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Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 177–180

Aminoimidazoles as bioisosteres of acylguanidines: novel, potent, selective and orally bioavailable inhibitors of the sodium hydrogen exchanger isoform-1

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Received 2 July 2003; revised 17 September 2003; accepted 27 September 2003

Abstract—Inhibition of the sodium hydrogen exchanger isoform-1 (NHE-1) has been shown to limit damage to the myocardium under ischemic conditions in animals. While most known NHE-1 inhibitors are acylguanidines, this report describes the design and synthesis of a series of heterocyclic inhibitors of NHE-1 including aminoimidazoles with undiminished in vitro activity and oral bioavailability.

The sodium hydrogen exchanger (NHE) isoforms are integral plasma membrane proteins that exchange extracellular sodium ions for intracellular protons. Currently there are at least six known isoforms of NHE. NHE-1 is ubiquitous and it plays a role in maintaining cellular pH, intracellular sodium ion concentration and cell volume. NHE-2 is present in all three major gastric epithelial cell types, and is expressed in the small intestine, colon and kidney.[1] It is believed to be involved in the regulation of acid secretion by the stomach.[2] NHE-3 is primarily found in renal epithelia, localized to the apical membrane, where it has been implicated in the absorption of sodium.[3] NHE-4 is found in the stomach[4] and the collecting tubule of the renal inner medulla, where it has been proposed to play a specialized role in volume regulation.[5] NHE-5 is present in a select number of non-epithelial tissues, including brain, spleen and skeletal muscle and NHE-6 is found in mitochondria. The roles of NHE-5 and NHE-6 are unknown.

Intracellular pH changes have been implicated in a variety of pathophysiological conditions including hypertension and myocardial ischemia. NHE-1 is the predominant isoform in myocardial cells and is believed to be responsible for exchanging intracellular protons generated by anaerobic metabolism for extracellular sodium ions during ischemia.[6] It is implicated in the increase in intracellular sodium that underlies calcium overload and contractile dysfunction observed during ischemia and reperfusion. NHE-1 inhibition limits ischemic damage by indirectly inhibiting calcium overload.[7] A potential major advantage of an NHE-1 inhibitor compared to other therapies is that since NHE-1 is believed to be inactive in the normal myocardium, any effects due to inhibition of NHE-1 should be specific for the ischemic region. Several NHE-1 inhibitors with an acylguanidine group are currently undergoing clinical development for various indications including myocardial infarction, ischemic heart disease and angina (Fig. 1). Among them are Cariporide (Hoechst Marion Roussel),[8] Sabiporide (Boehringer Ingelheim)[9] and Zoniporide (Pfizer).[10]

Most known NHE inhibitors contain an acylguanidine group.[11] It has been suggested that a charged acylguanidine group at physiological pH may mimic a sodium ion at the binding site of the exchanger.[12] We had previously reported a series of cyclopropyl acylguanidines represented by compounds **1a** (BMS-284640), **1b** and **1c** as potent and selective inhibitors of NHE-1.[13] During the course of our efforts to discover novel inhibitors of NHE-1, we synthesized a series of analogues of these

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doi:10.1016/j.bmcl.2003.09.066

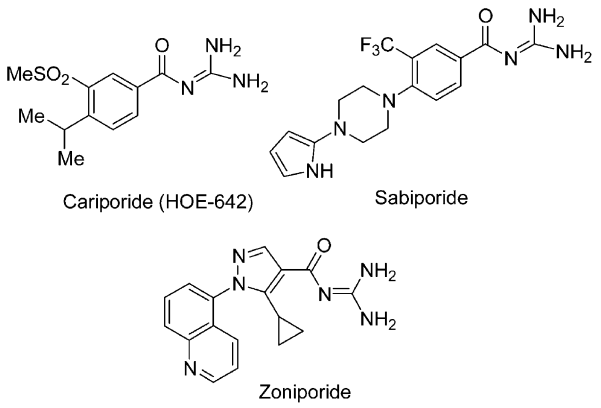


Figure 1.

compounds with heterocyclic rings replacing the acylguanidine functionality (Fig. 2). The selection of heterocycles included various five- and six-membered rings with a wide range of pK_a values including rings with pK_a values similar to that of the acylguanidine group. Herein we describe a summary of these efforts that lead to the discovery of aminoimidazoles as potent, selective and orally bioavailable inhibitors of NHE-1.

Synthesis of the various heterocyclic analogues was carried out as outlined in Schemes 1–3. The aminooxadiazole **3** was prepared by treating the cyclopropyl ester **2**[13] with hydroxyguanidine in refluxing methanol. The aminothiazoles **10a–c** were synthesized from the cyclopropyl acids **4a–c**.[13] Thus, treatment of **4a–c** with *N*,*O*-dimethylhydroxylamine hydrochloride in the presence of carbonyldiimidazole (CDI) in THF afforded the corresponding Wienreb amides **5a–c**, which were converted to the ketones **6a–c** and **7a–c** by treatment with methyl- or ethylmagnesiun bromides in THF. Conversion of the ketones to the corresponding α-bromoketones **8a–c** and **9a–c** was carried out by sequential treatment with lithium bis-trimethylsilylamide and *N*-bromosuccinimide in THF at −78 °C. The aminothiazoles **10a–c** were prepared in 70–80% yield by treating the corresponding α-bromoketones with thiourea in refluxing methanol.

Synthesis of the aminopyrazole **13** and diaminopyrimidine **14** is outlined in Scheme 2. The α-cyanoketone **11** was prepared by treating the amide **5c** with lithium anion of acetonitrile at −78 °C. Treatment of **11** with diazomethane in ether at room temperature for 3 days

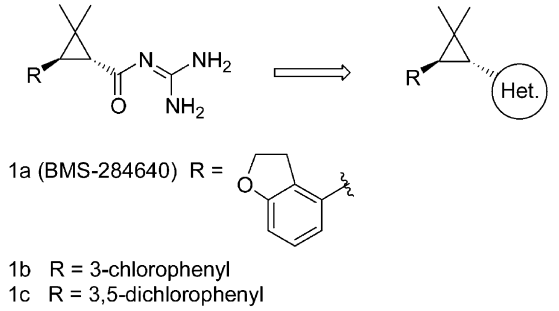
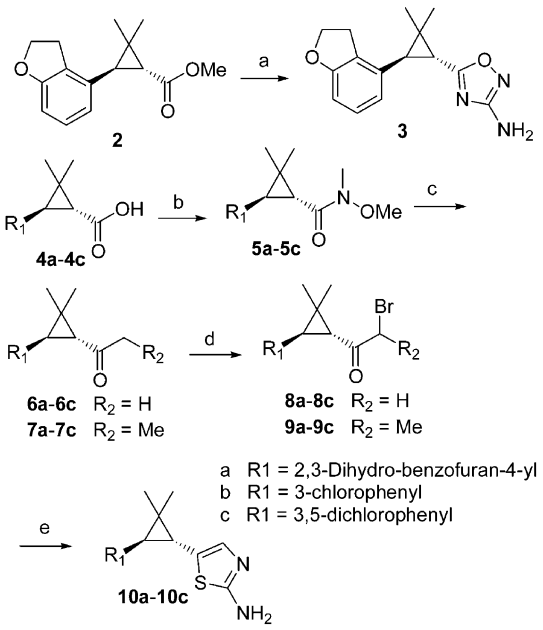


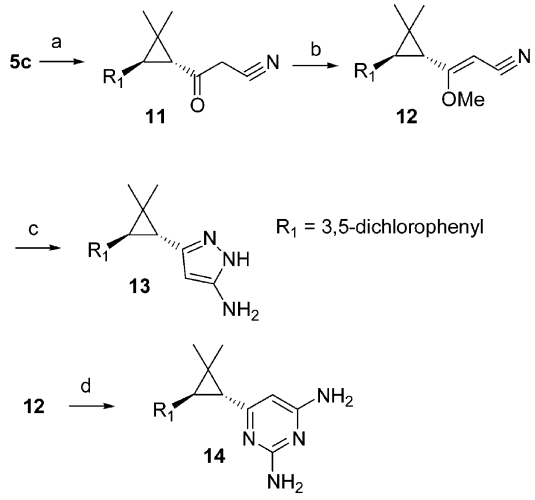
Figure 2.



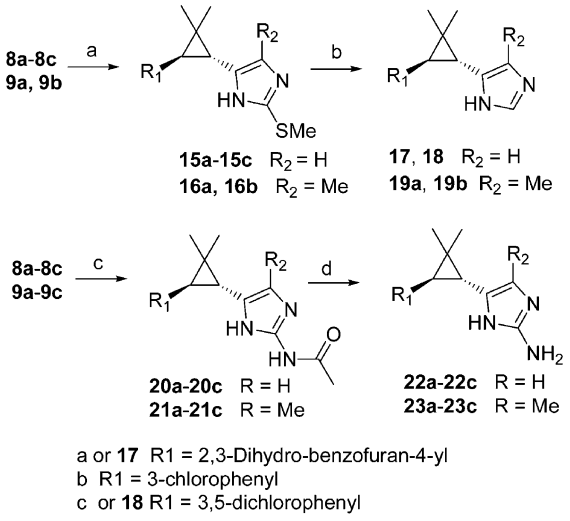
Scheme 1. (a) Hydroxyguanidine/methanol/reflux/65% yield; (b) CDI/CH_3ONHCH_3/THF/80–85% yield; (c) R_2CH_2MgBr/THF/−78 °C/80–85% yield; (d) lithium bis-trimethylsilylamide/*N*-bromosuccinimide/THF/−78 °C/70–75% yield; (e) thiourea/methanol reflux/70–80% yield.

afforded the methyl ether **12** (quantitative yield) which was converted to the aminopyrazole **13** or the diaminopyrimidine **14**, respectively, by reactions with hydrazine or guanidine (Scheme 2).

Synthesis of the various imidazole derivatives is outlined in Scheme 3. Treatment of the α-bromo ketones with *S*-methylisothiouronium sulfate in refluxing ethanol in the presence of stoichiometric amounts of sodium ethoxide afforded the methylthioimidazole derivatives **15a–c**, **16a** and **b** in 20–30% yield. These compounds were readily converted to the imidazole analogues **17**, **18**, **19a** and **19b** via reaction with Ra–Ni in methanol in 80–85% yield. The aminoimidazole analogues **22a–c** and **23a–c** were



Scheme 2. (a) CH_3CN/lithium bis-(trimethylsilyl)amide/−78 °C/90–95%; (b) CH_2N_2/ether/3 days at RT; (c) hydrazine; (d) 5 equivalents guanidine hydrochloride/equivalents potassium *t*-butoxide/ethanol/heat/5% yield over two steps.



Scheme 3. (a) *S*-Methylisothiourea/ethanol/reflux/20–30% yield; (b) Ra-Ni/methanol/80–85% yield; (c) acetylguanidine/DMF/60 °C/30–50% yield; (d) catalytic sulfuric acid/MeOH/water/75–80% yield.

similarly prepared from the corresponding α-bromoketones via the acetamidoimidazoles **20a–c** and **21a–c** by treatment with acetylguanidine in DMF at 60 °C followed by hydrolytic cleavage of the acetyl group using catalytic sulfuric acid in methanol–water.[14]

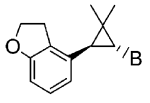
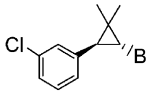
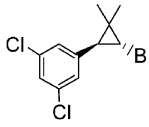
The various heterocyclic analogues described here were evaluated for NHE-1 inhibitory activity in AP1 cell line (devoid of NHE activity) expressing the human NHE isoform-1.[13,15] The IC_{50} values (see Table 1) were determined by measuring the ability of compounds to inhibit 50% of the sodium dependent recovery of pH following an imposed acidosis.[16] As described previously,[13] using this protocol the IC_{50} values for cariporide and eniporide were measured as 3.4 and 0.38 μM, respectively.

The aminooxadiazole group has been used previously as a bio-isostere of the acylguanidine group.[17] However, replacement of the acylguanidine group of the lead compound BMS-284640 (compound **1a**, $IC_{50} = 0.009$ μM) with this heterocycle resulted in the loss of NHE inhibitory activity (compound **3**, $IC_{50} = 30$ μM). Similarly, when the acylguanidine group of **1a** and the mono- and dichlorophenyl compounds **1b** and **1c** ($IC_{50} = 0.021$ and 0.006 μM, respectively) was replaced with aminothiazole, the resulting analogues **10a–c** displayed poor biological activity ($IC_{50} = 8 - > 30$ μM). The aminopyrazole analogue **13** was inactive against NHE-1 ($IC_{50} > 30$ μM) and the weakly basic diaminopyrimidine **14** showed only modest activity ($IC_{50} = 3.6$ μM).

Substantial efforts were made to investigate the imidazole ring to mimic the acylguanidine group. Various substituents were used to modulate the basisity of these analogues to increase the binding affinity at the active site. The methylthioimidazoles **15a** and **15b** had only weak to modest NHE-1 inhibitory activity ($IC_{50} = 10$ and 4.85 μM, respectively). However, 5-methylimidazol-4-yl analogue **19b** ($IC_{50} = 0.95$ μM) was significantly more potent than the unsubstituted imidazole analogues **17** and **18** ($IC_{50} > 30$ and 4.0 μM, respectively). Also notable is the enhanced inhibitory activity of aminoimidazoles **22b** and **22c** ($IC_{50} = 0.75$ and 0.18 μM, respectively) when compared to the unsubstituted imidazoles and the methylimidazoles. The improved potency of aminoimidazoles may be attributed to increased basicity (when compared to imidazoles) as well as the isosteric nature of aminoimidazoles and acylguanidines. Finally, combining the effects of the 4-methyl and the 2-amino groups afforded **23a–c** as the most potent heterocyclic NHE-1 inhibitors ($IC_{50} = 2.6$, 0.039 and 0.008 μM, respectively).

Most analogues were also evaluated for NHE-2 activity. As in the case of the corresponding acylguanidines,[13] the heterocyclic analogues exhibited only modest NHE-2 inhibitory activity. As an example, compound **23b** with an NHE-2 $IC_{50} = 1.2$ μM was found to be ca. 30-fold selective. Selected analogues were also evaluated

Table 1. NHE-1 inhibitory activity[a] of the various heterocyclic cyclopropanes and the corresponding acylguanidine analogues

B	Compd	IC_{50} (μM)	Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
Acylguanidine	**1a**[b]	0.009	**1b**	0.021	**1c**[c]	0.006
Aminooxadiazole	**3**[b]	30.0	—	—	—	—
Aminothiazole	**10a**[b]	> 30	**10b**	8.0	**10c**	> 30
2-Methythioimidazole	**15a**[b]	10.0	**15b**	4.85	—	—
3-Aminopyrazole	—	—	—	—	**13**	> 30
2,4-Diaminopyrimidine	—	—	—	—	**14**	3.6
Imidazole	**17**[b]	> 30	—	—	**18**	4.0
4-Methylimidazole	**19a**[b]	11.0	**19b**	0.95	—	—
2-Aminoimidazole	**22a**[b]	3.8	**22b**	0.75	**22c**	0.18
2-Amino-4-methylimidazole	**23a**[b]	2.6	**23b**	0.039	**23c**	0.008

[a] Compounds were screened in an AP1 cell line (devoid of NHE activity) expressing the human NHE-1 isoform, IC_{50} values ($n \geq 2$) were determined to measure the ability of compounds to inhibit recovery of pH following imposed acidosis.[13]
[b] *R,R*-Enantiomer.
[c] (+)-Enantiomer.

for NHE-3 activity. Similar to the acylguanidines, these analogues showed poor inhibitory activity for NHE-3. Compound **23b** was evaluated in rats for pharmacokinetic properties and oral bioavailability. After a single intravenous and an oral dose of 2.8 mg/kg body weight, **23b** exhibited a plasma half-life of 5 h and an oral bioavailability of 26%. The corresponding acylguanidine analogue **1b** showed similar oral bioavailability (29%) and a plasma half-life (5 h) at the same dosage. In conclusion, it has been demonstrated that the aminoimidazole group can be used to replace the acylguanidine group of the cyclopropyl series of NHE-1 inhibitors.

Acknowledgements

We greatly appreciate the support of Bristol Myers Squibb Department of Analytical Sciences.

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