** appeared to be more active than the other substituted aryl compounds, but were not as good as the halogen or alkyl substituted compounds. The 3,5-dichlorophenyl compound **66** showed an excellent activity (IC_{50} = 0.68 μM), which consistently indicates that the meta substitution at the phenyl ring may be beneficial to improve the activity compared with the other substitution patterns.

Next, the compounds showing a good NHE-1 inhibitory activity were evaluated for their cardioprotective efficacy against ischemia/reperfusion injury both in vitro and in vivo (Table 2). In the isolated rat heart model,[15] each isolated rat heart was treated with 10 μM concentration of the compound for 10 min, and subjected to 30 min global ischemia, followed by 30 min reperfusion. For the evaluation of their cardioprotective effect, the % recovery of rate pressure product (RPP, HR × LVDP, heart rate × left ventricular developing pressure) at the end of reperfusion to the pre-ischemic value was measured as an index of cardiac contractile function. Additionally left ventricular end diastolic pressure (LVEDP) as an indicator of cardiac contracture, and lactate dehydrogenase (LDH) released into the effluent perfusate during reperfusion as an indicator of myocyte injury were determined. In this model, cariporide significantly improved the recovery of contractile function (44% RPP), diminished LVEDP (44 mmHg), and reduced the release of LDH (12 IU/g), compared with the vehicle group (13% RPP, 55 mmHg LVEDP, and 28 IU/g LDH release). Cardioprotective in vivo efficacy was determined by measuring a ratio of myocardial infarction size to area at risk (IS/AAR), using rat myocardial infarction model,[16] which was stabilized for 20 min after a left thoracotomy operation, subjected to 45 min coronary artery occlusion, following 90 min reperfusion. The compounds (0.1 mg/kg) were administered by bolus intravenous injection at 5 min before onset of ischemia. In this in vivo rat myocardial infarct model, cariporide represented 41% of IS/AAR, which indicates the significant reduction of the infarct size compared with the vehicle group (59% of IS/AAR).

The aryl substituted compounds **66**, **67** did not show any improvement of cardiac dysfunction in isolated rat heart model regardless their strong inhibition on NHE-1. However, the other potent analogs significantly improved the cardiac function and reduced myocyte damage against ischemia/reperfusion injury, which was comparable to cariporide. Especially the iodo **18**, nitro **20**, chloro **21**, trifluoromethyl **23**, and cyano **50** compounds, excellently recovered the cardiac contractility above the 60% of basal RPP value as well as the significant attenuation of cardiac contracture and protection of myocyte damage in the isolated rat heart ischemia model. Furthermore, the nitro **20** and cyano **50** compounds

greatly limited the infarct size (36% and 38% IS/AAR) in the in vivo rat myocardial infarction model.

In summary, a series of benzo[*b*]thiophene-2-carbonylguanidine analogs with various 4-substituents including halogen, nitrile, nitro, amine, alkyl, and aryl group, were synthesized and evaluated for their in vitro and in vivo cardioprotective efficacy in addition to the NHE-1 inhibitory activity. The 4-nitro **20** and 4-cyano **50** compounds excellently improved the cardiac function and reduced infarct size against ischemia/reperfusion injury as well as strong inhibition on NHE-1. We will continuously study on this series of compounds including their advanced pharmacology, pharmacokinetic and safety profiles.

Acknowledgments

This research was supported by a grant (CBM-01-A100-001-1-0-0) from the Center for Biological Modulators of the 21st Century Frontier R&D program, the Ministry of Science and Technology, Korea.

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