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Synthesis and biological activity of 5-aryl-4-(4-(5-methyl-1*H*-imidazol-4-yl)piperidin-1-yl)pyrimidine analogs as potent, highly selective, and orally bioavailable NHE-1 inhibitors

Karnail S. Atwal,[a,*] Steven V. O'Neil,[a] Saleem Ahmad,[a,*] Lidia Doweyko,[a] Mark Kirby,[b] Charles R. Dorso,[b] Gamini Chandrasena,[c] Bang-Chi Chen,[a] Rulin Zhao[a] and Robert Zahler[a]

[a]*Department of Discovery Chemistry, Bristol-Myers Squibb, Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-5400, USA*
[b]*Department of Cardiovascular Biology, Bristol-Myers Squibb, Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-5400, USA*
[c]*Department of Drug Metabolism, Bristol-Myers Squibb, Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-5400, USA*

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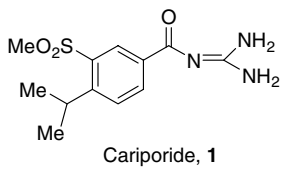
Abstract—A series of potent inhibitors of the sodium hydrogen exchanger-1 (NHE-1) is described. Structure–activity relationships identified the 3-methyl-4-fluoro analog **9t** as a highly potent (IC_{50} = 0.0065 μM) and selective (NHE-2/NHE-1 = 1400) non-acylguanidine NHE-1 inhibitor. Pharmacokinetic studies showed that compound **9t** has an oral bioavailability of 52% and a plasma half life of 1.5 h in rats. Because of its promising potency, selectivity, and a good pharmacokinetic profile, compound **9t** was selected for further studies.

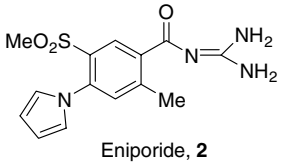
Sodium hydrogen exchanger-1 (NHE-1) has attracted considerable attention over the past several years.[1] This is largely because NHE-1 inhibitors have the potential to treat myocardial ischemia, a leading cause of death in the western world.[2] Several NHE-1 inhibitors have been evaluated preclinically and clinically for indications such as myocardial infarction, ischemic heart disease, and angina. The results of a phase II/III clinical trial of cariporide (**1**) in patients with acute coronary syndrome were less than encouraging.[3] The lack of positive clinical data for cariporide (**1**) may be due to the design of the trial or the modest potency (IC_{50} = 3.5 μM) of the compound; however, the results from clinical trials on a follow-up compound, eniporide (**2**, IC_{50} = 0.4 μM), have also been disappointing.[4]

The salient feature of most known NHE-1 inhibitors is an acylguanidine functionality. We believed that the acylguanidine group has the potential to undergo cleavage under metabolic conditions, releasing guanidine, a known cause of toxicity.

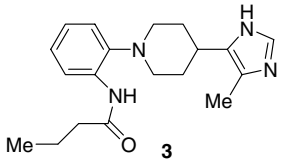
In our efforts to discover more potent, efficacious, and safe NHE-1 inhibitors, we sought to investigate compounds with an acylguanidine surrogate group.[5] Several imidazole containing NHE-1 inhibitors, represented by **3**, had previously been reported in the literature.[6] Evaluation of these compounds showed only modest potency



Cariporide, **1**



Eniporide, **2**



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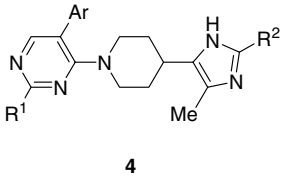

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Keywords: NHE; NHE-1; Sodium hydrogen exchanger.
* Corresponding authors. Tel.: +1 609 252 6955; fax: +1 609 252 6804; e-mail: saleem.ahmad@bms.com

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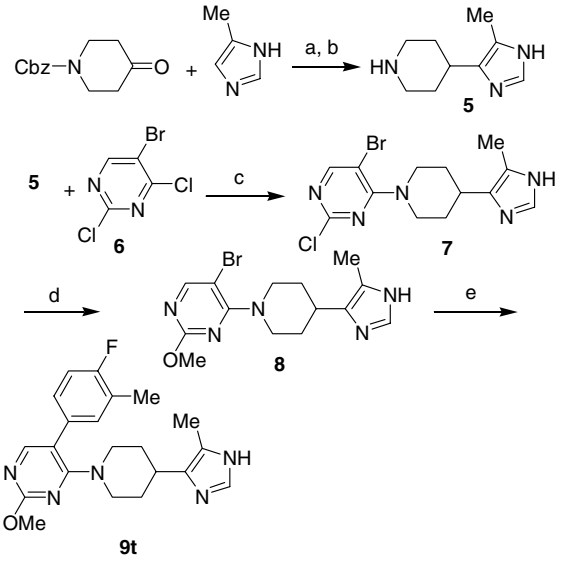


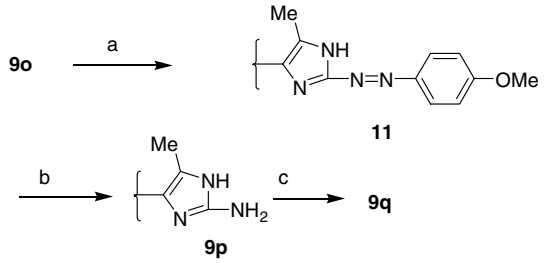
Scheme 1. Reagents and conditions: (a) KO^{t}Bu/neat/140 °C; (b) H_2/Pd–C/EtOH; (c) K_2CO_3/acetonitrile; (d) NaOMe/MeOH; (e) 4-fluoro-3-methylphenylboronic acid/$Pd(Ph_3P)_4$/Na_2CO_3/DMF/80 °C.

for NHE-1 (**3**, IC_{50} = 0.25 μM). Using piperidinylimidazole as a core template, we systematically introduced a variety of groups at the 1-position of piperidine ring as well as the imidazole group. This paper describes our efforts to optimize piperidinylimidazoles with arylpyrimidine substituents (**4**).

Synthesis of the various analogs described herein started with the readily available piperidinylimidazole core **5** as outlined in Scheme 1. Thus treatment of *N*-cbz-piperidone with excess 4-methylimidazole (5 equiv) in the presence of potassium *tert*-butoxide at 140 °C (melt) afforded the corresponding 4-hydroxypiperidine derivative in 40% yield after silica gel chromatography. This material could be readily converted to compound **5** (isolated as HCl salt) via catalytic hydrogenation using 10% palladium on carbon. Condensation of **5** with the trihalopyrimidine **6** provided compound **7** in 80% yield (Scheme 1). The chlorine in **7** was displaced with a methoxy group (NaOMe/MeOH) to provide the 2-methoxy analog **8** in 81% yield. The pyrimidinyl bromide **8** was then converted to the biaryl derivative **9t** via Suzuki reaction in 94% yield. Thus, all three halides in **6** were conveniently displaced in a stepwise fashion to provide the requisite compound.

Synthesis of the aminoimidazole **9p** is summarized in Scheme 2. The imidazole analog **9o** (see Scheme 3 for synthesis of **9o**) was treated with 4-methoxybenzenediazonium tetrafluoroborate to give the diazo compound **11**, which upon hydrogenation over platinum oxide provided the desired product **9p** in 62% overall yield. Acetylation of **9p** with acetic anhydride in pyridine gave the *N*-acetyl compound **9q**.

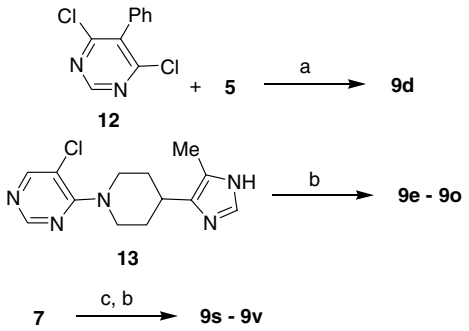
The ethoxy analog **9b** was prepared from the corresponding chloro analog **9d** by treatment with sodium ethoxide in ethanol at room temperature (80%). Similarly the morpholine derivative **9c** was prepared from **9d** by heating it in neat morpholine at 80 °C. Compound **9d** could be prepared via condensation of 4,6-dichloro-5-phenylpyrimidine (**12**) with piperidinylimidazole **5** in diglyme in the presence of potassium carbonate at 150 °C for 30 min (Scheme 3). Compounds **9e–o** were prepared via Suzuki coupling of the corresponding chloropyrimidinylimidazole **13** (prepared via coupling of compound **5** with the corresponding dichloropyrimidine) with an appropriate arylboronic acid (Scheme 3). Methylation of the imidazole **9o** with potassium carbonate and methyl iodide in acetone gave predominantly the *N*3-methylated product **9r**. Compounds **9s–v** were synthesized by displacement of the chloride of **7** by the appropriate nucleophile (MeO^-, morpholine, and $^iPrO^-$) prior to Suzuki coupling (Scheme 3).



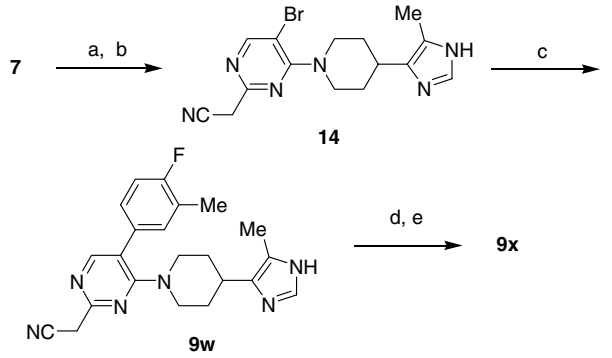
Scheme 2. Reagents and conditions: (a) 4-methoxybenzenediazonium tetrafluoroborate; (b) H_2/PtO_2/ethanol; (c) Ac_2O/Py.

Synthesis of the nitrile **9w** and the amide **9x** was carried out as outlined in Scheme 4. The nitrile **14** was prepared by heating the chloride **7** with *tert*-butylcyanoacetate in THF followed by decarboxylation (CF_3COOH, 60 °C). The bromide **14** was converted to **9w** via Suzuki coupling in 80% yield. Hydrolysis of the nitrile **9w** (2% NaOH and MeOH) and coupling of the resulting acid with isopropylamine provided the amide **9x** (78%).

The NHE-1 and NHE-2 activities were assessed as described.[7] These experiments were carried out in AP1 cell line expressing human NHE isoforms. This cell line has no endogenous NHE activity. The IC_{50} values were determined by measuring the ability of the compounds to inhibit 50% of the sodium dependent recovery of pH following imposed acidosis. Using this protocol,



Scheme 3. Reagents and conditions: (a) K_2CO_3/diglyme/150 °C; (b) arylboronic acids/$Pd(Ph_3P)_4$/Na_2CO_3/DMF/80 °C; (c) NaOMe or NaO^{i}Pr or morpholine.



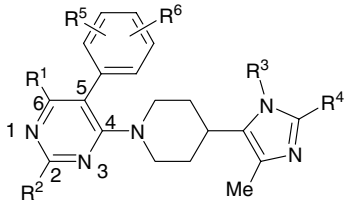
Scheme 4. Reagents and conditions: (a) *tert*-butyl cyanoacetate/NaH/THF/heat/2 days; (b) TFA/60 °C; (c) 4-fluoro-3-methylphenylboronic acid/$Pd(Ph_3P)_4/Na_2CO_3$/DMF/80 °C; (d) NaOH/MeOH; (e) isopropylamine/carbonyldiimidazole/triethylamine.

the NHE-1 IC_{50} of cariporide (**1**) and eniporide (**2**) were measured as 3.5 and 0.4 μM, respectively. The IC_{50} of the imidazole analog **3** was 0.25 μM, while that of the baseline compound **9a** was 0.52 μM. Starting from **9a**, we optimized the SAR by elaborating the aryl, the pyrimidine, and the imidazole rings.

Replacing a pyrimidine proton in **9a** with an ethoxy group (compound **9b**) did not have an effect on the potency. However, significant activity was lost when the ethoxy group in **9b** was replaced by a morpholino group (**9c**). Compound **9b**, which contains electron releasing ethoxy group, has similar potency to the analog with an electron withdrawing chloro substituent (**9d**). The potency appears to be sensitive to the size of the R^1 group, as shown by comparison of the morpholino analog **9c** with compounds having relatively smaller substituents at this position (**9a**, **9b**, and **9d**).

While introduction of a chlorine atom at the 4-position of the pendant phenyl ring (compound **9e**) had no effect on potency, introduction of a 3-chloro on the phenyl ring (**9f**, $IC_{50} = 0.061$ μM) resulted in a nearly 6-fold improvement in potency. The 2-chloro analog (**9g**, $IC_{50} = 0.061$ μM) showed potency similar to the 3-chloro compound **9f**. The 3- and 2-methoxy analogs (**9h** and **9i**) showed no significant improvement in potency relative to the starting compound **9a**. The lack of potency of the methylenedioxy analog **9j** may reflect a sterically restricted binding site. While the compounds with 3-methyl (**9m**, $IC_{50} = 0.027$ μM), and 2-chloro groups

Table 1. NHE-1 inhibitory activity of the various imidazole derived analogs



Compound	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50}[a] (μM)
9a	H	H	H	H	H	H	0.52
9b	OEt	H	H	H	H	H	0.82
9c	1-Morpholino	H	H	H	H	H	8.6
9d	Cl	H	H	H	H	H	0.58
9e	H	H	H	H	H	4-Cl	0.35
9f	H	H	H	H	H	3-Cl	0.061
9g	H	H	H	H	H	2-Cl	0.061
9h	H	H	H	H	H	3-OMe	0.35
9i	H	H	H	H	H	2-OMe	0.94
9j	H	H	H	H	H	(3,4)-OCH_2O–	45.5% at 1 μM
9k	H	H	H	H	H	2-Me	0.25
9l	H	H	H	H	H	4-Me	1.0
9m	H	H	H	H	H	3-Me	0.027
9n	H	H	H	H	3-Me	4-F	0.021
9o	H	H	H	H	3-Cl	4-F	0.041
9p	H	H	H	NH_2	3-Cl	4-F	0.0054
9q	H	H	H	NH-Ac	3-Cl	4-F	1.9
9r	H	H	Me	H	3-Cl	4-F	11
9s	H	–OMe	H	H	3-Cl	4-F	0.015
9t	H	–OMe	H	H	3-Me	4-F	0.0065
9u	H	1-Morpholine	H	H	3-Me	4-F	0.0079
9v	H	–O^{i}Pr	H	H	3-Me	4-F	0.0074
9w	H	–CH_2CN	H	H	3-Me	4-F	0.0046
9x	H	$CH_2C(O)NH^iPr$	H	H	3-Me	4-F	0.087
1	Cariporide	—	—	—	—	—	3.5
2	Eniporide	—	—	—	—	—	0.40
3	—	—	—	—	—	—	0.25

[a] Each value is an average of at least two determinations.

Table 2. NHE-2 activities of compound **9t**, cariporide (**1**), and eniporide (**2**)

Compound	NHE_2 (IC_{50}, μM)	NHE_2/NHE_1
9t	9	1400
1	62	18
2	17	43

(**9g**, $IC_{50} = 0.061$ μM) showed good potency, compounds with 2- and 4-methyl groups (**9k** and **9l**, respectively) were significantly less active. The presence of a 4-fluoro group adjacent to the 3-methyl (**9n**) or 3-chloro (**9o**) groups had no significant impact on potency.

Introduction of an amino group at the 2-position (R^4) of the imidazole of **9o** gave **9p**, possessing 8-fold greater potency over the parent. Acetylation of the amino group eroded potency (**9q**, $IC_{50} = 1.9$ μM), as did N-methylation ($R^3 = Me$) of the imidazole nitrogen (**9r**, $IC_{50} = 11$ μM).

Further modifications were made at the 2-position (R^2) of the pyrimidine ring. Introduction of a 2-methoxy group to compound **9o** improved NHE-1 inhibitory activity by 2-fold (compound **9s**, $IC_{50} = 0.015$ μM). Similarly, introduction of a methoxy group to the 3-methyl-4-fluorophenyl analog **9n** resulted in ca. 3-fold enhancement in potency (**9t**, $IC_{50} = 0.0065$ μM). Introduction of other substituents to the 2-position of the pyrimidine, such as morpholino (**9u**, $IC_{50} = 0.0079$ μM), *O*-isopropoxy (**9v**, $IC_{50} = 0.0074$ μM), and cyanomethyl (**9w**, $IC_{50} = 0.0046$ μM), retained nanomolar potency (see Table 1).

Most compounds described herein showed good selectivity for NHE-1 over NHE-2.[8] Table 2 displays NHE-2 activities for **9t**, cariporide, and eniporide. The IC_{50} for inhibition of NHE-2 activity was 9 μM for **9t**. Thus, compound **9t** is one of the most selective (1400-fold) NHE-1 inhibitors known and, accordingly, may possess a significantly improved safety profile.[9]

To further differentiate between these highly potent NHE-1 inhibitors, we determined their oral bioavailabilities in rats. Compounds with IC_{50} values <0.020 μM were initially evaluated in a coarse rat PK study. Based on the data from the coarse PK studies, compound **9t** was selected for further evaluation in a full rat PK study.[10] After a single oral or intraarterial dose, compound **9t** had an oral bioavailability of 52% and a plasma half life of 1.5 h in rats.

In summary, we have identified compound **9t**, with excellent NHE-1 inhibitory activity ($IC_{50} = 0.0065$ μM) and significantly greater selectivity for NHE-1 over NHE-2 (1400-fold) than either cariporide (**1**) or eniporide (**2**). In addition, **9t** is 60-fold more potent against NHE-1 than eniporide (**2**) and nearly 500-fold more potent than cariporide (**1**). It has a good oral bioavailability (52%) and modest plasma half life (1.5 h). Compound **9t** was thus selected for further studies.

Acknowledgments

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10. The test compounds were administered individually to male rats as a solution in PEG–EtOH–H_2O (1:1:1). Rats were dosed orally ($n = 3$ and 10 μmol/kg) or intraarterially ($n = 3$ and 5 μmol/kg) for full PK studies. Serial samples were withdrawn at 0, 5, 10, 20, and 40 min (ia) and at 1, 2, 4, 6, 8, 12, and 24 h (po) after dosing. Plasma was prepared from each sample by centrifugation and analyzed by valid LC/MS/MS procedure on a reverse-phase C18 column using 0.02 M ammonium acetate, pH 5.1/acetonitrile (65:5, v/v) as a mobile phase. Pharmacokinetic parameters were calculated by linear regression to determine the equation of the biexponential curve which best fit the data.