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Identification of a Potent Sodium Hydrogen Exchanger Isoform 1 (NHE1) Inhibitor with a Suitable Profile for Chronic Dosing and Demonstrated Cardioprotective Effects in a Preclinical Model of Myocardial Infarction in the Rat

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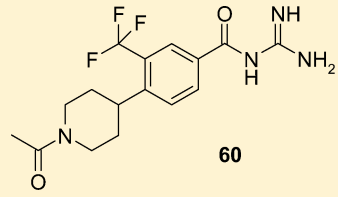
ABSTRACT: Sodium−hydrogen exchanger isoform 1 (NHE1) is a ubiquitously expressed transmembrane ion channel responsible for intracellular pH regulation. During myocardial ischemia, low pH activates NHE1 and causes increased intracellular calcium levels and aberrant cellular processes, leading to myocardial stunning, arrhythmias, and ultimately cell damage and death. The role of NHE1 in cardiac injury has prompted interest in the development of NHE1 inhibitors for the treatment of heart failure. This report outlines our efforts to identify a compound suitable for once daily, oral administration with low drug−drug interaction potential starting from NHE1 inhibitor sabiporide. Substitution of a piperidine for the piperazine of sabiporide followed by replacement of the pyrrole moiety and subsequent optimization to improve potency and eliminate off-target activities resulted in the identification of *N*-[4-(1-acetyl-piperidin-4-yl)-3-trifluoromethyl-benzoyl]-guanidine (**60**). Pharmacological evaluation of **60** revealed a remarkable ability to prevent ischemic damage in an ex vivo model of ischemia reperfusion injury in isolated rat hearts.



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INTRODUCTION

Sodium−hydrogen exchanger isoform 1 (NHE1) is a ubiquitously expressed transmembrane ion channel responsible for the regulation of intracellular pH via the electroneutral exchange of sodium ions and protons.[1] NHE1 is a member of a family of such proteins which encompasses nine variously expressed isoforms. While NHE1 is ubiquitously expressed,[2] it is the predominant NHE present in myocardial tissue[3a] where it plays a central role in the regulation of intracellular pH in cardiomyocytes. Under conditions of ischemia-induced acidosis, a decrease in intracellular pH leads to the activation of NHE1, which functions to restore pH to normal levels through the efflux of protons with concomitant influx of sodium ions. Under normal conditions, the elevation of sodium ion concentration is counteracted by a number of transporters and exchangers, primarily the ATP-driven sodium/potassium exchanger and the sodium/bicarbonate exchanger (which also plays a role in regulating pH). However, under hypoxic conditions, reduced ATP levels decrease the activity of the sodium/potassium exchanger, resulting in prolonged increases in intracellular sodium ions levels, which in turn causes the sodium calcium exchanger (NCX), normally a calcium extruder, to run in reverse, resulting in an increase in intracellular calcium ion levels.[3b−f] This increased calcium ion level results in aberrant cellular processes leading to myocardial stunning, arrhythmias, and ultimately cell damage and death. Inhibition of NHE1 is expected to prevent the initial influx of sodium ions, block the resultant calcium overload due to NCX activity, and thereby prevent subsequent cellular damage.[4]

The central role of NHE1 in myocardial pH regulation has prompted considerable interest in the development of NHE1 inhibitors for the treatment of ischemia-reperfusion injury. However, despite strong preclinical validation,[5−8] clinical trials have provided mixed results in this indication. The recent emergence of data to support a role for NHE1 inhibition in heart failure[9−15] led to our interest in pursuing NHE1 inhibition as a mechanism to treat chronic heart failure.

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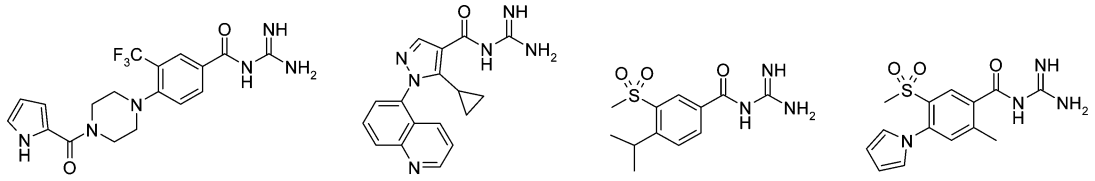

1 2 3 4

Figure 1. Clinically investigated NHE1 inhibitors.

However, the shift in focus from an acute to a chronic indication prompted us to re-evaluate the profiles of the compounds evaluated in clinical studies as prospective starting points for optimization. Four NHE1 inhibitors, sabiporide[16] (**1**), zoniporide[17] (**2**), cariporide[18] (**3**), and eniporide[19] (**4**) (Figure 1) were advanced to clinical evaluation for the treatment of ischemia reperfusion injury. Compound **1** was discovered in our laboratories in a discovery program directed toward the identification of NHE1 inhibitors for the treatment of neuronal injury following ischemic stroke. Subsequently, **1** was demonstrated to have significant cardioprotective effects in vivo in a variety of animal studies and was advanced into phase I studies as an oral agent for the treatment of acute myocardial infarction (MI).[8,16,20] The other three clinical compounds, **2**–**4**, were advanced to clinical evaluation for acute MI, and were studied as iv formulations. These compounds display pharmacokinetic properties that would render them unsuitable for oral administration (Table 1), with mean residence times

Table 1. Rat Pharmacokinetics of Clinical NHE1 Compounds

compd	clearance (%Q_H)	MRT (h)	Vdss (L/kg)	*F* (%)
1	24	1.3	1.3	42
2	105	0.6	2.8	5
3	82	0.4	1.3	37
4	28	0.4	0.5	65

(MRTs) markedly lower than that of **1**. Considering the intrinsic advantage provided by the pharmacokinetic profile of sabiporide, we chose to take this molecule as the starting point for our optimization efforts to identify a compound for the treatment of chronic heart failure.

The minimization of the potential for drug–drug interactions (DDIs) is of importance for chronic indications, in particular indications such as heart failure for which patients are typically receiving a large number of comedications. **1** is a moderate inhibitor of CYP 3A4 (IC_{50} 7 μM) and 2C19 (IC_{50} 9 μM), and it was deemed desirable to decrease the inhibitory potency against these isoforms. In addition, an improvement in potency was viewed to be desirable as another means to decrease the potential for off-target activity. It was also desirable to further improve the pharmacokinetic profile to enable once-daily dosing. A final goal for optimization efforts was the replacement of the pyrrole substructure, as this substructure may be oxidatively metabolized to electrophilic species implicated in adverse drug reactions.[21,22]

We describe here the discovery of *N*-[4-(1-acetyl-piperidin-4-yl)-3-trifluoromethyl-benzoyl]-guanidine (**60**), a potent NHE1 inhibitor with a pharmacokinetic profile suitable for chronic administration in humans and remarkable efficacy in a preclinical model of MI in the rat. Discovery efforts leading ultimately to compound **60** initiated from sabiporide. From this starting point, a change of the piperazine linker to a piperidine was found to provide uncompromised potency against NHE1. Subsequent replacement of the pyrrole amide substituent to avoid a potential for formation of reactive metabolites was followed by optimization focused on improvements in potency and elimination of off-target activities, including CYP inhibition. Optimization resulted in the identification of **60**, the pharmacological evaluation of which revealed a remarkable ability to prevent ischemic damage in an isolated heart model of ischemia reperfusion injury (Langendorff isolated heart preparation).

CHEMISTRY

The arylacylguanidine substructure of compounds **1**–**4** is considered a privileged pharmacophore for NHE1 inhibition and was left unaltered in the optimization efforts described herein.

Taking **1** as our starting point, our first set of compounds examined the effect of a change to the piperazine substructure. The piperazine was replaced with racemic 3-aminopyrrolidine, racemic 3-aminopiperidine, or 4-aminopiperidine (Scheme 1). Compounds **5**–**7** were synthesized by a nucleophilic aromatic substitution of the methyl ester **8** with the BOC-protected amines **9a**–**c** to provide the BOC-protected amino esters **10a**–**c**. The BOC group was removed under standard acidic conditions, and the amine was coupled with 1*H*-pyrrole-2-carboxylic acid chloride to provide the pyrrole amides **11a**–**c**. The esters of compounds **11a**–**c** were hydrolyzed to the free acids **12a**–**c**, which were subsequently coupled with CBZ-guanidine or BOC-guanidine using a Mukuyama coupling.[23] The resulting CBZ- or BOC-protected acylguanidines **13a**–**c** were deprotected under standard conditions to afford the desired 3-aminopyrrolidine, 3-aminopiperidine, and 4-aminopiperidine derivatives **5**–**7**.

The further examination of the effect of changes to the sabiporide piperazine substructure was enabled by the synthesis of the corresponding piperidine derivative **14** (Scheme 2). The synthesis of **14** took its starting point in the methyl ester **15**, which underwent Suzuki coupling with commercially available pinacol boronate **16** to provide the BOC-protected tetrahydropyridine **17**. Catalytic hydrogenation using platinum(IV) oxide in acetic acid afforded the BOC-protected piperidine **18**, which was hydrolyzed to provide the corresponding carboxylic acid derivative **19**. Condensation with CBZ-protected guanidine and subsequent removal of the BOC protecting group provided the amino derivative **20a**. Condensation of the amine **20a** with pyrrole-2-carboxylic acid, using carbonyl diimidazole (CDI) in dimethylformamide, was followed by the removal of the CBZ protecting group under standard conditions to provide the piperidine **14**.

In a next set of compounds (**21**–**62**) the effect of a replacement of the pyrrole substructure with unsubstituted or

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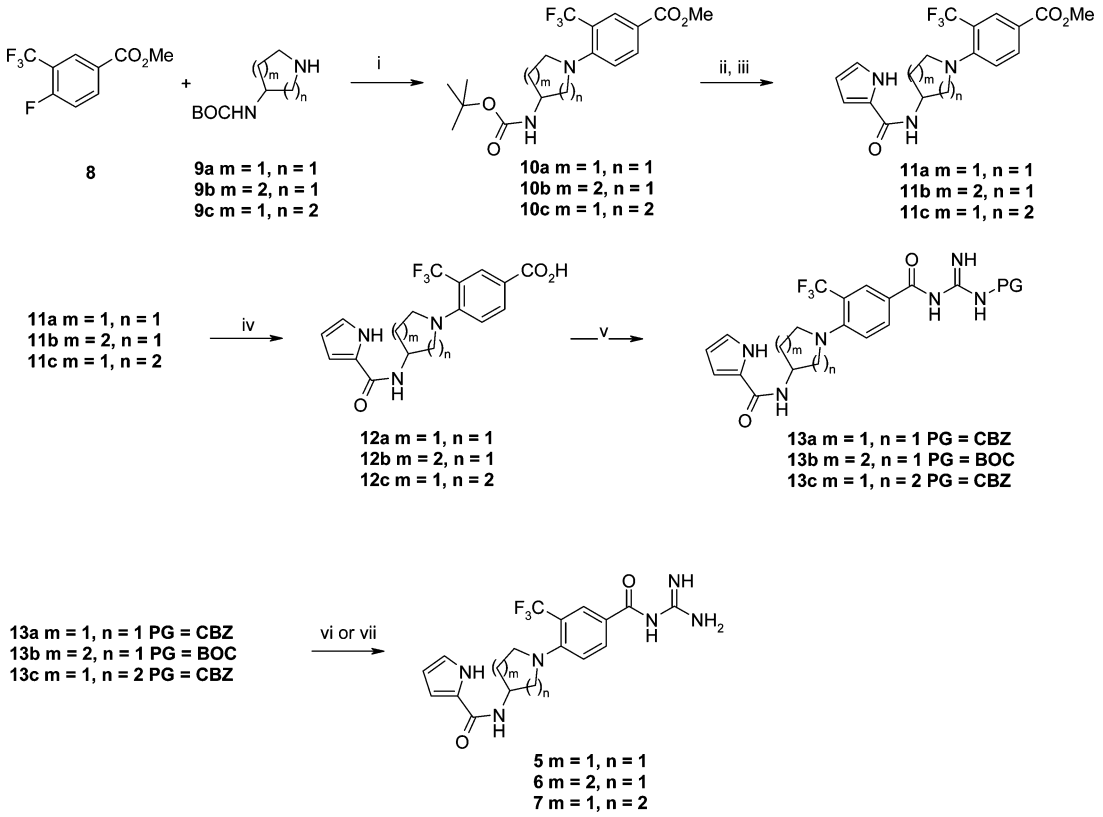
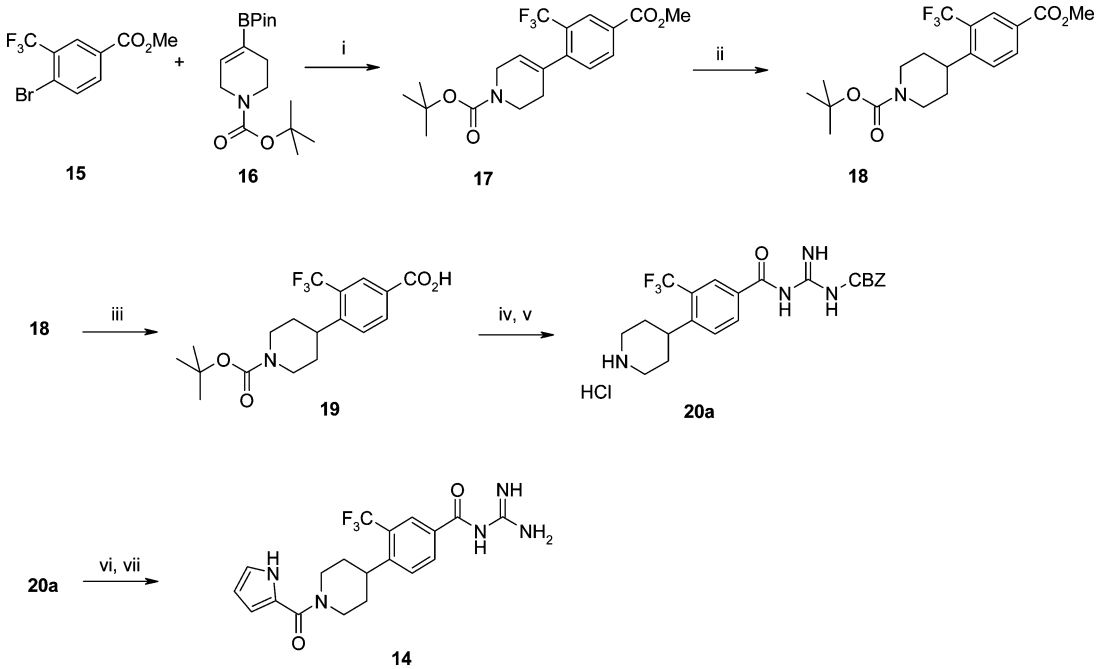
Scheme 1[a]



[a]Reagents and conditions: (i) diisopropylethylamine (DIEA), DMSO, 90 °C; (ii) NaOH, MeOH, reflux, 2.5 h; (iii) 1-methyl-2-chloropyridinium iodide, 1-methyl pyrrolidinone (NMP), 90 min, then CBZ-guanidine, DIEA, 16 h; (iv) HCl, dioxane, methanol, 4.5 h; (v) 1*H*-pyrrole-2-carboxylic acid chloride, DIEA, CH_2Cl_2, 16 h; (vi) Pd/C, 1 atm H_2, EtOH, 16 h.

Scheme 2[a]



[a]Reagents and conditions: (i) $Pd(PPh_3)_4$, 140 °C, 4 h; (ii) PtO_2, 1 atm H_2, acetic acid, 60 h; (iii) K_2CO_3, $MeOH/H_2O$ (10/1), 60 °C, 2 h; (iv) 1-methyl-2-chloropyridinium iodide, NMP, 90 min, then CBZ-guanidine, DIEA, 16 h; (v) HCl, 1,4-dioxane, CH_2Cl_2, 2 h; (vi) 2-pyrrole-carboxylic acid, CDI, DMF, 90 min, then **20a**, DIEA, 16 h; (vii) Pd/C, 1 atm H_2, EtOH, 4−16 h.

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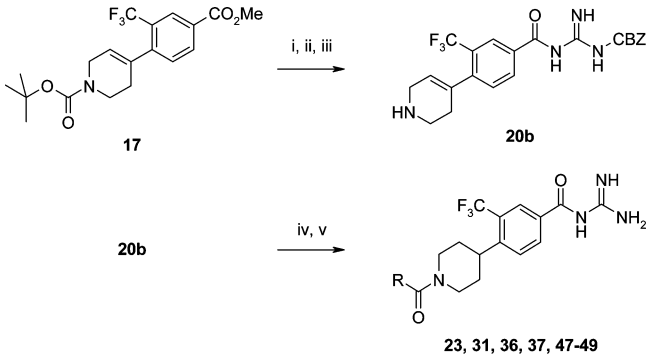
substituted aryl was examined. The majority of these compounds were synthesized using a similar strategy as that used in the synthesis of compound **14** (Scheme 2). However, for compounds **23**, **31**, **36**, **37**, and **47**−**49**, the intermediate **17** was carried forward with its alkene functionality intact to provide the tetrahydropyridine intermediate **20b** (Scheme 3),

Scheme 3[a]



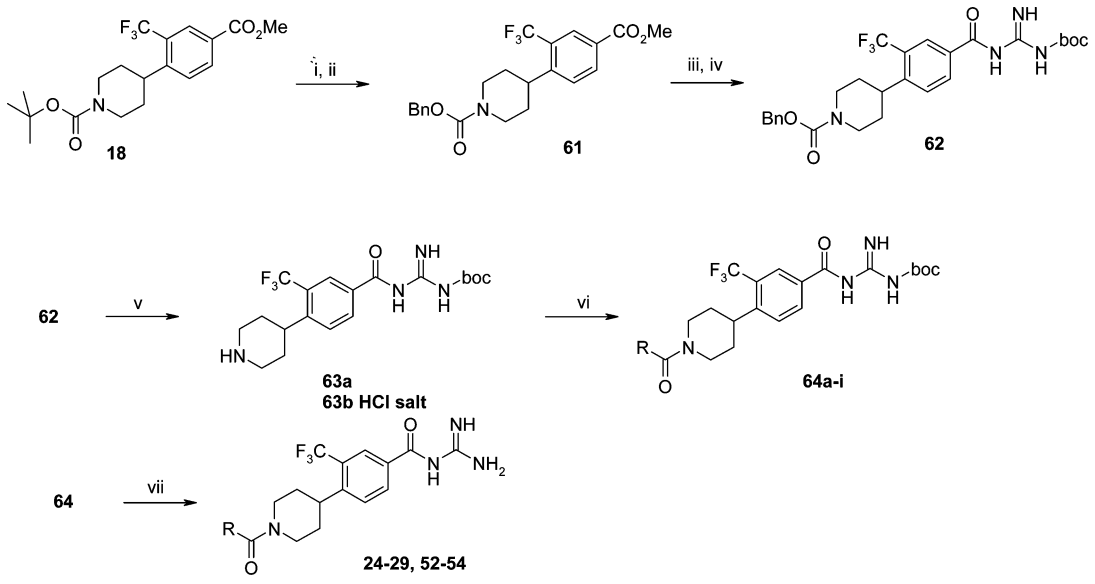
[a]Reagents and conditions: (i) K_2CO_3, MeOH/H_2O (10/1), 60 °C, 2 h; (ii) 1-methyl-2-chloropyridinium iodide, NMP, 90 min, then CBZ-guanidine, DIEA, 16 h; (iii) HCl, 1,4-dioxane, CH_2Cl_2, 2 h; (iv) RCO_2H, CDI, DMF, 90 min, then **20b**, DIEA, 16 h; (v) Pd/C, 1 atm H_2, EtOH, 4−48 h.

which was acylated under the conditions used for compound **14**, followed by the hydrogenation of the CBZ group and alkene moieties in the final step. This synthetic sequence, however, suffered from low synthetic reproducibility, likely a result of the poorly reactive alkene being extremely difficult to hydrogenate completely. For the alkyl amide compounds **55**−**60**, a synthetic strategy similar to that employed for compound **14** was used, albeit using an acid chloride in place of the CDI-mediated coupling of the acid derivative.

To enable the further exploration of changes to the pyrrole in a parallel format, the BOC-protected acylguanidine intermediates **63a** and **63b** were prepared. These intermediates were synthesized starting from the intermediate **18** by the conversion of this BOC-protected piperidine to the corresponding CBZ-protected piperidine **61**. Hydrolysis of **61** under basic conditions and subsequent subjection to a Mukuyama type coupling with BOC-guanidine provided the CBZ-protected acylguanidine **62**, which was deprotected by hydrogenation to give the desired intermediate amino derivative **63a**. This intermediate or its hydrochloride salt **63b** was condensed with a variety of aryl acids using TBTU to give the BOC-protected amides **64a**−**i**, which upon BOC removal provided the desired compounds **24**−**29** and **52**−**54** (Scheme 4).

To examine the effect of aryl core substitution, compounds **78**−**83** were prepared from their corresponding carboxylic acid ester intermediates **69a**−**c** using a similar synthetic strategy as applied for compound **14** (Scheme 5). The 2-methyl-5-trifluoromethyl core of **69a** was synthesized by initiating from the iodophenol **65**, which was converted to its benzyl protected derivative **66**. Subjection of **66** to a copper-promoted trifluoromethylation provided **67**, which was deprotected by hydrogenation to provide the phenol derivative **68**. Conversion of **68** to the corresponding triflate followed by a Suzuki coupling with **16** provided the tetrahydropyridine intermediate **69a**. The 2-methyl-5-methylsulfonyl core of compounds **69b** was synthesized by initiating from the benzoic acid **70**. Methsulfonylation to provide the arylmethylsulfone **71** was followed by esterification to provide the bromo ester intermediate **72**, which was subsequently subjected to Suzuki coupling with **16** to provide the tetrahydropyridine intermediate **69b**. The 2-methoxy-5-trifluoromethyl core of **69c** was synthesized from the ester **73**. Selective benzyl protection of the phenol to give the benzyloxy compound **74** followed by bromination to provide the bromophenyl **75**, which was methylated to provide the methoxy bromide **76**. Subsequent

Scheme 4[a]



[a]Reagents and conditions: (i) HCl, 1,4-dioxane, 18 h, then 50 °C, 6 h; (ii) benzyl chloroformate, DIEA, CH_2Cl_2, 0 °C to rt, 16 h; (iii) K_2CO_3, MeOH/H_2O (3/1), 16 h; (iv) 1-methyl-2-chloropyridinium iodide, NMP, 90 min, then BOC-guanidine, DIEA, 16 h; (v) $Pd(OH)_2$/C, 1 atm H_2, EtOH, 16 h, then for **63b**:HCl in ether; (vi) RCO_2H, TBTU, NMM, DMA, 1 h; (vii) 4N HCl in dioxane, CH_2Cl_2. For R groups see tables 3, 5, and 6.

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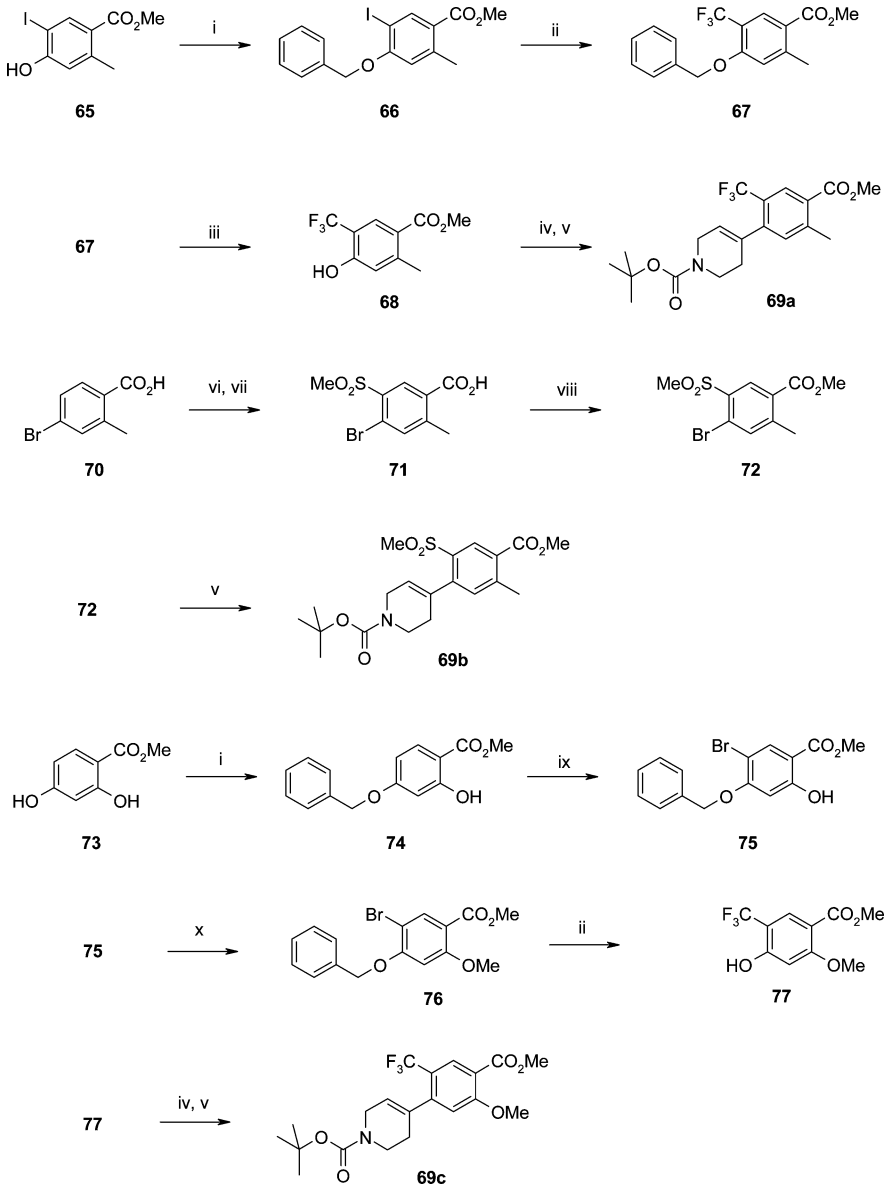
Scheme 5[a]



[a]Reagents and conditions: (i) BnBr, K_2CO_3, DMF, 80 °C, 2h; (ii) $KOC(O)CF_3$, CuI, NMP, 150 °C, 5h; (iii) Pd/C, H_2, EtOH, o/n; (iv) *N*-phenyltrifluoromethanesulfonimide, DIEA, DMF, o/n; (v) **16**, 2M K_2CO_3, $Pd(Ph_3P)_4$, 110 °C, 30 min; (vi) $ClSO_3H$, 0 to 100 °C, 2h; (vii) Na_2SO_3, $NaHCO_3$, H_2O, THF, 70 °C, 1 h then MeI, 50 °C, o/n; (viii) AcCl, MeOH, 50 °C, o/n; (ix) Br_2, $CHCl_3$, 30 min; (x) KOtBu, MeI, THF, 72 h.

trifluoromethylation and deprotection gave the phenol **77** (Scheme 5), which was subjected to triflation and Suzuki reaction with **16** to provide the tetrahydropyridine intermediate **69c**. The required intermediate for the 5-methylsulfonyl core was synthesized according to literature procedures.[21]

RESULTS AND DISCUSSION

The superior pharmacokinetic profile of **1** led us to take this molecule as the starting point for discovery of an NHE1 inhibitor suitable for once daily, oral administration with minimal potential for drug−drug interactions. **1** can be viewed as consisting of four structural domains: a left-hand side heteroaryl amide, a piperazine linker, a substituted aryl core, and an acylguanidine headgroup (Figure 2). Although some NHE1 inhibitors incorporate replacements for the aryl core and acylguanidine,[25] the aryl core and right-hand side acylguanidine

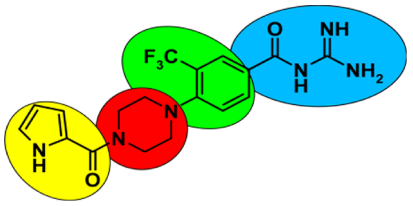


Figure 2. Chemical structure of the NHE1 inhibitor **1** illustrating its four structural domains. From left to right, these are the heteroaryl amide (yellow), piperazine linker (red), aryl core (green), and the acylguanidine (blue).

is present in the vast majority of NHE1 inhibitors and hence can be considered to be a privileged pharmacophore for NHE1 inhibition. During the discovery of **1**, the trifluoromethylphenyl core was demonstrated to provide good pharmacokinetic

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properties. In light of this experience, and considering the extensive prior art for arylacylguanidines, which includes a vast number of claims to aryl core structures other than phenyl, our initial efforts focused on the exploration of changes to the piperazine substructure with the goal to identify structural changes likely to provide patentability and address the optimization goals outlined above.

Close examination of the minimized structures of the clinical benchmark compounds **1**–**4**, assuming identical positioning of the aryl acylguanidine moiety, revealed different positioning of the substituents of the core aryl, hence suggesting some flexibility with respect to the identity of the core aryl substituents (Figure 3). Initial optimization efforts therefore

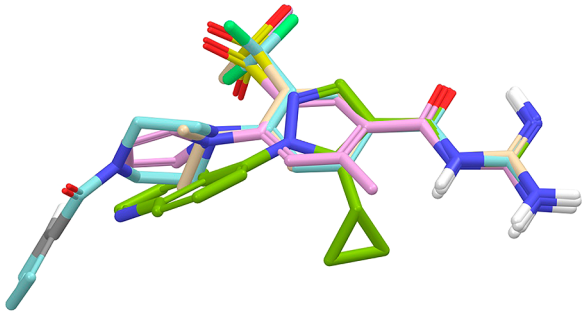


Figure 3. Overlay of the minimized conformations of the four NHE-1 inhibitors **1** (turquoise), **2** (green), **3** (yellow), and **4** (pink), illustrating the different positioning of the left-hand side aryl moiety.

focused on changes to the piperazine linker in order to identify an alternative to the piperazine while maintaining or improving the favorable properties of sabiporide. A change of the piperazine substructure to aminopyrrolidine, 3-aminopiperidine, and 4-aminopiperidine was investigated by the synthesis of compounds **5**, **6**, and **7**. The overlay of the minimized conformations of these compounds with that of **1** suggested a relatively close agreement in the placement of their left and right-hand sides (Figure 4). However, these compounds (EC_{50}

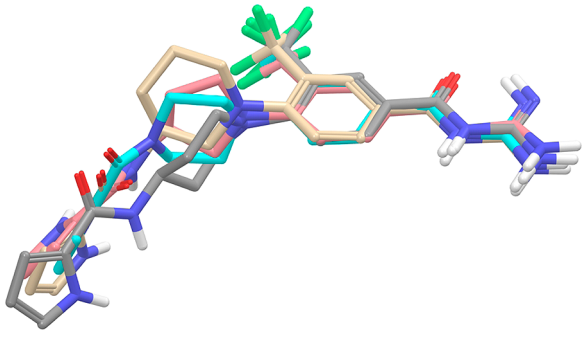
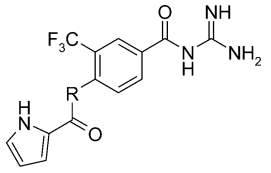


Figure 4. Overlay of the minimized conformations of **1** (turquoise) with **5** (salmon), **6** (beige), and **7** (gray), illustrating the relatively good overlap of the core aryl and far left-hand side substructures.

172 nM) resulted in reduced potency in the functional platelet swelling assay (see Experimental Section), with EC_{50}s ranging from 950 to 3477 nM for compounds **5**–**7** as compared to 172 nM for **1** (Table 2). In contrast, IC_{50} values in the pH_i assay were largely uncompromised, with IC_{50}s ranging from 36 to 172 nM for compounds **5**–**7** compared to the 28 nM IC_{50} for **1**. More radical departures from the piperazine core such as those based on bicyclic systems, rings fused to the aryl ring, or

Table 2. pH_i and Human Platelet Swelling Inhibition Potencies for Piperazine Replacements 5–7 and 14



Compound	R	pH_i change IC_{50} (nM)	hPSA IC_{50} (nM)
1		28	172
5		36	1586
6		172	3477
7		41	950
14		45	241

bridged heterocycles resulted in compounds which completely lacked inhibitory potency in the human platelet-swelling assay (data not shown). Examination of structural overlays again did not reveal the basis for the complete lack of inhibitory potency displayed by these compounds. In contrast, our evaluation of the piperidine derivative **14** revealed a near-perfect overlay with sabiporide and the compound displayed comparable potency to sabiporide in the platelet swelling assay as well as in the pH_i assay (IC_{50} 45 nM and EC_{50} 241 nM for compound **14** compared to IC_{50} 28 nM and EC_{50} 172 nM for **1**) (Figure 5).

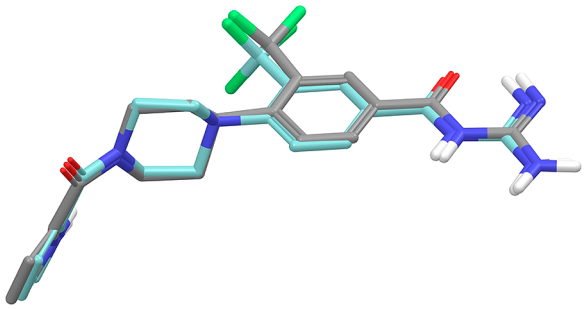


Figure 5. Overlay of the minimized conformations of the piperidine derivative **14** (gray) with **1** (turquoise), illustrating near-perfect overlay for both the right-hand side aryl acylgaunidine and the left-hand side aryl amide.

For the majority of poorly active compounds (such as **5**, **6**, and **7**), the activity in the human platelet swelling assay was markedly inferior to that of sabiporide, while a significant degree of potency frequently was still observed in the pH_i assay. Various possibilities were examined in an effort to understand the basis for the differences in potencies observed in these assays. The observed differences could be due to protein binding in the human platelet swelling assay which would not occur in the pH_i assay. To examine this possibility, the pH_i assay was conducted with plasma protein (data not shown). However, the potencies observed in this assay did not shift remarkably from those observed without plasma, hence suggesting that plasma protein binding is not the underlying

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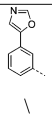
Table 3. pH_i and hPSA Potencies for Aryl Replacements for the Pyrrole Moiety in 14 and xLogP Values Illustrating the Improvement in Potency for More Polar Left-Hand Sides

Compound	R	pH_i change IC_{50} (nM)	hPSA IC_{50} (nM)	xlogP
14		45	241	2.07
21		68	109	3.26
22		109	184	3.42
23		320	539	4.18
24		306	1130	3.88
25		385	989	3.88
26		108	495	3.88
27		4705	>10000	5.2
28		102	368	3.04
29		33	353	3.04
30		65	169	3.07
31		21	138	2.85

Compound	R	pH_i change IC_{50} (nM)	hPSA IC_{50} (nM)	xlogP
32		24	152	2.98
33		35	111	2.98
34		61	200	2.98
35		15	93	2.78
36		60	50	2.78
37		58	269	2.78
38		18	70	2.01
39		18	32	2.01
40		18	79	2.1
41		26	36	2.15
42		19	25	2.33
43		14	23	1.61
44		27	115	1.99
45		14	111	1.52
46		18	62	1.52

reason for the observed differences in potencies observed in the pH_i and platelet swelling assays. Examination of physiochemical properties of the molecules that displayed significant differences in potency in the pH_i and human platelet swelling assays, such as polar surface area, log *P*, log *D*, and hydrogen bond acceptor/donor count, also did not reveal a correlation that might explain the observed shift in potencies between the assays.

Consequently, our further optimization efforts were driven by the human platelet swelling assay as this assay was viewed to provide a greater extent of physiologically relevance in its measure of NHE1 inhibition (see Experimental Section). However, the platelet swelling assay measures the inhibition of osmotic swelling due to increased intracellular sodium concentration. Therefore, this indirect measurement of NHE1 activity was complemented by potency assessments in the pH_i assay to ensure a mechanistic link to the intracellular pH restoration.

Having identified the piperidine derivative **14** as a novel replacement of the piperazine structure of sabiporide, which provided uncompromised potency in both the pH_i and platelet swelling assays, we shifted our attention to the optimization of the heteroaryl left-hand side. We initially sought to replace the pyrrole moiety as this substructure has been associated with adverse drug reactions.[21,22]

In our examination of potential aryl replacements for the pyrrole substructure, the phenyl derivative **21** was found to provide a slight potency improvement in the platelet swelling assay (EC_{50} 109 nM compared to EC_{50} 241 nM for **1**) (Table 3). Evaluation of derivatives in which the aryl was substituted in the 4-position with nonpolar substituents, or which incorporated a biphenyl moiety, revealed reduced potency in the platelet swelling assay (EC_{50} 539, 1130, and 989 nM for compounds **23**, **24**, and **27**, respectively) (Table 3). In contrast, compounds **28**, **29**, and **30**, where the additional ring in biphenyl **27** was replaced by more polar five-membered heterocycles, showed improvement in potency relative to **27** (EC_{50} 368, 353, and 169 nM for **28**, **29**, and **30**, respectively), suggesting the low potency of the larger nonpolar substituents is due to a polarity mismatch rather than a steric clash with the ion channel. On the basis of this data, it was hypothesized that increased polarity (as can be measured by xlogP,[26] see Table 3) may improve the potency. Following this hypothesis, the compounds **31** through **43** were evaluated to reveal modest improvements in potency, with the compounds incorporating the most polar left-hand sides being the most potent compounds in this class (EC_{50} 36, 25, and 23 nM, for **41**, **42**, and **43** compared to sabiporide EC_{50} 172 nM). Several five-membered heterocycle replacements were also explored, giving modestly improved potency (**44**–**46**). To determine if there was any regioisomeric preference for the aryl substitution, several compounds were synthesized in all three regioisomers. The potencies of these compounds suggested no strong preference for placement of the left-hand side aryl substituent

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Table 4. CYP450 Isoform Inhibition, Stability in Rat and Human Liver Microsomes, and PAMPA Permeability Rating for Pyrrole Replacement Compounds with Potency 200 nM or Better in the hPSA Assay Showing Improved Overall Profiles with Increasing Polarity of Left-Hand Side Amide

Compound	R	hPSA IC_{50} (nM)	CYP inhibition IC_{50} (2C19/2C9/2D6/3A4) (μM)	rLM/hLM ($\%Q_H$)	PAMPA Rating[a]	xlogP
21		109	5/7/>30/>30	46/67	HIGH	3.26
22		184	11/8/>30/6	30/32	HIGH	3.42
31		138	NT	11/27	LOW	2.85
32		152	15/13/>30/11	20/<11	HIGH	2.98
33		112	7/19/>30/22	<5.6/<11	HIGH	2.98
34		200	16/4/10/>30	<6/40	HIGH	2.98
35		93	>30/>30/>30/15	<5.6/<11	LOW	2.78
36		50	>30/>30/>30/>30	14/15	LOW	2.78
38		70	0.06/0.4/11/>30	12/<11	HIGH	2.01
39		32	>30/13/>30/>30	<6/33	LOW	2.01
40		79	26/3/27/>30	<6/49	HIGH	2.1
41		36	28/4/12/24.5	<5.6/24	LOW	2.15
42		25	>30/29/>30/>30	10/18	LOW	2.33
43		23	>30/16/>30/24	7/24	LOW	1.61
44		115	NT	78/68	LOW	1.99
45		111	16/18/>30/26	12/47	HIGH	1.52
46		62	15/25/>30/3	<5.6/23	LOW	1.52

[a]PAMPA rating was assigned as LOW if the permeation velocity was $<10^{-6}$ cm/s.

(compounds **24**–**26**, **32**–**34**, and **35**–**37**). The apparent flat structure–activity relationship suggested to us a lack of specific interactions such as hydrogen bonds or dipole–dipole attractions between the left-hand side aryl moiety and the NHE1 channel.

Compounds with potencies in the platelet swelling assay of less than 200 nM were examined for their propensity to inhibit cytochrome P450 enzymes (CYP 450) and for their metabolic stability in human and rat liver microsomes (Table 4). In general, selectivity against CYP 450 enzyme inhibition and metabolic stability in liver microsomal fractions was improved with increasing polarity of the amide substituent. Notable exceptions were provided by the pyrazole **44** (likely due to facile pyrazole *N*-demethylation in microsomes) and the pyridine **38** (for which the pyridine moiety was likely a source of potent CYP inhibition).[27] The 4-methylsulfonyl derivative **35** was selected as a class representative for evaluation of its pharmacokinetic profile in the rat based on its low degree of CYP450 inhibition (IC_{50}s >30, >30, >30, and 15 μM for the 2C19, 2C9, 2D6, and 3A4 isoforms, respectively), acceptable potency (EC_{50} 93 nM), and high metabolic stability in microsomes (<5.6% Q_h in rat liver microsomes and <11 Q_h in human liver microsomes). Surprisingly, **35** was rapidly cleared in the rat with a rate of 66% of hepatic blood flow (Q_h) and its bioavailability (F) was somewhat low at 15%. A lack of cellular permeability, possibly due to the polar left-hand side, could explain the observed low bioavailability of **35**. To test this hypothesis, **35** was profiled in the PAMPA and Caco-2 permeability assays, which revealed low permeability and a high efflux ratio of 8 for this methylsulfonyl derivative.

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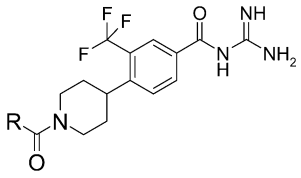
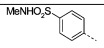
Subsequently, compounds with good metabolic stability and CYP inhibition were therefore tested in the PAMPA assay, and it was found that the more polar analogues generally displayed low permeability (Table 4).

At this point, we faced the rather discouraging proposition that the SAR for NHE-1 potency appeared to be divergent with that of acceptable membrane permeability and oral bioavailability. The examination of the compounds for which permeability in the PAMPA assay was poor (**31**, **35**, **36**, **41**–**44**, and **46**) revealed that the majority of these contained hydrogen bond donor moieties such as sulfonamides and amides. Analogues of these compounds were therefore synthesized in which these NH and OH groups were "capped" with methyl groups to determine if permeability could be rescued without negatively affecting potency (Table 5).

Table 5. pH_i and hPSA Potencies and PAMPA Permeability Ratings for Methylated Analogues of Phenol 31, Primary Amide 41, and Sulfonamide 43 Illustrating That Capping of the Hydrogen-Bond Donor Group Failed to Balance Potency and Permeability



Compound	R	pH_i change IC_{50} (nM)	hPSA IC_{50} (nM)	PAMPA Rating
47		132	498	HIGH
48		52	214	LOW
49		50	77	LOW
50		30	88	LOW
51		36	241	HIGH

However, in cases where the permeability was in fact restored, potency was found to be attenuated to unacceptable levels (**47** and **51** methyl capped analogues of **31** and **43**). In the other cases, an improvement in permeability was not achieved with capping of the hydrogen-bond donor group.

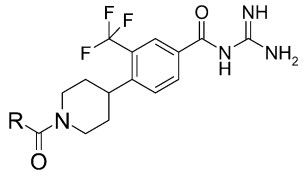
Increased polarity of substituents on the left-hand side arylamide afforded improvements in CYP inhibition and microsomal stability and resulted in more potent inhibition of NHE1. However, polarity on the left-hand side was found to negatively affect the permeability and oral bioavailability. Attempts to modify compounds with low permeability had not resulted in compounds with acceptable potency and permeability, and therefore a change in strategy was necessary. Considering the totality of the data available, we hypothesized that moving away from the aryl amides to alkyl amides may offer an advantage. The alkyl amides should allow for more compact structures that would allow for higher polarity, as was seen to be beneficial for potency, without large increases in polar surface area which was likely a contributor to the permeability problems faced by the more polar aryl amides.

To examine the effect of alkyl amides as a replacement for the left-hand side pyrrole of **1**, compounds **52**–**60** were evaluated in the platelet swelling assay and the pH_i assay. More extended alkyl amides exhibited only moderate potency (EC_{50} 201–416 nM for compounds **52**–**54**, Table 6). However, our evaluation of truncated alkyl amides revealed these to be potent inhibitors of NHE1 both in the platelet swelling assay and in the pH_i assay (EC_{50} 127, 79, 50, 31, and 31 nM for compounds **55**, **56**, **57**, **59**, and **60**, respectively) with the methyl and ethyl amides (**59** and **60**) exhibiting the most potent inhibition of NHE1 so far observed. To understand the potential of the alkyl amide derivatives to provide acceptable permeability, these compounds were evaluated in the PAMPA assay, which revealed permeability to be high for all compounds tested (Table 6). Interestingly, once again, the inhibitory potency against NHE1 as assessed in the platelet swelling assay could be improved modestly by the addition of polarity to the lipophilic alkyl group as in the case of the methoxyacetyl **58** (EC_{50} 48 nM) versus the *n*-propyl compound **54** (EC_{50} 201 nM). The CYP inhibition profiles and microsomal stability were acceptable for the *tert*-butyl and methoxyacetyl amides **55** and **58** and excellent for the ethyl and acetyl amides **59** and **60** (Table 6), with acetyl amide **60** having an IC_{50} >30 μM across all CYP isoforms tested. In contrast, for the *iso*-propyl amide derivative **56**, inhibition of the CYP isoforms 2C9 and 3A4 was significant (7 and 0.4 μM, respectively), and for cyclopropyl **57**, CYP2C19 and 2C9 displayed significant inhibition (5 and 6 μM, respectively).

Having identified the potent and permeable alkyl amides **59** and **60**, several core substituents were explored in an attempt to expand the structural scope of compounds for advancement. The efforts by others, leading to the discovery of cariporide, had demonstrated that the inclusion of a methyl substituent in the 6-position of the core aryl resulted a modest improvement in potency.[24] In our case, the inclusion of a 6-methyl substituent did not provide a similar improvement (compound **78** as compared to **60**; EC_{50} 28 nM versus 31 nM, respectively). The inclusion of a 6-methoxy substituent was also examined (compound **79**), resulting in a decrease in potency of more than 10-fold compared to the parent compound **60** (EC_{50} 432 nM versus 31 nM, respectively). The cariporide core substituent SAR had been examined in the context of a methylsulfone rather than a trifluoromethyl core. It was therefore hypothesized that the potency may be improved by the incorporation of such a methylsulfone in combination with the 6-methyl substituent. The replacement of the trifluoromethyl of compounds **59** and **60** with a methylsulfone (compounds **80** and **81**), however, was found to result in compounds of lower potency (EC_{50} 31 and 31 nM for compounds **59** and **60**, respectively, versus 266 and 189 nM for **80** and **81**, respectively). However, as observed in the context of the cariporide SAR, the incorporation of the 6-methyl substituent now resulted in an approximately 3-fold potency improvement (compound **82**, EC_{50} 88 nM and compound **83** EC_{50} 56 nM) (Table 7).

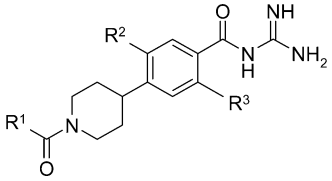
During the earlier optimization efforts leading to the discovery of **1**, it was found that methylsulfone bearing cores suffered from very low oral bioavailability which was attributed to rapid efflux of the compounds (data not shown). Before undertaking further exploration of the 6-methyl methylsulfone core compounds, compound **82** was tested in the Caco-2 permeability assay and found to have a high efflux ratio of 14. The pharmacokinetic profiles of compounds **82** and **83** were

Table 6. pH_i Potencies, hPSA Potencies, CYP450 Isoform Inhibition Potencies, Stability in Rat and Human Liver Microsomes, and PAMPA Permeability Ratings for Alkyl Amide Left-Hand Side Compounds



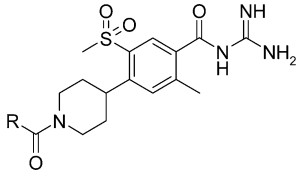
Compound	R	pH_i change IC_{50} (nM)	hPSA IC_{50} (nM)	CYP inhibition IC_{50} (2C19/2C9/2D6/3A4) (μM)	rLM/hLM (%Q_H)	PAMPA Rating
52		64	416	—	—	—
53	*i*-Bu	47	221	—	—	—
54	*n*-Pr	29	201	—	—	—
55	*t*-Bu	72	127	13/17/>30/13	<5.6/38	HIGH
56	*i*-Pr	28	79	ND/7/>30/0.4	39/<11	HIGH
57		16	50	5/6/17/16	<5.6/<11	HIGH
58		10	48	8/25/>30/>30	36/6	HIGH
59	Et	11	31	18/21/>30/26	26/<11	HIGH
60	Me	6	31	>30/>30/>30/>30	11/<11	HIGH

Table 7. pH_i and hPSA Potencies for Core Modifications of Compounds 59 and 60 as well as Caco-2 Efflux Ratio for 2-Methyl-5-methylsulfone Core Compound 82



compd	R^1	R^2	R^3	pH_i change IC_{50} (nM)	IC_{50} (nM) hPSA	Caco-2 efflux ratio (AB/BA)
78	Me	CF_3	Me	5	28	–
79	Me	CF_3	MeO	19	432	–
80	Et	$MeSO_2$	H	29	266	–
81	Me	$MeSO_2$	H	22	189	–
82	Et	$MeSO_2$	Me	15	88	14
83	Me	$MeSO_2$	Me	15	56	–

Table 8. Rat Pharmacokinetic Profiles of Methylsulfone Core Containing Compounds 82 and 83 Showing Low Bioavailability, Attributed to High Efflux



compd	clearance (%Q_H)	MRT (h)	Vdss (L/kg)	*F* (%)
82	54	0.3	0.95	1
83	47	0.36	1	4

evaluated in rat, and both compounds were found to suffer from very low oral bioavailability (Table 8, 4% and 1% *F*, respectively). This problem of efflux-driven low bioavailability was found to be general for the methylsulfone-containing cores (data not shown), and therefore work on these compounds did not proceed further. The 3-trifluoromethyl-6-methyl core compound (**78**), which maintains the potency of **60**, was next examined in CYP and hERG inhibition assays. For CYPs 3A4, 2C9, and 2D6, there was no significant inhibition (IC_{50} >30 μM), however, a modest inhibition of 2C19 did appear to be caused by the addition methyl group (IC_{50} 22 μM). More concerning, however, was that compound **78** showed significant hERG inhibition in the PatchXpress assay (IC_{50} 7 μM). As a result, this compound was not further pursued and efforts to explore the core SAR were abandoned.

Having identified a potent class of NHE1 inhibitors with potential for high permeability and selectivity against metabolic enzymes, we evaluated compounds from this class which had revealed acceptable CYP inhibition and both RLM and HLM stability for their clearance profiles in rat following iv administration and their propensity to inhibit the hERG channel (PatchExpress) (Table 9). The *para*-benzonitrile compound **32** exhibited rather potent hERG inhibition with an EC_{50} of 4 μM and was therefore eliminated from further profiling. From the remaining compounds, the ethyl and methyl amides **59** and **60** displayed low clearance (4.4 and 5.7% Q_h, respectively), while the *meta*-benzonitrile derivative **33** displayed a MRT similar to those of **59** and **60**, albeit with a substantial increase in volume of distribution (3.3 L/kg for compound **33** versus 1.0 and 0.76 L/kg for compounds **59** and **60**). Compounds **33**, **59**, and **60** were evaluated for their pharmacokinetic profiles following oral administration in both rats and dogs (Table 10). The bioavailabilities in rats for all three compounds were acceptable (ranging from 49% for compound **59** to 72% and 73% for compounds **33** and **60**),

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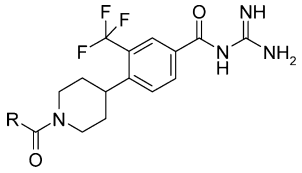
Table 9. hERG Inhibition and Rat i.v. Pharmacokinetics of Compounds with Acceptable in Vitro Profiles



			Rat i.v. PK		
Compound	R	hERG PatchExpress IC_{50} (μM)	Clearance (%Q_H)	Vdss (L/kg)	MRT (h)
32		4	19	6.5	8.5
33		>30	16	3.3	4.9
45		>30	31	1.4	1.0
55	*t*-Bu	27	29	1.8	1.5
58		>30	21	1.3	1.4
59	Et	>30	4.4	1.0	5.3
60	Me	>30	5.7	0.76	3.2

albeit with **59** displaying somewhat lower bioavailability compared to **33** and **60**. In dogs, **33** and **60** displayed reduced but still acceptable bioavailability (36% and 33%, respectively). Overall, the pharmacokinetic profiles in rat and dog were viewed to support the further advancement of compounds **33**, **59**, and **60**.

The attractive overall profiles of compounds **59** and **60** prompted the evaluation of these compounds for their selectivity against NHE2 and NHE3 using a pH_i assay format (Table 10). Both compounds, displayed >30-fold selectivity against NHE2 and with no measurable inhibitory activity against the NHE3 isoform (up to 16 μM concentration). While both **59** and **60** were attractive compounds based on their overall profile to this point, the modestly superior CYP inhibition profile of **60** resulted in the selection of this compound for further advanced profiling in preparation for in vivo pharmacology and toxicology studies. The potential for this compound to induce CYP 3A4 expression was assessed using the PXR induction assay in which **60** showed no significant induction of CYP3A4 (6% of rifampin at 10 μM). Compound **60** was also free of time-dependent CYP3A4 inactivation up to 50 μM. Testing in several in vitro toxicology assays also supported the further advancement of compound **60**, as it was found to be free of mutagenicity potential in the Ames assay both directly and after S9 incubation up to 5000 μg/plate, free of cytotoxicity up to 100 μM, and free of phospholipidosis potential in the Nile Red assay up to 50 μM.

Compound **60** was therefore examined in the Langendorff isolated perfused rat heart model[28] ex vivo to examine its ability to prevent or attenuate ischemia-reperfusion injury. Compound **60** was found to have a profound efficacy in this model, providing recovery of left ventricular developed pressure (LVDP) to levels of 100% and 36% of baseline pressure at perfusate concentrations of 100 and 10 nM, as measured 30 min postreperfusion. Furthermore, the administration of compound **60** provided a significantly beneficial effect on left-ventricular end diastolic pressure, preventing much of the increase seen postreperfusion for the vehicle control (Figure 6).

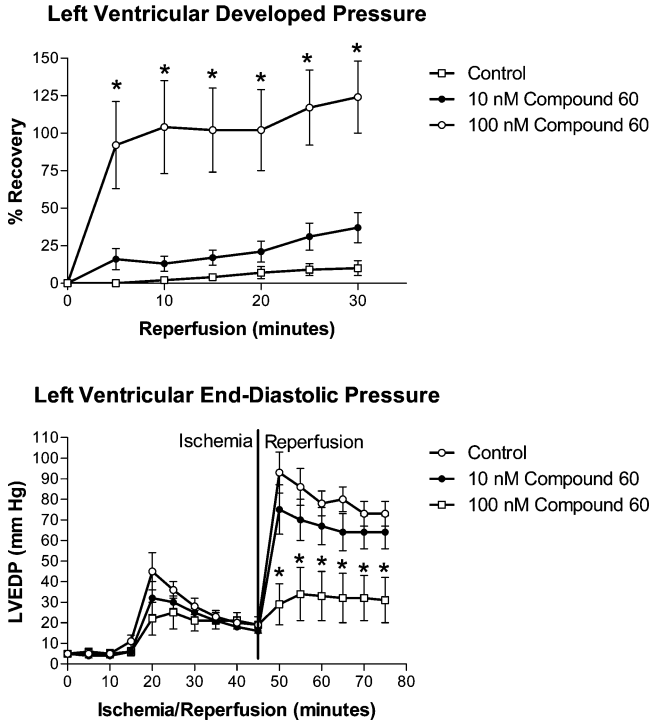


Figure 6. Left ventricular developed pressure (LVDP) and left ventricular end-diastolic pressure (LVEDP) for compound **60**. Data were analyzed using a repeated-measures ANOVA followed by a post hoc Tukey's test, $N = 6$, for all groups. For LVDP, values indicate the percentage recovery from baseline values obtained immediately before initiating ischemia. Preischemic baseline values for LVDP (in mm Hg) were 63 ± 5, 61 ± 7, and 59 ± 5 nM for control, and 10 and 100 nM compound **60**-treated hearts, respectively. Absolute values are shown for LVEDP, which were initially adjusted to approximately 5 mmHg as indicated in Experimental Section. *$P < 0.05$ from compound **60**-free group.

These effects were particularly remarkable in light of the fact that the platelet-swelling assay using rat platelets (rPSA) had revealed an EC_{50} for compound **60** of only 138 nM,

Table 10. Full Rat and Dog Pharmacokinetic Profile of Compounds with Acceptable hERG Potencies and MRT >3 h in Rat i.v. Pharmacokinetics

	rat				dog			
compd	clearance (%Q_H)	MRT (h)	Vdss (L/kg)	*F* (%)	clearance (%Q_H)	MRT (h)	Vdss (L/kg)	*F* (%)
33	16	3.3	4.9	72	18	17		36
59	4.4	5.3	1	49	8.1	14	2.0	49
60	5.7	3.2	0.76	73	13	6.2	1.4	33

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demonstrating that compound **60** exerts profound beneficial effects at concentrations approximating its EC_{50} value.

In summary, optimization of **1** beginning with replacement of the piperazine with a piperidine linker followed by optimization of the left-hand side amide afforded the acetyl compound **60** with high NHE1 potency, low DDI potential as measured by CYP inhibition, CYP 3A4 inactivation, and PXR mediated CYP 3A4 induction, low hERG potency with concomitant absence of effects in lengthening action potential duration, excellent pharmacokinetics in rat and dog, and remarkably potent activity in the isolated heart model of ischemia-reperfusion injury. Further pharmacological and toxicological profiling of this compound will be reported in due course.

EXPERIMENTAL SECTION

General Procedures. All reactions were performed at ambient temperature where not otherwise indicated. Unless otherwise indicated, extraction and washing steps were performed a single time. ^{1}H NMR spectra were recorded on a Bruker Avance (400 MHz unless otherwise indicated) spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard ($CDCl_3$, 7.24 ppm; DMSO-d_6, 2.52 ppm; CD_3OD, 3.34 ppm). Data are reported as follows: Chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, dm = doublet of multiplets, br = broad, vbr = very broad, m = multiplet, app = apparent), coupling constants (Hz), and integration. Flash chromatography was performed using prepacked silica gel columns (Analogix Flash chromatograph RS-12 cartridges) of the indicated solvent system unless otherwise indicated. Separation was achieved with a CombiFlash SQ16X chromatographer by Isco. HPLC purification was performed on a Gilson GX280 platform fitted with a Waters SunFire Prep C-18 OBD column (20 mm × 150 mm, 5 μm), Gilson 322 binary pump, and Gilson UV/vis 155 detector using a 20 mL/min flow rate, detection at 254 nM, and the indicated gradient unless otherwise indicated. For compounds **24**−**29** and **52**−**54**, HPLC purification was performed on a Waters 2767 platform fitted with a Waters 2525 gradient module, Waters 515 binary pump, Waters 2996 DAD, and Waters Micromass ZQ mass spectrometer fitted with a SunFire Prep C18 OBD column (19 mm × 150 mm, 5 μm) and running the following gradient (expressed as a percentage of acetonitrile in water with 0.1% formic acid) at a 20 mL/min flow rate: 0−3.5 min 10%, 3.5−13.0 min 10−85%, 13.0−13.5 min 85−95%, 13.5−15.0 min isocratic 95%. All compounds described were of >95% purity unless otherwise indicated. Compounds dosed in vivo were of >98% purity. Purity was confirmed by analytical LC/MS recorded on a system consisting of a Waters ZQ mass spectrometer, an Agilent 1100 DAD, Sedex 85 ELS detector, and an Agilent 1100 HPLC system, equipped with a Zorbax SB-C18 rapid resolution cartridge (4.6 mm × 30 mm, 3.5 μm). Elution started with 95% water (0.1% formic acid)/5% acetonitrile (0.1% formic acid) and ended with 95% acetonitrile (0.1% formic acid)/5% water (0.1% formic acid) and used a linear gradient at a flow rate of 2.5 mL/min. Purity of final compounds submitted to biological testing was assessed by HPLC recorded on a system consisting of an Agilent 1100 HPLC system equipped with a Zorbax Eclipse XDB-C8 column (4.6 mm × 150 mm, 3.5 μm) with a flow rate of 1 mL/min and a column temperature of 50 °C using the following two gradient conditions for all compounds: (1) 5−70% acetonitrile/water/0.1%TFA from 0 to 18 min, 18−26 min run isocratic at 70% acetonitrile/water/0.1%TFA; (2) 3090% acetonitrile/water/0.1% TFA from 0 to 14 min, 14−16 min 90% acetonitrile/water/0.1% TFA.

The following compounds were synthesized according to available literature procedures: *N*-carbobenzyloxyguanidine;[29] 6-benzyloxynicotinic acid,[30] pyrrole-2-carboxylic acid chloride,[31] 4-fluoro-3-trifluoromethylbenzoic acid methyl ester,[32] 4-bromo-3-methylsulfonyl-benzoic acid.[24] All commercially available chemicals were used as received and were purchased from Sigma-Aldrich except where indicated.

Computational Methods. Structural overlays were generated using Cresset Fieldalign (Fieldalign 3.0.0, revision 14780, copyright 2006−2010, Cresset Biomolecular Discovery Ltd., BioPark, Hertfordshire, Broadwater Road, Welwyn Garden City, Herts AL7 3AX, United Kingdom) and matching the acylguanidine functionality in each molecule. The graphics were generated using Maestro 9.1. from Schrödinger LLC, New York, New York, USA, (2010).

Synthetic Methods. *4-{3-[(1H-Pyrrole-2-carbonyl)-amino]-pyrrolidin-1-yl}-3-trifluoromethyl-benzoic Acid Methyl Ester (**11a**).* To a solution of 4-fluoro-3-trifluoromethyl-benzoic acid methyl ester (**8**, 5.0 g, 22.5 mmol) in dry DMSO (50 mL) was added K_2CO_3 (3.08 g, 23.3 mmol) and *tert*-butyl-pyrrolidin-3-yl-carbamate (**9a**) (TCI, 4.2 g, 22.5 mmol) at room temperature. The reaction mixture was heated at 95 °C overnight. The reaction mixture was diluted with water and extracted three times with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4, and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography eluting 20% ethyl acetate/petroleum-ether to give the desired product **10a** as a white solid with an unknown impurity still present. The impure residue was dissolved in methanol (50 mL) and saturated methanolic HCl (50 mL) was added at 0 °C. The reaction mixture was stirred overnight at room temperature, after which time the solvent was removed in vacuo and the residue was washed with diethyl ether (100 mL) to afford the piperidine hydrochloride salt product (5 g, 68% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.26 (br s, 2H), 8.13 (d, J = 1.8 Hz, 1H), 7.97 (dd, J = 1.9 Hz, 9.0 Hz, 1H), 7.06 (d, J = 9.0 Hz, 1H), 3.94−3.91 (m, 1H), 3.82 (s, 3H), 3.79−3.75 (m, 1H), 3.69−3.63 (m, 1H), 3.55−3.49 (m, 2H), 2.32−2.26 (m, 1H), 2.11−2.08 (m, 1H). MS (ES+): calcd for $C_{13}H_{14}F_3NO_3$ + H^+ 289.26, found 289.28.

To a partially dissolved mixture of the product from the previous step (1 g, 3.08 mmol) in dry THF (30 mL) was added HATU (1.2 g, 3.16 mmol) and pyrrole-2-carboxylic acid (0.324 g, 3.06 mmol), and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was cooled to 0 °C and diisopropylamine (1.2 mL, 15.40 mmol) was added, and the mixture was stirred overnight at room temperature. The solvent was removed in vacuo, and the residue was diluted with H_2O (50 mL) and extracted three times with ethyl acetate. The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (50 mL), dried over Na_2SO_4, and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography eluting 10% ethyl acetate/petroleum ether to afford the desired product as a white solid contaminated with 5 wt % TMU (0.75 g, 59% yield accounting for presence of TMU). ^{1}H NMR ($CDCl_3$): δ 9.31 (br s, 2H), 8.31 (s, 1H), 8.01 (d, J = 8.8 Hz, 1H), 6.94−6.90 (m, 2H), 6.55 (s, 1H), 6.24 (d, J = 3.5 Hz, 1H), 6.00 (d, J = 7.6 Hz, 1H), 4.76 (d, J = 5.9 Hz, 1H), 3.90 (s, 3H), 3.79−3.75 (m, 1H), 3.71−3.66 (m, 1H), 3.54−3.39 (m, 1H), 2.40−2.35 (m, 1H), 2.10−2.04 (m, 1H). MS (ES+): calcd for $C_{18}H_{17}F_3N_3O_3$ + H^+ 382.36, found 382.26.

*4-{3-[(1H-Pyrrole-2-carbonyl)-amino]-pyrrolidin-1-yl}-3-trifluoromethyl-benzoic Acid (**12a**).* To a solution of **11a** (0.75 g, 1.97 mmol) in THF/H_2O (9/1) was added LiOH (0.25 g, 5.91 mmol), and the resulting mixture was stirred overnight. The solvent was removed in vacuo, and the residue was diluted with H_2O and extracted with ether. The aqueous phase was acidified with 10% aqueous citric acid, and the resulting precipitate was collected by filtration and washed with ether to give pure desired product as a white solid (0.65 g, 89%). ^{1}H NMR (DMSO-d_6): δ 13.0−12.4 (vbr s, 1H), 11.4 (s, 1H), 8.11−8.09 (m, 2H), 7.93 (d, J = 8.8 Hz, 1H), 7.03 (d, J = 7.0 Hz, 1H), 6.86 (s, 1H), 6.82 (s, 1H), 6.08 (d, J = 2.3 Hz, 1H), 4.52−4.49 (m, 1H), 3.75−3.71 (m, 1H), 3.58−3.53 (m, 2H), 3.44−3.40 (m, 1H), 2.24−2.20 (m, 1H), 2.19−2.04 (m, 1H). MS (ES+): calcd for $C_{17}H_{15}F_3N_3O_3$ + H^+ 368.33, found 368.26.

General Procedure for Mukuyama Coupling. To a solution of the desired acid (1 equiv) in NMP (approximately 0.2 M concentration) was added 2-chloro-1-methylpyridinium iodide (1.2 equiv), and the resulting solution was stirred for 30 min at room temperature and then

at 50 °C for 30 min. The dark-orange solution was cooled to room temperature, and either BOC or CBZ protected guanidine (1.3 equiv) and DIEA (3 equiv) were added. The resulting mixture was stirred overnight, after which time the solvent was removed in vacuo. The mixture was partitioned between water and ethyl acetate, and the phases were separated. The organic phase was washed twice with water, once with brine, dried over Na_2SO_4, and filtered, and the solvent was removed in vacuo. The crude material was purified via silica gel chromatography using the indicated eluent.

*1H-Pyrrole-2-carboxylic Acid [1-(4-(N-Carbobenzyloxy)-guanidinocarbonyl-2-trifluoromethyl-phenyl)-pyrrolidin-3-yl]-amide (**13a**).* Synthesized according to the Mukuyama coupling general procedure using **12a** (50.0 mg, 0.136 mmol). The crude material was purified via silica gel chromatography using a gradient elution of 0−80% ethyl acetate/hexanes to afford the desired product (46.2 mg, 63%) as a glassy solid. ^{1}H NMR (DMSO-d_6): δ 11.5 (s, 1H), 8.37 (s, 1H), 8.12−8.06 (m, 2H), 7.42−7.35 (m, 5H), 7.02 (d, 1H, J = 8.9 Hz), 6.88−6.86 (m, 1H), 6.85−6.81 (m, 1H), 6.08 (app dd, J = 2.4 Hz, 5.9 Hz, 1H), 5.19 (s, 1H), 4.50 (app quintet, J = 6.0 Hz, 1H), 3.74−3.70 (m, 1H), 3.59−3.50 (m, 1H), 3.44−3.40 (m, 1H), 2.25−2.17 (m, 1H), 2.09−2.03 (m, 1H). MS (ES+): calcd for $C_{26}H_{24}F_3N_6O_4$ + H^+ 543.52, found 543.47.

General Procedure for the Removal of the CBZ Protecting Group. To a solution of CBZ-guanidine compound (1 equiv) in ethanol under an argon atmosphere was added 20 wt % $Pd(OH)_2$ on carbon (3 mol %) or 10 wt % Pd on carbon (10 mol %), and the mixture was stirred under a hydrogen atmosphere for 3−16 h. The mixture was filtered through Celite, and the solvent was removed in vacuo. The resulting residues were purified by the methods indicated for the specific compounds.

*1H-Pyrrole-2-carboxylic Acid [1-(4-Guanidinocarbonyl-2-trifluoromethyl-phenyl)-pyrrolidin-3-yl]-amide (**5**).* The CBZ protecting group of **13a** was removed according to the general procedure using $Pd(OH)_2$ on carbon in ethanol (4.0 mL) for 4 h. The crude residue was dissolved in minimal 10% methanol/CH_2Cl_2 and precipitated using ether. The resulting precipitate was collected by filtration and triturated with ether to give the desired product as a white solid (10.5 mg, 28%). ^{1}H NMR (DMSO-d_6): δ 11.41 (s, 1H), 11.36 (s, 1H), 8.47−8.22 (br m, 4H), 8.18 (d, J = 2.1 Hz, 1H), 8.07 (d, J = 6.5 Hz, 1H), 8.05−8.00 (m, 1H), 6.81−6.77 (m, 1H), 6.77−6.71 (m, 1H), 6.04−5.98 (m, 1H), 4.52−4.40 (m, 1H), 3.76−3.66 (m, 1H), 3.64−3.47 (m, 2H), 3.45−3.37 (m, 1H), 2.27−2.10 (m, 1H), 2.10−1.96 (m, 1H). MS (ES+): calcd for $C_{18}H_{19}F_3N_6O_2$ + H^+ 409.39, found 409.40.

*4-{3-[(1H-Pyrrole-2-carbonyl)-amino]-piperidin-3-yl}-3-trifluoromethyl-benzoic Acid Methyl Ester (**11b**).* Following the procedure for the first two steps of the synthesis of compound **11a** using as the starting material **8** (7 g, 31.5 mmol) and *tert*-butyl piperidin-3-yl-carbamate (**9b**) (Oakwood, 6.31 g, 31.5 mmol) afforded the desired product as a white solid (4.00 g, 41% over two steps) ^{1}H NMR (DMSO-d_6): δ 8.21 (d, J = 8.4 Hz, 1H), 8.16 (s, 1H), 7.99 (br s, 2H), 7.60 (d, J = 8.4 Hz, 1H), 3.88 (s, 3H), 3.24 (m, 1H), 3.02 (d, J = 11.8 Hz, 1H), 2.81−2.79 (m, 2H), 2.03 (m, 1H), 1.86−1.83 (m, 1H), 1.64−1.61 (m, 1H), 1.48 (m, 1H). MS (ES+): calcd for $C_{14}H_{17}F_3N_2O_2$ + H^+ 303.30, found 303.26.

Using the product of the previous step as starting material (1 g, 2.95 mmol) and employing the procedure for **11a** step 3 afforded the desired product as a white solid contaminated with 9 wt % tetramethylurea (TMU) (0.9 g, 71% yield accounting for presence of TMU). ^{1}H NMR ($CDCl_3$): δ 9.27 (s, 1H), 8.35 (d, J = 1.8 Hz, 1H), 8.21 (dd, J = 1.6 Hz, 8.2 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 1.1 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H), 6.63 (s, 1H), 6.27 (dd, J = 2.5 Hz, 6.2 Hz, 1H), 3.94 (s, 3H), 3.21−3.18 (m, 1H), 3.14 (d, J = 10.7 Hz, 1H), 3.03−3.00 (m, 1H), 2.76−2.73 (m, 1H), 2.04−1.97 (m, 2H), 1.69−1.64 (m, 2H). MS (ES+): calcd for $C_{19}H_{20}F_3N_3O_3$+ H^+ 396.38, found 382.26 (pdt − Me + H^+).

*4-{3-[(1H-Pyrrole-2-carbonyl)-amino]-piperidin-3-yl}-3-trifluoromethyl-benzoic Acid (**12b**).* Following the procedure for **12a** using as the starting material **11b** (0.90 g, 2.12 mmol) afforded the desired product as a white solid (0.75 g, 87%). ^{1}H NMR (DMSO-d_6): δ 11.4 (s, 1H), 8.13 (m, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.53 (d, J = 9.0 Hz, 1H), 6.84 (s, 1H), 6.77 (s, 1H), 6.07 (d, J = 3.5 Hz, 1H), 3.17 (d, J = 4.9 Hz, 1H), 3.11−3.09 (m, 1H), 2.80−2.72 (m, 1H), 2.69−2.57 (m, 1H), 1.92−1.89 (m, 1H), 1.84−1.81 (m, 1H), 1.69−1.66 (m, 1H), 1.54−1.51 (m, 1H). MS (ES+): calcd for $C_{18}H_{18}F_3N_3O_3$ + H^+ 382.36, found 382.31

*1H-Pyrrole-2-carboxylic Acid [1-(4-Guanidinocarbonyl-2-trifluoromethyl-phenyl)-piperidin-3-yl]-amide (**6**).* The required protected acyl guanidine intermediate **13b** was synthesized according to the Mukuyama coupling general procedure using **12b** (100 mg, 0.262 mmol) and was used without purification in the next step. The residue was dissolved in 1,4-dioxane (2.0 mL), and 4 M HCl in 1,4-dioxane (1.5 mL) was added. The reaction was stirred for 60 h, at which time the solvent was removed in vacuo. The residue was purified by preparative HPLC eluting 0−75% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (44 mg, 40% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.44 (s, 1H), 11.38 (s, 1H), 8.18 (s, 1H), 8.13 (dd, J = 2.2 Hz, 8.6 Hz, 1H), 7.77 (d, J = 7.8 Hz, 9.0 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H,), 6.85 (s, 1H), 6.78 (s, 1H), 6.08 (s, 1H), 4.04−4.02 (m, 1H), 3.34 (d, J = 8.6 Hz, 1H,), 3.19 (app d, J = 12.0 Hz, 1H,), 2.83 (t, J = 9.3 Hz, 1H,), 2.73 (t, J = 10.4 Hz, 1H,), 1.94−1.83 (m, 2H), 1.72−1.51 (m, 2H). MS (ES +): calcd for $C_{19}H_{21}F_3N_6O_2$ + H^+ 423.41, found 423.44.

*4-{4-[(1H-Pyrrole-2-carbonyl)-amino]-piperidin-1-yl}-3-trifluoromethyl-benzoic Acid (**12c**).* Following the procedure for the first two steps of the synthesis of **11a** using as the starting material piperidin-4-yl-carbamic acid *tert*-butyl ester (Astatech, **9c**, 0.43 g, 2.25 mmol) and **8** (0.50 g, 2.25 mmol) afforded crude **10c** as a yellow solid. HCl gas was bubbled through a solution of crude **10c** in methanol (25 mL) at 0 °C for 5 min. The reaction mixture was then stirred overnight at room temperature. The solvent was removed in vacuo, and the residue was washed with diethyl ether (100 mL) to afford the title compound as a yellow solid (0.30 g, 44% over two steps). ^{1}H NMR (DMSO): δ 8.24 (partially obscured br s, 2H), 8.20−8.11 (m, 2H), 7.57 (d, J = 8.40 Hz, 1H), 3.87 (s, 3H), 3.25−3.12 (m, 3H), 2.92−2.80 (m, 2H), 2.09−1.99 (m, 2H), 1.80- 1.60 (m, 2H). MS (EI): calcd for $C_{14}H_{17}F_3N_2O_2$ + H^+ 303.30, found 303.10.

Following the procedure for the third step of the synthesis of **11a** using as starting material the product of the previous step (1.00 g, 2.95 mmol) afforded crude **11c** as a white solid. Crude **11c** (1.00 g, 2.52 mmol) was subjected to the same procedure as for **12a** to afford the title compound as an off-white solid (0.75 g, 67% over two steps). ^{1}H NMR (DMSO): δ 13.19 (broad, 1H), 11.39 (s, 1H), 8.15 (partially obscured d, J = 8.0 Hz, 1H), 8.14 (s, 1H), 7.90−7.82 (m, 1H), 7.58 (d, J = 8.4 Hz, 1H), 6.83 (s, 2H), 6.07 (s, 1H), 3.95 (br s, 1H), 3.22−3.12 (m, 2H), 3.10−2.85 (m, 2H), 1.95−1.82 (m, 2H), 1.80−1.60 (m, 2H). MS (EI): calcd for $C_{18}H_{18}F_3N_3O_3$ + H^+ 382.34, found 382.1.

*1H-Pyrrole-2-carboxylic Acid [1-(4-Guanidinocarbonyl-2-trifluoromethyl-phenyl)-piperidin-4-yl]-amide (**7**).* Following the procedure for **13a** using as the starting material **12c** (23.0 mg, 0.06 mmol) afforded crude **13c** as a white solid. This crude material (0.02 g, 0.32 mmol) was treated as in the procedure for **6** to the title compound as a white solid (10.0 mg, 47% over two steps). ^{1}H NMR (400 MHz, DMSO): δ 11.72−11.41 (m, 2H), 8.75−8.32 (m, 4H), 8.25 (d, J = 8.4 Hz, 1 H), 8.20 (s, 1H) 7.68- 758 (d, J = 8.92 Hz, 1H), 6.90 (s, 1H), 5.62−5.40 (m, 1H), 4.15−3.90 (m, 1H), 3.85−3.60 (m, 2H), 3.60−3.18 (obscured by water peak, m, 1H), 2.45−2/07 (m, 2H). MS (EI): calcd for $C_{18}H_{18}F_3N_5O_3$ + H^+ 410.37, found 410.36.

*4-Bromo-3-trifluoromethyl-benzoic Acid Methyl Ester (**15**).* To a solution of 4-bromo-3-trifluoromethyl-benzoic acid (3B Scientific, 115 g, 428 mmol) in methanol (400 mL) was added concentrated sulfuric acid (2 mL). The mixture was sealed and heated at 80 °C overnight. The mixture was cooled to room temperature, and the solvent was removed in vacuo. The residue was treated with water, and the resulting solid was collected by filtration to afford the desired product as a colorless solid (121 g, 98%). ^{1}H NMR (CDCl3): δ 8.21 (d, J = 2.0 Hz, 1H), 7.90 (dd, J = 2.0 Hz, 8.1 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 3.82 (s, 3H). MS (EI): calcd for $C_9H_6BrF_3O_2$ + H^+ 282.95, 284.95, found 283.00 and 285.00.

*4-(4-Methoxycarbonyl-2-trifluoromethyl-phenyl)-3,6-dihydro-2H-pyridine-1-carboxylic Acid tert-Butyl Ester (**17**).* To a solution of

15 (17.3 g, 61.2 mmol) in 1,4-dioxane (250 mL) were added 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-3,6-dihydro-2*H*-pyridine-1-carboxylic acid *tert*-butyl ester (**16**, Digital Specialty Chemicals, 18.9 g, 61.2 mmol), 2 M aqueous potassium carbonate (61.2 mL, 122 mmol), and tetrakistriphenylphosphinepalladium(0) (Strem, 7.07 g, 6.10 mmol). The reaction vessel was sealed and heated to 140 °C for 4 h. The mixture was cooled to room temperature, and the dioxane was removed in vacuo. The resulting residue was diluted with water and ethyl acetate and filtered to remove insoluble material, and the phases were separated. The aqueous phase was extracted twice with ethyl acetate, the combined organics were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo. The brown residue was then passed through a short bed of silica rinsing first with 10% ethyl acetate in hexane, then 20% ethyl acetate in hexane. The material collected from the 20% ethyl acetate fraction was evaporated to afford the desired product as a light-yellow solid (19.6 g, 83%). ^{1}H NMR (DMSO-d_6): δ 8.35 (d, J = 1.5 Hz, 1H), 8.17 (dm, J = 7.6 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 5.64 (s, 1H), 4.06 (q, J = 3.0 Hz, 2H), 3.98 (s, 3H), 3.65 (t, J = 5.6 Hz, 2H), 2.34 (br s, 2H), 1.52 (s, 9H). MS (ES+): calcd for $C_{19}H_{22}F_3NO_4$ − tBu + CH_3CN + H^+ 371.11, found 371.51.

*4-(4-Methoxycarbonyl-2-trifluoromethyl-phenyl)-piperidine-1-carboxylic Acid tert-Butyl Ester (**18**).* To a solution of **17** (13.6 g, 35.3 mmol) in acetic acid (180 mL) under an argon atmosphere was added platinum(IV) oxide (750 mg, 3.3 mmol), and the mixture was stirred under a hydrogen atmosphere for 60 h. The mixture was filtered through Celite, and the solvent was removed in vacuo to give the desired product as a foam (13.5 g, 99%). ^{1}H NMR (DMSO-d_6): δ 8.16 (s, 1H), 8.14 (s, 1H), 7.82 (d, J = 8.8 Hz, 1H), 4.11−4.08 (m, 2H), 3.02−3.01 (m, 1H), 2.94−2.70 (m, 2H), 1.67−1.64 (m, 4H), 1.42 (s, 9H). MS (ES+): calcd for $C_{19}H_{25}F_3N_2O_4$ − Me + H^+ 373.39, found 373.33.

*4-(4-Carboxy-2-trifluoromethyl-phenyl)-piperidine-1-carboxylic Acid tert-Butyl Ester (**19**).* To a solution of **18** (5.10 g, 13.2 mmol) in methanol (39 mL) and water (13 mL) was added potassium carbonate (3.64 g, 26.3 mmol), and the reaction was stirred for 16 h at room temperature. The methanol was removed in vacuo, the residue was poured into dilute aqueous hydrochloric acid, and the desired product was isolated by filtration (5.0 g, 100%). ^{1}H NMR (DMSO-d_6): δ 8.14 (s, 1H), 8.12 (s, 1H), 7.77 (d, J = 8.0 Hz, 1H), 4.10 (br d, J = 10.7 Hz, 2H), 3.16−3.00 (m, 1H), 2.90−2.75 (m, 2H), 1.67−1.61 (m, 4H), 1.42 (s, 9H). MS (ES+): calcd for $C_{18}H_{22}F_3NO_4$ + H^+ 374.38, found 374.60.

*N-(4-Piperidin-4-yl-3-trifluoromethyl-benzoyl)-(N′-(carbobenzyloxy)-guanidine (**20a**).* Synthesized according to the Mukuyama coupling general procedure using **19** (2.60 g, 6.96 mmol). The crude residue was purified via flash chromatography eluting 0−80% ethyl acetate/hexanes to give the desired CBZ-guanidine as a pale-orange oil (3.06 g, 80%). ^{1}H NMR (DMSO-d_6): δ 11.22 (br s, 1H), 9.80 (br s, 1H), 8.74 (br s, 1H), 8.41 (d, J = 1.6 Hz, 1H), 8.26 (dd, J = 1.2 Hz, 8.2 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.46−7.34 (m, 5H), 5.23 (s, 2H), 4.11 (br d, J = 10.0 Hz, 2H), 3.12−2.96 (m, 1H), 2.90−2.72 (m, 2H), 1.68−1.59 (m, 4H), 1.43 (s, 9H). MS (ES+): calcd for $C_{27}H_{31}F_3N_4O_5$ + H^+ 549.57, found 549.20.

To a solution of the product of the previous step (3.06 g, 5.58 mmol) in 1,4-dioxane (25 mL) was added 4 M hydrogen chloride in 1,4-dioxane (14.0 mL, 55.8 mmol), and the mixture was stirred for 16 h. The mixture was concentrated to give pure product as a white powder as the hydrochloride salt (2.70 g, 100%). ^{1}H NMR (DMSO-d_6): δ 10.16 (br s, 1H), 9.40−9.60 (m, 2H), 9.21−9.19 (m, 1H), 8.46 (s, 1H), 8.44 (d, J = 8.2 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.47−7.36 (m, 5H), 5.30 (s, 2H), 3.38−3.35 (m, 2H), 3.23 (t, J = 11.8 Hz, 1H), 3.06 (app q, J = 11.9 Hz, 2H), 2.22−2.19 (m, 2H), 1.84−1.81 (m, 2H). MS (ES+): calcd for $C_{22}H_{23}F_3N_4O_3$ + H^+ 449.57, found 449.20.

General Procedure for Amide Bond Coupling with CDI. To a solution of benzoic acid derivative (1.10 equiv) in DMF was added CDI (1.10 equiv), and the mixture was stirred for 30−60 min. **20a** or **20b** was then added (1.00 equiv), followed by DIEA (3.0 equiv), and the reaction was stirred overnight at room temperature. The resulting mixture was either diluted with water or dilute HCl solution and filtered to give the desired the product or diluted with ethyl acetate and washed with water three times followed by brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting coupling products were either used crude in the next step or purified by flash chromatography as indicated.

*N-{4-[1-(1H-Pyrrole-2-carbonyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine, Hydrochloride (**14**).* The CBZ-protected amide intermediate was obtained using the CDI coupling general procedure from pyrrole-2-carboxylic acid (75.7 mg, 0.682 mmol) in *N*,*N*-dimethylformamide (10 mL) and **20a** (300 mg, 0.619 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using Pd on carbon in a mixture of ethanol and ethyl acetate (10:2, 4 mL) for 16 h. The residue was purified by two serial flash chromatography purifications eluting 0−40% methanol in dichloromethane. The resulting pure residue was treated with 4N HCl in 1,4-dioxane to give the desired compound as a white solid (54 mg, 20% over two steps). ^{1}H NMR (400 MHz, MeOD): δ 8.28−8.21 (m, 1H), 8.26−8.12 (m, 1H), 7.63 (d, J = 8.16 Hz, 1H), 6.90−6.80 (m, 1H), 6.66−6.58 (m, 1 H), 6.26−6.17 (m, 1H), 4.70−4.62 (m, 2H), 3.40−3.25 (m, 1H), 3.10−2.95 (m, 2H), 1.91−1.74 (m, 4H). MS (EI): calcd for $C_{19}H_{20}F_3N_5O_2$+ H^+ 408.40, found 408.56.

*N-[4-(1,2,3,6-Tetrahydro-pyridin-4-yl)-3-trifluoromethyl-benzoyl]-N′-(carbobenzyloxy)-guanidine (**20b**).* To a solution of **17** (2.74 g, 7.10 mmol) in methanol (40.0 mL) was added water (4.00 mL) and potassium carbonate (2.46 g, 17.8 mmol), and the reaction mixture was heated at 60 °C for 2 h. The mixture was then diluted with ethyl acetate (200 mL) and water (100 mL). The aqueous phase was brought to a pH of about 4 using 1N aqueous hydrochloric acid. The phases were separated, and the aqueous phase was extracted twice with ethyl acetate. The combined organic phase were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo to yield the desired carboxylic acid as a colorless solid (2.48 g, 94%). ^{1}H NMR ($CDCl_3$): δ 8.33 (d, J = 1.3 Hz, 1H), 8.15 (dd, J = 1.5 Hz, 8.0 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 5.57 (s, 1H), 3.99−3.98 (m, 2H), 3.56 (t, J = 5.6 Hz, 2H), 2.31−2.30 (m, 2H), 1.44 (s, 9H). MS (ES+): calcd for $C_{16}H_{14}F_3N_2O_4$ − tBu + CH_3CN + H^+ 357.10, found 357.46.

The required CBZ-protected guanidine intermediate was synthesized according to the Mukuyama coupling general procedure using the product of the previous step (2.48 g, 6.68 mmol). The crude material was purified via silica gel chromatography using a gradient elution of 0−40% ethyl acetate/hexanes to afford the desired CBZ-protected guanidine intermediate (3.37 g, 92%) as a glassy solid. ^{1}H NMR ($CDCl_3$): δ 8.47−8.40 (m, 2H), 7.47−7.38 (m, 6H), 5.63 (br s, 1H), 4.06 (s, 2H), 3.73−3.63 (m, 2H), 2.47−2.29 (m, 2H), 1.52 (s, 9H). MS (ES+): calcd for $C_{27}H_{30}F_3N_4O_5$ + H^+ 548.22, found 547.83.

To a solution of the product of the previous step (3.37 g, 6.20 mmol) in dichloromethane (24 mL) was added 4 N hydrogen chloride in 1,4-dioxane (25 mL, 100 mmol), and the reaction was stirred for 2 h. The mixture was diluted with ether (200 mL) and filtered to give the desired product as a colorless solid (2.80 g, 94%). ^{1}H NMR (CD_3OD): δ 8.41 (s, 1H), 8.34 (br s, 1H), 7.64 (br s, 1H), 7.56−7.38 (m, 5H), 5.77 (s, 1H), 5.37 (s, 2H), 3.85 (br s, 2H), 3.47 (br s, 2H), 2.66 (br s, 2H). MS (ES+): calcd for $C_{22}H_{21}F_3N_4O_3$ + H^+ 447.43, found 447.77.

*N-{4-[1-(4-Trifluoromethyl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**23**).* The required CBZ-protected acylguanidine was obtained using the CDI coupling general procedure from 4-trifluoromethyl-benzoic acid (43.3 mg, 0.228 mmol) in *N*,*N*-dimethylformamide (3 mL) and **20b** (100 mg, 0.207 mmol). The desired product was obtained following aqueous workup and flash chromatography eluting 0−60% ethyl acetate/hexanes (103 mg, 80%). ^{1}H NMR (DMSO-d_6): δ 9.03 (br s, 1H), 8.62 (br s, 1H), 8.45 (s, 1H), 8.22 (d, J = 7.2 Hz, 1H), 7.71 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 8.0 Hz, 1H), 7.42−7.37 (m, 5H), 7.24−7.20 (m, 1H, partially obscured by $CHCl_3$ peak), 5.74−5.50 (m, 1H), 5.24 (s, 2H), 4.38 (s, 1H), 4.01 (s, 2H), 3.57 (s, 1H), 2.51−2.38 (m, 2H). MS (ES+): calcd for $C_{30}H_{27}F_3N_4O_6S$ + H^+ 619.53, found 619.46.

The product of the previous step (103 mg, 0.167 mmol) was treated according to the CBZ removal general procedure using Pd on carbon in ethanol (4.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 10−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (64.0 mg, 64%). ^{1}H NMR (DMSO-d_6): δ 11.67 (br s, 1H), 8.79−8.43 (m, 4H), 8.22−8.20 (m, 2H), 8.05 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 8.0 Hz, 2H), 4.70 (d, J = 10.8 Hz, 1H), 3.60 (d, J = 13.6 Hz, 1H), 3.27−3.17 (m, 2H), 2.94−2.89 (m, 1H), 1.91−1.76 (m, 3H), 1.66−1.63 (m, 1H). MS (ES+): calcd for $C_{22}H_{20}F_3N_4O_2$ + H^+ 487.41, found 487.74.

*N-{4-[1-(4-Hydroxy-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**31**).* The required CBZ-protected acylguanidine was obtained using the CDI coupling general procedure from 4-benzyloxy-benzoic acid (Lancaster, 52.0 mg, 0.228 mmol) in *N*,*N*-dimethylformamide (3 mL) and **20b** (100 mg, 0.207 mmol). The desired product was obtained following aqueous workup and flash chromatography eluting 0−60% ethyl acetate/hexanes (78 mg, 57%). ^{1}H NMR (DMSO-d_6): δ 9.42 (vbr s, 1H), 8.66 (br s, 1H), 8.42 (s, 1H), 8.19 (d, J = 8.2 Hz, 1H), 7.45−7.21 (m, 13H), 7.00 (dd, J = 2.0 Hz, 6.9 Hz, 2H), 6.79−5.41 (m, 1H), 5.23 (s, 2H), 5.11 (s, 2H), 4.41−3.64 (m, 4H), 2.44 (br s, 2H). MS (ES+): calcd for $C_{36}H_{31}F_3N_4O_5$ + H^+ 657.65, found 657.60.

The product of the previous step (78 mg, 0.118 mmol) was treated according to the CBZ removal general procedure using Pd on carbon in ethanol (3.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 10−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (39.0 mg, 60%). ^{1}H NMR (DMSO-d_6): δ 11.71 (br s, 1H), 9.87 (br s, 1H), 8.64−8.45 (m, 4H), 8.22 (d, J = 1.5 Hz, 1H), 8.18 (dd, J = 1.7 Hz, 8.2 Hz, 1H), 8.02 (d, J = 8.3 Hz, 1H), 7.34 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 4.96−3.78 (vbr s 2H), 3.97 (s, 1H), 3.21−2.76 (m, 3H). MS (ES+): calcd for $C_{21}H_{21}F_3N_4O_3$ + H^+ 435.41, found 435.72.

*N-{4-[1-(3-Methylsulfonyl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**36**).* The required CBZ-protected acylguanidine was obtained using the CDI coupling general procedure from 3-methylsulfonyl-benzoic acid (Acros, 36.9 mg, 0.228 mmol) in *N*,*N*-dimethylformamide (3 mL) and **20b** (100 mg, 0.207 mmol). The desired product was obtained following aqueous workup and flash chromatography eluting 0−100% ethyl acetate/hexanes (110 mg, 85%). ^{1}H NMR (DMSO-d_6): δ 11.62 (br s, 1H), 8.69−8.41 (m, 4H), 8.21−8.19 (m, 2H), 8.07−8.01 (m, 3H), 7.85 (dt, J = 1.2 Hz, 7.7 Hz, 1H), 7.76 (t, J = 7.7 Hz, 1H), 4.69 (d, J = 11.8 Hz, 1H), 3.63 (d, J = 11.7 Hz, 1H), 3.30 (s, 3H), 3.28−3.21 (m, 2H, partially obscured by singlet at 3.30), 3.07−2.93 (m, 1H), 1.99−1.76 (m, 3H), 1.73−1.64 (m, 1H). MS (ES+): calcd for $C_{30}H_{27}F_3N_4O_6S$ + H^+ 629.62, found 629.41.

The product of the previous step (110.0 mg, 0.175 mmol) was treated according to the CBZ removal general procedure using Pd on carbon in ethanol (3.0 mL) for 48 h. The residue was purified via preparative HPLC eluting 10−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (60.0 mg, 56%). ^{1}H NMR (DMSO-d_6): δ 11.16 (br s, 1H), 9.86 (br s, 1H), 8.73 (br s, 1H), 8.46 (s, 1H), 8.28 (d, J = 7.2 Hz, 1H), 8.03 (d, J = 7.7 Hz, 1H), 7.97 (s, 1H), 7.83−7.75 (m, 2H), 7.52 (br s, 1H), 7.45−7.39 (m, 5H), 5.74−5.55 (m, 1H), 4.26 (s, 1H), 4.03 (s, 1H), 3.88 (s, 1H), 3.52 (s, 1H), 3.29 (s, 3H), 2.42−2.36 (m, 2H). MS (ES+): calcd for $C_{22}H_{23}F_3N_4O_4S$ + H^+ 497.49, found 497.73.

*N-{4-[1-(2-Methylsulfonyl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**37**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling general procedure from 2-methylsulfonyl-benzoic acid (Acros, 46.0 mg, 0.228 mmol) in *N*,*N*-dimethylformamide (2 mL) and **20b** (100 mg, 0.207 mmol). The desired product was obtained following aqueous workup and was used crude in the following step.

The CBZ protecting group was removed according to the general procedure using Pd on carbon (20 mol %) in ethanol (4.0 mL) for 48 h at 40 °C. The residue was purified via preparative HPLC eluting 10−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (37.0 mg, 29% over two steps). ^{1}H NMR (CD_3OD) mixture of 2 rotamers in a 3:1 ratio, shifts reported for major rotamer: δ 8.26 (d, J = 1.6 Hz, 1H), 8.18 (dd, J = 1.8 Hz, 8.3 Hz, 1H), 8.12 (dd, J = 1.0 Hz, 8.9 HZ, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.81 (dt, J = 1.1 Hz, 7.5 Hz, 1H), 7.71 (dt, J = 1.2 Hz, 7.7 Hz, 1H), 7.49 (dd, J = 1.1 Hz, 7.6 Hz, 1H), 4.83−4.81 (m, 1H), 3.58−3.55 (m, 1H), 3.36−3.33 (m, 1H, partially obscured by methyl singlet), 3.31 (s, 3H), 2.99 (dt, J = 2.6 Hz, 13.0 Hz, 1H), 2.19 (dq, J = 4.2 Hz, 12.4 Hz, 1H), 2.04 (dq, J = 3.7 Hz, 12.6 Hz, 1H, partially obscured), 2.00−1.95 (m, 1H, partially obscured), 1.81−1.77 (m, 1H), 1.58−1.55 (m, 1H). MS (ES+): calcd for $C_{22}H_{23}F_3N_4O_4S$ + H^+ 497.50, found 497.38.

*N-{4-[1-(4-Methoxy-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**47**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 4-methoxy-benzoic acid (34.7 mg, 0.228 mmol) in *N*,*N*-dimethylformamide (2 mL) and **20b** (100 mg, 0.207 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using Pd on carbon in ethanol (4.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 10−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (60.0 mg, 52% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.68 (br s, 1H), 8.78−8.44 (m, 4H), 8.22 (s, 1H), 8.18 (dd, J = 1.6 Hz, 8.3 Hz, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 8.8 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 4.68−4.57 (m, 1H), 3.81 (s, 4H), 3.28−3.06 (m, 1H), 3.02−2.84 (m, 2H), 1.91−1.63 (m, 4H). MS (ES+): calcd for $C_{22}H_{23}F_3N_4O_3$ + H^+ 449.44, found 449.73.

*4-[4-(4-Guanidinocarbonyl-2-trifluoromethyl-phenyl)-piperidine-1-carbonyl]-N-methyl-benzamide (**48**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from *N*-methyl-terephthalamic acid (Matrix, 61.0 mg, 0.340 mmol) in *N*,*N*-dimethylformamide (4 mL) and **20b** (150 mg, 0.311 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using Pd on carbon (20 mol %) in ethanol (4.0 mL) for 48 h. The residue was purified via preparative HPLC eluting 10−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (50.0 mg, 28% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.57 (br s, 1H), 8.71−8.26 (m, 5H), 8.21−8.18 (m, 2H), 8.05 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.55 (d, J = 7.9 Hz, 2H), 4.79−4.54 (m, 1H), 3.66−3.64 (m, 1H, partially obscured by H_2O peak), 2.95−2.84 (m, 2H), 2.80 (d, J = 4.2 Hz, 3H), 2.00−1.55 (m, 4H). MS (ES+): calcd for $C_{23}H_{24}F_3N_5O_3$ + H^+ 476.46, found 476.67.

N,N-Dimethyl-terephthalamic Acid. To a solution of terephthalic acid monomethyl ester chloride (TCI, 1.00 g, 5.04 mmol) in CH_2Cl_2 (20 mL) was added dimethylamine (2 M solution in methanol, 5.29 mL, 10.6 mmol) dropwise over 15 min, and the reaction was stirred for 1 h. The resulting mixture was diluted with ethyl acetate and washed with water, 1 M HCl, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated to give the desired amide which was used directly in the next reaction. To a solution of the crude ester in methanol (30 mL) was added water (10 mL) followed by K_2CO_3 (1.58 g, 11.4 mmol), and the resulting mixture was heated at 50 °C for 3.5 h. The methanol was removed in vacuo, and the resulting aqueous solution was acidified with concentrated aqueous HCl and diluted with 25 mL of water. The cloudy aqueous solution was extracted twice with ethyl acetate, the combined organic phases were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo to give the desired product as a white solid (690 mg, 71% over two steps). ^{1}H NMR (DMSO-d_6): δ 13.16 (br s, 1H), 7.98 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 3.00 (s, 3H), 2.88 (s, 3H). MS (ES+): calcd for $C_{10}H_{11}NO_3$ + H^+ 194.20, found 194.32.

*4-[4-(4-Guanidinocarbonyl-2-trifluoromethyl-phenyl)-piperidine-1-carbonyl]-N,N-dimethyl-benzamide (**49**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from *N*,*N*-dimethyl-terephthalamic acid (65.7 mg, 0.340 mmol) in *N*,*N*-dimethylformamide (4 mL) and

20b (150 mg, 0.311 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using Pd on carbon (20 mol %) in ethanol (4.0 mL) for 48 h. The residue was purified via preparative HPLC eluting 10−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (100 mg, 53% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.69 (br s, 1H), 8.87−8.30 (m, 4H), 8.21−8.18 (m, 2H), 8.05 (d, J = 8.3 Hz, 1H), 7.53 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 4.69 (br s, 1H), 3.70 (br s, 1H), 3.30−3.10 (m, 2H), 3.00 (s, 3H), 2.92 (s, 3H), 2.90−2.80 (m, 1H, partially obscured by methyl peak), 1.90−1.57 (m, 4H). MS (ES+): calcd for $C_{23}H_{24}F_3N_5O_3$ + H^+ 490.49, found 490.70.

*N-[4-(1-Benzoyl-piperidin-4-yl)-3-trifluoromethyl-benzoyl]-guanidine (**21**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from benzoic acid (27.7 mg, 0.227 mmol) in *N*,*N*-dimethylformamide (3 mL) and **20a** (100 mg, 0.206 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using $Pd(OH)_2$ on carbon in ethanol (3.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 10−100% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (68 mg, 62% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.62 (br s, 1H), 8.71−8.41 (m, 4H), 8.21 (s, 1H), 8.17 (d, J = 8.3 Hz, 1H), 8.05 (d, J = 8.3 Hz, 1H), 7.47 (s, 5H), 4.69 (br s, 1H), 3.72 (br s, 1H), 3.39−3.31 (m, 2H), 3.00−2.90 (m, 1H), 1.99−1.58 (m, 4H). MS (ES+): calcd for $C_{21}H_{21}F_3N_4O_2$ + H^+ 419.42, found 419.30.

*N-{4-[1-(4-Fluoro-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**22**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling general procedure from 4-fluorobenzoic acid (1.85 g, 13.2 mmol) in *N*,*N*-dimethylformamide (120 mL) and **20a** (5.82 g, 12.0 mmol). The desired product was obtained by diluting the mixture with ice-cold dilute hydrochloric acid solution followed by filtration and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using Pd on carbon in ethanol (75 mL) for 16 h. The residue was dissolved in dioxane (100 mL) and treated with excess 4 M hydrogen chloride in 1,4-dioxane. The solvent was removed in vacuo, and the resulting residue was triturated with ether. The resulting solid was recrystallized from acetonitrile to give the desired product as a colorless solid (3.77 g, 65% over two steps). ^{1}H NMR (DMSO-d_6): δ 12.15 (s, 1H), 8.72−8.49 (m, 4H), 8.40−8.38 (d, J = 8.4 Hz, 1H), 8.33 (s, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.57−7.53 (m, 2H), 7.32−7.27 (m, 2H), 4.70 (br s, 1H), 3.73 (br s, 1H), 3.19−3.17 (m, 2H), 2.95−2.75 (m, 1H), 1.90−1.67 (m, 4H), 1.84−1.81 (m, 2H). MS (ES+): calcd for $C_{21}H_{20}F_4N_4O_2$ + H^+ 437.40, found 437.20.

*N-{4-[1-(4-Imidazol-1-yl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**30**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 4-(1*H*-imidazol-1-yl)benzoic acid (42.7 mg, 0.227 mmol) in *N*,*N*-dimethylformamide (3 mL) and **20a** (100 mg, 0.206 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using Pd on carbon in ethanol (3.0 mL) for 16 h. The residue was redissolved in methanol/CH_2Cl_2 and treated with an excess of 1N HCl in ether. The resulting mixture was concentrated, and the resulting white solid was suspended in acetonitrile and boiled for 15 min. The resulting precipitate was collected by filtration to give the desired product as a crystalline white solid (73 mg, 64% over two steps). ^{1}H NMR (DMSO-d_6): δ 12.56 (br s, 1H), 9.73 (s, 1H), 8.88 (br s, 2H), 8.70 (br s, 2H), 8.54 (dd, J = 1.3 Hz, 8.2 Hz, 1H), 8.40 (d, J = 1.5 Hz, 1H), 8.34 (t, J = 1.7 Hz, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.93−7.91 (m, 3H), 7.77 (d, J = 8.6 Hz, 2H), 4.77−4.61 (m, 1H), 3.72−3.58 (m, 1H), 3.32−3.16 (m, 2H), 2.99−2.83 (m, 1H), 2.02−1.58 (m, 4H). MS (ES+): calcd for $C_{24}H_{23}F_3N_6O_2$ + H^+ 484.49, found 485.25.

*N-{4-[1-(4-Cyano-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**32**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling general procedure from 4-cyanobenzoic acid (33.4 mg, 0.227 mmol) in *N*,*N*-dimethylformamide (15 mL) and **20a** (100 mg, 0.206 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using $Pd(OH)_2$ on carbon in ethanol (4.0 mL). The residue was purified via preparative HPLC using a gradient elution from 10% to 80% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (66 mg, 57% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.57 (br s, 1H), 8.70−8.30 (m, 4H), 8.25−8.18 (m, 2H), 8.05 (d, J = 8.3 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.4 Hz, 2H), 4.68 (d, J = 11.7 Hz, 1H), 3.57−3.54 (m, 1H), 3.26−3.23 (m, 2H), 2.91 (app t, J = 11.6 Hz, 1H), 1.92−1.76 (m, 3H), 1.72−1.65 (m, 1H). MS (ES+): calcd for $C_{22}H_{20}F_4N_5O_2$ + H^+ 444.43, found 444.40.

*N-{4-[1-(3-Cyano-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**33**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 3-cyanobenzoic acid (33.4 mg, 0.227 mmol) in *N*,*N*-dimethylformamide (3 mL) and **20a** (100 mg, 0.206 mmol). The desired product was obtained following aqueous workup and was used crude in the following step (115 mg, 80%). The CBZ protecting group was removed according to the general procedure using $Pd(OH)_2$ on carbon in ethanol (4.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 10−80% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (71.7 mg, 62% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.60 (br s, 1H), 8.69−8.36 (m, 4H), 8.21−8.19 (m, 2H), 8.06 (d, J = 8.2 Hz, 1H), 7.99 (m, 1H), 7.95 (dt, J = 1.3 Hz, 7.7 Hz, 1H), 7.82 (dt, J = 1.3 Hz, 7.9 Hz, 1H), 4.68 (d, J = 11.3 Hz, 1H), 3.60 (d, J = 12.4 Hz, 1H), 3.30−3.15 (m, 2H), 2.98−2.85 (m, 1H), 1.88−1.82 (m, 3H), 1.66−1.55 (m, 1H). MS (ES+): calcd for $C_{22}H_{20}F_4N_5O_2$ + H^+ 444.43, found 444.37.

*N-{4-[1-(2-Cyano-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**34**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 2-cyanobenzoic acid (33.4 mg, 0.227 mmol) in *N*,*N*-dimethylformamide (3 mL) and **20a** (100 mg, 0.206 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using $Pd(OH)_2$ on carbon in ethanol (4.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 10−80% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (78 mg, 68% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.54 (br s, 1H), 8.66−8.28 (m, 4H), 8.22−8.20 (m, 2H), 8.00−7.92 (m, 2H), 7.83 (td, J = 1.2 Hz, 7.7 Hz, 1H), 7.72−7.65 (m, 2H), 4.72 (d, J = 13.1 Hz, 1H), 3.46−3.43 (m, 1H), 3.33−3.16 (m, 2H), 3.09−2.90 (m, 1H), 1.86−1.76 (m, 3H), 1.69−1.66 (m, 1H). MS (ES+): calcd for $C_{22}H_{20}F_4N_5O_2$ + H^+ 444.43, found 444.39.

*N-{4-[1-(4-Methylsulfonyl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**35**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 4-methylsulfonyl-benzoic acid (136 mg, 0.681 mmol) in *N*,*N*-dimethylformamide (8 mL) and **20a** (300 mg, 0.619 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using $Pd(OH)_2$ on carbon in ethanol (8.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 10−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (253 mg, 67% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.58 (br s, 1H), 8.81−8.36 (m, 4H), 8.22−8.19 (m, 2H), 8.11−8.02 (m, 3H), 7.75 (d, J = 8.4 Hz, 2H), 4.70 (d, J = 12.6 Hz, 1H), 3.58 (d, J = 13.0 Hz, 1H), 3.28 (s, 3H), 3.25−3.20 (m, 2H), 2.92 (app t, J = 11.2 Hz, 1H), 1.98−1.83 (m, 3H), 1.66−1.50 (m, 1H). MS (ES+): calcd for $C_{22}H_{23}F_4N_4O_4S$ + H^+ 496.50, found 497.34.

*N-{4-[1-(Pyridine-4-carbonyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**38**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the

general procedure from isonicotinic acid (28.2 mg, 0.227 mmol) in DMF (2.0 mL) and **20a** (100 mg, 0.206 mmol) using TEA as the base. The desired product was obtained by dilution with water and filtration of the resulting precipitate. The CBZ protecting group was removed according to the general procedure using Pd on carbon (15 mol %) in ethyl acetate (1.0 mL) and methanol (1.0 mL) for 16 h. The crude material was purified by column chromatography eluting 0−10% methanol/dichloromethane to give the desired product as a white solid (68 mg, 79% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.69 (dd, J = 1.5 Hz, 4.4 Hz, 2H), 8.36 (d, J = 1.6 Hz, 1H), 8.25 (d, J = 8.1 Hz, 1H), 8.02 (s, 2H), 7.79 (d, J = 8.2 Hz, 1H), 7.47 (dd, J = 1.6 Hz, 4.4 Hz, 2H), 6.75 (s, 2H), 4.66 (d, J = 13.0 Hz, 1H), 3.54 (d, J = 13.4 Hz, 1H), 3.26−3.07 (m, 2H), 2.95−2.79 (m, 1H), 1.89−1.73 (m, 3H), 1.63 (d, J = 11.9 Hz, 1H). MS (ES+): calcd for $C_{20}H_{20}F_3N_5O_2$ + H^+ 420.41, found 420.20.

N-{4-[1-(Pyridine-3-carbonyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (***39***). The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from nicotinic acid (28 mg, 0.23 mmol) in DMF (2.0 mL) and **20a** (100 mg, 0.206 mmol) using TEA as the base. The desired product was obtained by dilution with water and filtration of the resulting precipitate. The CBZ protecting group was removed according to the general procedure using Pd on carbon (20 mol %) in ethyl acetate (1.0 mL) and methanol (1.0 mL) for 16 h. The crude hydrogenation was purified by flash chromatography eluting 0−10% ethanol/dichloromethane to give the desired product as a white solid (71 mg, 82% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.69 (d, J = 2.1 Hz, 1H), 8.66 (dd, J = 4.8, 1.6 Hz, 1H), 8.36 (d, J = 1.5 Hz, 1H), 8.26 (d, J = 8.2 Hz, 1H), 7.96 (br s, 2H), 7.96−7.88 (m, 1H), 7.80 (d, J = 8.2 Hz, 1H), 7.50 (dd, J = 4.9 Hz, 7.8 Hz, 1H), 6.77 (s, 2H), 4.68 (d, J = 8.3 Hz, 1H), 3.64 (d, J = 11.3 Hz, 1H), 3.28−3.09 (m, 2H), 2.97−2.80 (m, 1H), 1.95−1.55 (m, 4H). MS (ES+): calcd for $C_{20}H_{20}F_3N_5O_2$ + H^+ 420.41, found 420.60.

N-{4-[1-(Pyridine-2-carbonyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (***40***). The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from picolinic acid (28.2 mg, 0.227 mmol) in DMF (2.0 mL) and **20a** (100 mg, 0.206 mmol) using TEA as the base. The desired product was obtained by dilution with water and filtration of the resulting precipitate. The CBZ protecting group was removed according to the general procedure using Pd on carbon (15 mol %) in ethyl acetate (1.0 mL) and methanol (1.0 mL) for 16 h. The crude hydrogenation was purified by flash chromatography eluting 0−10% methanol/dichloromethane to give the desired product as a white solid (68 mg, 79% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.60 (ddd, J = 0.9 Hz, 1.6 Hz, 4.8 Hz, 1H), 8.36 (d, J = 1.6 Hz, 1H), 8.26 (d, J = 8.1 Hz, 1H), 7.98 (br s, 2H), 7.94 (td, J = 1.7 Hz, 7.7 Hz, 1H), 7.71 (d, J = 8.2 Hz, 1H), 7.62 (dt, J = 1.0 Hz, 7.8 Hz, 1H), 7.48 (ddd, J = 1.2 Hz, 4.8 Hz, 7.6 Hz, 1H), 6.76 (s, 2H), 4.69 (d, J = 13.1 Hz, 1H), 3.79 (d, J = 13.4 Hz, 1H), 3.24−3.09 (m, 2H), 2.89 (td, J = 12.8, 3.3 Hz, 1H), 1.88−1.58 (m, 4H). MS (ES+): calcd for $C_{20}H_{20}F_3N_5O_2$ + H^+ 420.41, found 420.59.

4-[4-(4-Guanidinocarbonyl-2-trifluoromethyl-phenyl)-piperidine-1-carbonyl]-benzamide (***41***). The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from terephthalamic acid (37.5 mg, 0.227 mmol) in *N*,*N*-dimethylformamide (3 mL) and **20a** (100 mg, 0.206 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using $Pd(OH)_2$ on carbon in ethanol (6.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 20−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (35 mg, 30% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.63 (br s, 1H), 8.57−8.41 (m, 4H), 8.25−8.17 (m, 2H), 8.07−8.00 (m, 2H), 7.93 (d, J = 8.3 Hz, 2H), 7.54 (d, J = 8.3 Hz, 2H), 7.48 (s, 1H), 4.70−4.67 (m, 1H), 3.65−3.62 (m, 1H), 3.33−3.16 (partially obscured m, 2H), 2.99−2.75 (m, 1H), 1.89−1.65 (m, 4H). MS (ES+): calcd for $C_{22}H_{22}F_3N_5O_3$ + H^+ 462.45, found 462.70.

N-{4-[1-(6-Hydroxy-pyridine-3-carbonyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (***42***). The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 6-benzyloxy-nicotinic acid (52 mg, 0.23 mmol) in *N*,*N*-dimethylformamide (4 mL) and **20a** (100 mg, 0.206 mmol) using TEA as the base. The desired product was obtained by dilution with water and filtration of the resulting precipitate. The CBZ protecting group was removed according to the general procedure using Pd on carbon (20 mol %) in methanol (4.0 mL) for 48 h. The crude hydrogenation was dissolved in methanol, and Et_2O was added. A white solid was formed, and it was filtered and rinsed with more Et_2O. The material was recrystallized from methanol to give the desired product as a white solid (25 mg, 28% over two steps). ^{1}H NMR (DMSO-d_6): δ 12.09 (s, 1H), 8.62 (s, 2H), 8.51 (s, 2H), 8.37 (dd, J = 1.5 Hz, 8.3 Hz, 1H), 8.32 (d, J = 1.6 Hz, 1H), 8.01 (d, J = 8.3 Hz, 1H), 7.63 (d, J = 2.3 Hz, 1H), 7.56 (dd, J = 2.6 Hz, 9.5 Hz, 1H), 6.36 (d, J = 9.5 Hz, 1H), 4.22 (s, 2H), 3.14 (s, 1H), 3.01 (d, J = 15.8 Hz, 2H), 1.95−1.61 (m, 4H). MS (ES+): calcd for $C_{20}H_{20}F_3N_5O_3$ + H^+ 436.41, found 436.41.

N-{4-[1-(4-Sulfamoyl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (***43***). The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 4-sulfamoyl-benzoic acid (45.6 mg, 0.227 mmol) in *N*,*N*-dimethylformamide (3 mL) and **20a** (100 mg, 0.206 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using $Pd(OH)_2$ on carbon in ethanol (4.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 10−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (20.0 mg, 16% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.46 (br s, 1H), 8.60−8.45 (m, 6H), 7.99 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 7.42 (s, 1H), 4.63 (d, J = 10.5 Hz, 1H), 3.56−3.46 (m, 1H), 3.21−3.11 (m, 2H), 2.96−2.76 (m, 1H), 1.85−1.76 (m, 3H), 1.70−1.61 (m, 1H). MS (ES+): calcd for $C_{21}H_{22}F_4N_5O_4S$ + H^+ 498.49, found 498.61.

N-{4-[1-(1,5-Dimethyl-1H-pyrazole-3-carbonyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (***44***). The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 1,5-dimethyl-1*H*-pyrazole-3-carboxylic acid (Maybridge, 33.5 mg, 0.227 mmol) in DMF (2.0 mL) and **20a** (100 mg, 0.206 mmol) using TEA as the base. The desired product was obtained by dilution with water and filtration of the resulting precipitate. The CBZ protecting group was removed according to the general procedure using Pd on carbon (15 mol %) in ethyl acetate (2.0 mL) and methanol (1.0 mL) for 60 h. The crude hydrogenation was purified by column chromatography eluting 0−10% methanol/dichloromethane to give the desired product as a white solid (54 mg, 60% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.35 (d, J = 1.6 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.05 (s, 2H), 7.66 (d, J = 8.2 Hz, 1H), 6.75 (s, 2H), 6.35 (d, J = 0.6 Hz, 1H), 4.86 (d, J = 12.6 Hz, 1H), 4.65 (d, J = 11.8 Hz, 1H), 3.75 (s, 3H), 3.23−3.03 (m, 2H), 2.78 (t, J = 12.1 Hz, 1H), 2.27 (s, 3H), 1.85−1.59 (m, 4H). MS (ES+): calcd for $C_{20}H_{23}F_3N_6O_2$ + H^+ 437.44, found 437.20.

N-{4-[1-(1-Methyl-1H-imidazole-2-carbonyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (***45***). The required CBZ protected amide intermediate was obtained using the CDI coupling according to the general procedure from 1-methyl-1*H*-imidazole-2-carboxylic acid (Combi-Blocks, 28.6 mg, 0.227 mmol) in DMF (2.0 mL) and **20a** (100 mg, 0.206 mmol) using TEA as the base. The desired product was obtained by dilution with water and filtration of the resulting precipitate. The CBZ protecting group was removed according to the general procedure using Pd on carbon (20 mol %) in ethyl acetate (2.0 mL) for 16 h. The crude hydrogenation was purified by flash chromatography eluting 0−10% methanol/dichloromethane to give the desired product as a white solid (50 mg, 57% over two steps). ^{1}H NMR (400 MHz, DMSO): δ 8.36 (d, J = 1.6 Hz, 1H), 8.25 (d, J = 8.2 Hz, 1H), 8.03 (s, 2H), 7.68 (d, J = 8.2 Hz, 1H), 7.31 (d, J = 0.9 Hz, 1H), 6.98 (d, J = 1.1 Hz, 1H), 6.76 (s, 2H), 4.77 (d, J = 13.3 Hz, 1H), 4.67 (d, J = 12.7 Hz, 1H), 3.79 (s, 3H), 3.24 − 3.10 (m, 2H), 2.86 (t, J

= 11.6 Hz, 1H), 1.91−1.62 (m, 4H). MS (ES+): calcd for $C_{19}H_{21}F_3N_6O_2$ + H^+ 423.41, found 423.55.

*N-{4-[1-(3-Methyl-3H-imidazole-4-carbonyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**46**).* The required CBZ protected-amide intermediate was obtained using the CDI coupling according to the general procedure from 3-methyl-3*H*-imidazole-4-carboxylic acid (Maybridge, 28.6 mg, 0.227 mmol) in DMF (2.0 mL) and **20a** (100 mg, 0.206 mmol) using TEA as the base. The desired product was obtained by dilution with water and filtration of the resulting precipitate. The CBZ protecting group was removed according to the general procedure using Pd on carbon (15 mol %) in ethyl acetate (1.0 mL), methanol (1.0 mL), and dichloromethane (1.0 mL) for 16 h. The crude hydrogenation was purified by column chromatography eluting 0−10% methanol/dichloromethane to give the desired product as a white solid (68 mg, 78%). ^{1}H NMR (DMSO-d_6): δ 8.36 (d, J = 1.4 Hz, 1H), 8.30 (d, J = 8.2 Hz, 1H), 8.26 (br s, 2H), 7.83 (d, J = 8.2 Hz, 1H), 7.78 (s, 1H), 7.39 (br s, 2H), 7.27 (s, 1H), 4.45 (br s, 2H), 4.11 (d, J = 4.2 Hz, 1H), 3.72 (s, 3H), 3.14−2.89 (m, 2H), 1.90−1.69 (m, 4H). MS (ES+): calcd for $C_{19}H_{21}F_3N_6O_2$ + H^+ 423.41, found 423.76.

*N-{4-[1-(4-Methylsulfamoyl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**50**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 4-methylsulfamoyl-benzoic acid (Buttpark, 48.8 mg, 0.227 mmol) in *N*,*N*-dimethylformamide (2 mL) and **20a** (100 mg, 0.206 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The residue was dissolved in acetic acid (1.0 mL) and 30 wt % HBr in acetic acid (1.0 mL) was added, and the resulting mixture was stirred for 2 h and diluted with CH_2Cl_2 (2 mL) and then with ether. The resulting precipitate was collected by filtration and purified by preparative HPLC eluting 10−90% acetonitrile/water with 0.1% TFA to obtain the desired product as the trifluoroacetic acid salt (67.0 mg, 53.2% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.59 (br s, 1H), 8.78−8.40 (m, 4H), 8.27−8.25 (m, 2H), 8.12−8.10 (d, J = 8.2 Hz, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H), 7.64 (q, J = 5.0 Hz, 1H), 3.74−3.42 (m, 1H, partially obscured by H_2O peak), 3.33−3.23 (m, 2H), 3.12−2.98 (m, 1H), 2.51 (d, 3H, J = 5.0 Hz), 1.97−1.88 (m, 3H), 1.79−1.70 (m, 1H). MS (ES+): calcd for $C_{22}H_{24}F3N_5O_4S$ + H^+ 512.52, found 512.72.

*N-{4-[1-(4-Dimethylsulfamoyl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**51**).* The required CBZ-protected amide intermediate was obtained using the CDI coupling according to the general procedure from 4-dimethylsulfamoyl-benzoic acid (Buttpark, 52.0 mg, 0.227 mmol) in *N*,*N*-dimethylformamide (2 mL) and **20a** (100 mg, 0.206 mmol). The desired product was obtained following aqueous workup and was used crude in the following step. The CBZ protecting group was removed according to the general procedure using $Pd(OH)_2$ on carbon in ethanol (4.0 mL) for 16 h. The residue was purified via preparative HPLC eluting 20−90% acetonitrile/water with 0.1% trifluoroacetic acid to obtain the desired product as the trifluoroacetic acid salt (55.0 mg, 42% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.69 (br s, 1H), 8.80−8.44 (m, 4H), 8.22−8.21 (m, 2H), 8.04 (d, J = 8.3 Hz, 1H), 7.84 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 8.4 Hz, 2H), 4.69 (d, J = 12.4 Hz, 1H), 3.61 (d, J = 12.3 Hz, 1H), 3.28−3.17 (m, 2H), 2.95−2.90 (m, 1H), 2.66 (s, 6H), 1.99−1.83 (m, 3H), 1.76−1.64 (m, 1H). MS (ES+): calcd for $C_{23}H_{26}F_4N_5O_4S$ + H^+ 525.55, found 526.38.

General Procedure for Acid Chloride Coupling. To a suspension of **20a** (100 mg, 0.206 mmol) in dichloromethane (2.0 mL) was added triethylamine (0.072 mL, 0.52 mmol). To the resulting solution was added the required acid chloride (0.186 mmol) dropwise, and the mixture was stirred for 1 h at room temperature. The reaction was diluted with saturated $NaHCO_3$ and water and extracted three times with dichloromethane. The combined extracts were dried over Na_2SO_4, and the solvent was removed in vacuo and the products were used crude.

*N-{4-[1-(2,2-Dimethyl-propionyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**55**).* The required CBZ-protected amide intermediate obtained according to the general procedure for acid chloride coupling using 2,2-dimethyl-propionyl chloride (0.023 mL, 0.186 mmol) to give the crude product which was subjected to the general procedure for CBZ deprotection using Pd on carbon in ethyl acetate (2.0 mL) for 2 h to afford the pure product as a white amorphous solid (68 mg, 83% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1H), 8.23 (d, J = 8.1 Hz, 1H), 8.04 (s, 2H), 7.66 (d, J = 8.2 Hz, 1H), 6.76 (s, 2H), 4.46 (d, J = 12.9 Hz, 2H), 3.11 (t, J = 11.3 Hz, 1H), 2.87 (t, J = 11.9 Hz, 2H), 1.79−1.53 (m, 4H), 1.23 (s, 9H). MS (ES+): calcd for $C_{19}H_{25}F_3N_4O_2$ + H^+ 399.43, found 399.69.

*N-[4-(1-Isobutyryl-piperidin-4-yl)-3-trifluoromethyl-benzoyl]-guanidine (**56**).* The required CBZ-protected amide intermediate obtained according to the general procedure for acid chloride coupling using isobutyryl chloride (0.020 mL, 0.186 mmol) to give the crude product which was subjected to the general procedure for CBZ deprotection using Pd on carbon in ethyl acetate (2.0 mL) for 2 h to afford the pure product as a white solid (75 mg, 95% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.36 (d, J = 1.6 Hz, 1H), 8.23 (d, J = 9.2 Hz, 1H), 8.13 (s, 2H), 7.65 (d, J = 8.2 Hz, 1H), 6.76 (s, 2H), 4.60 (d, J = 12.8 Hz, 1H), 4.09 (d, J = 15.1 Hz, 1H), 3.15−3.01 (m, 2H), 2.97−2.83 (m, 1H), 2.58 (t, J = 11.9 Hz, 1H), 1.81−1.48 (m, 4H), 1.03 (dd, J = 20.3, 6.6 Hz, 6H). MS (ES+): calcd for $C_{18}H_{23}F_3N_4O_2$ + H^+ 385.40, found 385.58.

*N-[4-(1-Cyclopropanecarbonyl-piperidin-4-yl)-3-trifluoromethyl-benzoyl]-guanidine (**57**).* The required CBZ-protected amide intermediate was obtained according to the general procedure for acid chloride coupling using cyclopropane carboxylic acid chloride (0.019 mL, 0.206 mmol) to give the crude product which was subjected to the general procedure for CBZ deprotection using Pd on carbon in ethyl acetate (2.0 mL) for 2 h to afford the pure product as a white solid (65 mg, 83% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.35 (d, J = 1.6 Hz, 1H), 8.24 (d, J = 9.5 Hz, 1H), 8.02 (s, 2H), 7.67 (d, J = 8.2 Hz, 1H), 6.76 (s, 2H), 4.56 (d, J = 12.0 Hz, 1H), 4.41 (d, J = 13.0 Hz, 1H), 3.25−3.06 (m, 2H), 2.63 (t, J = 12.1 Hz, 1H), 2.01 (dt, J = 12.9, 6.3 Hz, 1H), 1.83−1.50 (m, 4H), 0.88 − 0.64 (m, 4H). MS (ES+): calcd for $C_{18}H_{21}F_3N_4O_2$ + H^+ 383.29, found 383.44.

*N-{4-[1-(2-Methoxy-acetyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**58**).* The required CBZ-protected amide intermediate obtained according to the general procedure for acid chloride coupling using 2-methoxyacetyl chloride (0.018 mL, 0.186 mmol) to give the crude product, which was subjected to the general procedure for CBZ deprotection using Pd on carbon in ethyl acetate (2.0 mL) for 2 h to afford the pure product as a white amorphous solid (46 mg, 58% over two steps). ^{1}H NMR (DMSO): δ 8.35 (d, J = 1.3 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.03 (s, 2H), 7.65 (d, J = 8.3 Hz, 1H), 6.76 (s, 2H), 4.52 (d, J = 13.3 Hz, 1H), 4.21−4.03 (m, 2H), 3.89 (d, J = 13.5 Hz, 1H), 3.31 (s, 3H), 3.14−3.01 (m, 2H), 2.64 (t, J = 12.1 Hz, 1H), 1.84−1.52 (m, 4H). MS (ES+): calcd for $C_{17}H_{21}F_3N_4O_3$ + H^+ 387.38, found 387.59.

*N-[4-(1-Propionyl-piperidin-4-yl)-3-trifluoromethyl-benzoyl]-guanidine (**59**).* The required CBZ-protected amide intermediate obtained according to the general procedure for acid chloride coupling using propionyl chloride (0.017 mL, 0.186 mmol) to give the crude product, which was subjected to the general procedure for CBZ deprotection using Pd on carbon in ethyl acetate (2.0 mL) for 2 h to afford the pure product as a white solid (67 mg, 88% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.36 (d, J = 1.5 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.13 (s, 2H), 7.66 (d, J = 8.2 Hz, 1H), 6.78 (s, 2H), 4.59 (d, J = 12.9 Hz, 1H), 4.00 (d, J = 13.4 Hz, 1H), 3.17−3.00 (m, 2H), 2.59 (t, J = 11.7 Hz, 1H), 2.36 (q, J = 7.4 Hz, 2H), 1.81−1.50 (m, 4H), 1.02 (t, J = 7.4 Hz, 3H). MS (ES+): calcd for $C_{17}H_{21}F_3N_4O_2$ + H^+ 371.38, found 371.62

*N-[4-(1-Acetyl-piperidin-4-yl)-3-trifluoromethyl-benzoyl]-guanidine (**60**).* The required CBZ-protected amide intermediate obtained according to the general procedure for acid chloride coupling using acetyl chloride (0.013 mL, 0.186 mmol) to give the crude product, which was subjected to the general procedure for CBZ deprotection using Pd on carbon in ethyl acetate (2.0 mL) for 2 h to afford the pure product as a white solid (41 mg, 56% over two steps). ^{1}H NMR (DMSO-d_6): δ 8.35 (s, 1H), 8.24 (d, J = 8.1 Hz, 1H), 8.03 (s, 2H), 7.66 (d, J = 8.2 Hz, 1H), 6.77 (s, 2H), 4.56 (d, J = 13.0 Hz, 1H), 3.94

(d, J = 13.4 Hz, 1H), 3.20−3.01 (m, 2H), 2.58 (t, J = 11.6 Hz, 1H), 2.04 (s, 3H), 1.83−1.49 (m, 4H). MS (ES+): calcd for $C_{16}H_{19}F_3N_4O_2$ + H^+ 357.35, found 357.59.

*4-(4-Methoxycarbonyl-2-trifluoromethyl-phenyl)-piperidine-1-carboxylic Acid Benzyl Ester (**61**).* To a solution of **19** (2.50 g, 6.45 mmol) in CH_2Cl_2/MeOH (8/2, 50 mL) was added 4N HCl in dioxane (8.07 mL, 32.3 mmol), and the resulting mixture was stirred for 16 h. The solvent was removed in vacuo to give the desired free piperidine product as a white solid as the hydrochloride salt (1.90 g, 91%). ^{1}H NMR (DMSO-d_6): δ 8.33 (s, 1H), 8.29 (d, J = 8.5 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 4.89 (s, 3H), 3.58−3.55 (m, 2H), 3.39−3.34 (partially obscured m, 1H), 3.28−3.12 (m, 2H), 2.27−1.98 (m, 4H). MS (ES+): calcd for $C_{14}H_{16}F_3NO_2$ + H^+ 288.28, found 288.58.

To a solution of the product of the previous step (1.90 g, 5.86 mmol) in CH_2Cl_2 (250 mL) at 0 °C was added benzylchloroformate (0.94 mL, 6.61 mmol), followed by *N*,*N*-diisopropylamine (2.62 mL, 10.1 mmol), and the mixture was warmed to room temperature and stirred 16 h. The reaction mixture was then washed three times with saturated $NaHCO_3$ (30 mL each) and brine (30 mL), the organics were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo. The resulting oily residue was purified by flash chromatography eluting 0−100% ethyl acetate/hexanes to afford the desired product as a colorless oil (2.20 g, 89%). ^{1}H NMR (DMSO-d_6): δ 8.20−8.12 (m, 2H), 7.84 (d, J = 8.8 Hz, 1H), 7.42−7.31 (m, 5H), 5.11 (s, 2H), 4.18 (d, J = 13.0 Hz, 2H), 3.89 (s, 3H), 3.09−2.82 (m, 3H), 1.86−1.69 (m, 4H). MS (ES+): calcd for $C_{22}H_{22}F_3NO_4$ + H^+ 422.41, found 422.31.

*4-(4-(N-tert-Butoxycarbonyl)-guanidinocarbonyl-2-trifluoromethyl-phenyl)-piperidine-1-carboxylic Acid Benzyl Ester (**62**).* To a solution of **61** (2.20 g, 5.22 mmol) in methanol (40 mL) was added water (4 mL) followed by K_2CO_3 (1.80 g, 13.1 mmol), and the reaction was heated at 60 °C for 5 h. The reaction was concentrated to a paste in vacuo, and the residue was partitioned between ethyl acetate and water and the pH of the aqueous phase was adjusted to pH 5 (pH paper) by addition of 1 N HCl. The phases were separated, and the aqueous was extracted with ethyl acetate three times. The combined organics were dried over Na_2SO_4, filtered, and concentrated to give the desired product as a white solid (1.91 g, 90%). ^{1}H NMR (DMSO-d_6): δ 13.38 (br s, 1H), 8.21−8.11 (m, 2H), 7.81 (d, J = 8.4 Hz, 1H), 7.45−7.31 (m, 5H), 5.11 (s, 2H), 4.18 (d, J = 13.0 Hz, 2H), 3.09−2.83 (m, 3H), 1.76−1.58 (m, 4H). MS (ES+): calcd for $C_{21}H_{20}F_3NO_4$ + H^+ 408.38, found 408.20.

To a solution of the product of the previous step (2.52 g, 4.59 mmol) in *N*-methylpyrrolidinone (20 mL) was added 2-chloro-1-methylpyridinium iodide (1.42 g, 5.56 mmol), and the mixture was stirred for 75 min. To this mixture was added *tert*-butoxycarbonyl-guanidine (0.960 g, 6.03 mmol) and diisopropylamine (2.30 mL, 13.9 mmol), and the resulting mixture was stirred for 16 h. The crude mixture was partitioned between water and ethyl acetate, the phases were separated, and the organics were washed 2 times with water and then once with brine. The organics were collected, dried over Na_2SO_4, filtered, and concentrated to pale-orange oil. The crude residue was purified by flash chromatography eluting 0−60% ethyl acetate/hexanes to give the desired product as a colorless oil (2.52 g, 99%). ^{1}H NMR (DMSO-d_6): δ 8.98 (br s, 2H), 8.43 (br s, 1H), 8.29 (s, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.36−7.15 (m, 5H), 5.01 (s, 2H), 4.20 (br s, 2H), 2.99 (t, J = 11.6 Hz, 1H), 2.90−2.74 (m, 2H), 1.73−1.39 (m, 4H). MS (ES+): calcd for $C_{27}H_{31}F_3N_4O_5$ + H^+ 549.55, found 549.74.

*N-(4-Piperidin-4-yl-3-trifluoromethyl-benzoyl)-N′-(tert-butoxycarbonyl)-guanidine (**63a**).* To a solution of **62** (11.0 g, 20.1 mmol) in ethanol (200 mL) was added $Pd(OH)_2$ on carbon (10 mol %, 500 mg, 0.356 mmol), and the reaction was stirred under a hydrogen atmosphere for 36 h. The reaction was diluted with ethyl acetate (200 mL) and filtered through Celite. The solvent was removed in vacuo, the resulting foam was triturated with ether, and the resulting solid was collected by filtration to give the desired product as a white solid (6.81 g, 82%). ^{1}H NMR (DMSO-d_6): δ 9.67 (br s, 1H), 8.62 (br s, 1H), 8.40 (d, J = 1.6 Hz, 1H), 8.27 (d, J = 8.2 Hz, 1H), 7.68 (d, J = 8.2 Hz, 1H), 3.03 (d, J = 12.1 Hz, 2H), 2.91 (t, J = 11.3 Hz, 1H), 2.67−2.66 (m, 2H), 1.69−1.56 (m, 4H), 1.48 (s, 9H). MS (ES+): calcd for $C_{19}H_{25}F_3N_4O_3$ + H^+ 415.43, found 415.72.

*N-(4-Piperidin-4-yl-3-trifluoromethyl-benzoyl)-N′-(tert-butoxycarbonyl)-guanidine hydrochloride salt (**63b**).* To a solution of **63a** (1.89 g, 4.55 mmol) in CH_2Cl_2 (10 mL) was added 1N HCl in ether (5.0 mL). The resulting mixture was diluted with hexanes to give a cloudy white suspension from which the solvent was removed in vacuo to give the desired product as a white solid (2.05 g, 100%). ^{1}H NMR (DMSO-d_6): δ 11.18 (br s, 1H), 9.82 (br s, 1H), 9.21−9.13 (m, 1H), 9.13−9.00 (m, 1H), 9.00−8.67 (m, 1H), 8.46 (d, J = 1.6 Hz, 1H), 8.37 (d, J = 8.2 Hz, 1H), 7.66 (d, J = 8.3 Hz, 1H), 3.42−3.36 (m, 2H), 3.24−3.21 (m, 1H), 3.18−3.03 (m, 2H), 2.21−2.05 (m, 2H), 1.85−1.74 (m, 2H). MS (ES+): calcd for $C_{19}H_{25}F_3N_4O_3$ + H^+ 415.43, found 415.39

General Procedure for Amide Bond Coupling with HATU. To the benzoic acid derivative (1.10 equiv when reacting with **63a** and 2.74 equiv when reacting with **63b**) and HATU (1.10 equiv) was added DMA (1 mL), and the mixture was shaken for 15 min. A solution of **63a** or **63b** (1.00 equiv) and diisopropylamine (2 equiv) in DMA (0.5 mL) was then added, and the reaction was shaken overnight at room temperature. The crude mixtures were filtered through an SPE cartridge containing 1 g of basic alumina. The alumina plug was eluted with a 10:1 mixture of DMA:MeOH (4 × 0.5 mL), then the solutions were concentrated in vacuo, and the resulting coupling products were used crude in the next step.

General Procedure for BOC Deprotection with TFA. The crude amide from the HATU coupling was dissolved in CH_2Cl_2 (200 μL), and then 1−2 mL of 20% TFA in CH_2Cl_2 was added. The resulting solution was shaken overnight at room temp. The solvent was then removed in vacuo, and the residue was purified by reverse phase mass triggered LCMS.

*N-{4-[1-(4-Chloro-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**24**).* Using the general procedure for HATU amide bond coupling with 4-chlorobenzoic acid (38.2 mg, 0.244 mmol) in *N*,*N*-dimethylformamide (2 mL) and **63a** (92 mg, 0.222 mmol) followed by the general BOC deprotection procedure afforded the title compound as the formate salt after purification by preparative HPLC (60.1 mg, 60%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.53 (br s, 1H), 8.31 (s, 1H), 8.22 (d, J = 8.1 Hz, 1H), 8.20−8.00 (br s, 1H), 8.13 (s, 1H), 7.85 (br s, 1H), 7.51 (q, J = 8.6 Hz, 4H), 6.90 (s, 1H), 4.65 (br s, 1H), 3.65 (br s, 1H), 3.21−3.07 (m, 2H), 2.89 (br s, 1H), 1.87−1.58 (m, 4H). MS (ES+): calcd for $C_{21}H_{20}ClF_3N_4O_2$ + H^+ 452.86, found 453.92.

*N-{4-[1-(3-Chloro-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**25**).* Using the general procedure for HATU amide bond coupling with 3-chlorobenzoic acid (38.2 mg, 0.244 mmol) in *N*,*N*-dimethylformamide (2 mL) and **63a** (92 mg, 0.222 mmol) followed by the general BOC deprotection procedure afforded the title compound as the formate salt after purification by preparative HPLC (74 mg, 74%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.71 (br s, 0.5H), 11.44 (br s, 0.5H), 8.60 (s, 1H), 8.30 (s, 1H), 8.24 (d, J = 8.1 Hz, 1H), 8.19−8.14 (m, 1H), 7.98−7.84 (m, 2H), 7.78 (s, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 7.6 Hz, 1H), 6.91 (br s, 0.5H), 6.58 (br s, 0.5H), 4.71 (br s, 1H), 3.75 (br s, 1H), 3.23−3.13 (m, 2H), 2.90 (br s, 1H), 1.92−1.59 (m, 4H). MS (ES+): calcd for $C_{21}H_{20}ClF_3N_4O_2$ + H^+ 453.86, found 454.13.

*N-{4-[1-(2-Chloro-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**26**).* Using the general procedure for HATU amide bond coupling with 3-chlorobenzoic acid (38.2 mg, 0.244 mmol) in *N*,*N*-dimethylformamide (2 mL) and **63a** (92 mg, 0.222 mmol) followed by the general BOC deprotection procedure afforded the title compound as the formate salt after purification by preparative HPLC (74.5 mg, 74.1%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.75 (br s, 1H), 8.49 (s, 1H), 8.35 (s, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.13 (s, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.79 (s, 1H) 7.59 (d, J = 8.2 Hz, 2H), 6.90 (s, 1H), 4.69 (br s, 1H), 3.74 (br s, 1H), 3.20−3.11 (m, 2H), 2.90 (br s, 1H), 1.90−1.59 (m, 4H). MS (ES+): calcd for $C_{21}H_{20}ClF_3N_4O_2$ + H^+ 453.86, found 453.99.

*N-{4-[1-(Biphenyl-4-carbonyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**27**).* Using the general procedure for HATU amide bond coupling with biphenyl-4-carboxylic acid (48.3 mg, 0.244 mmol)) in DMA (1 mL) and **63b** (40 mg, 0.089 mmol) followed by

the general BOC deprotection procedure afforded the title compound as the formate salt after preparative HPLC purification (12.8 mg, 27%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.73 (br s, 1H), 8.34 (s, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.14 (s, 1H), 8.00 (br s, 1H), 7.90−7.82 (br m, 1H), 7.76 (d, J = 8.2 Hz, 2H), 7.72 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 8.1 Hz, 2H), 7.50 (t, J = 7.6 Hz, 2H), 7.41 (t, J = 7.3 Hz, 1H), 6.90 (s, 1H), 4.70 (br s, 1H), 3.82 (br s, 1H), 3.22−3.07 (m, 2H), 3.04−2.78 (m, 1H), 1.92−1.61 (m, 4H). MS (ES+): calcd for $C_{27}H_{25}F_3N_4O_2$ + H$^+$ 495.20, found 495.01.

*N-{4-[1-(4-Oxazol-5-yl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**28**).* Using the general procedure for HATU amide bond coupling with 4-(1,3-oxazol-5-yl)benzoic acid (46.2 mg, 0.244 mmol) in DMA (2 mL) and **63a** (92 mg, 0.222 mmol) followed by the general BOC deprotection procedure afforded the title compound as the formate salt after preparative HPLC purification (74 mg, 66%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.80 (br s, 1H), 8.50 (s, 1H), 8.36 (d, J = 1.3 Hz, 1H), 8.26 (d, J = 8.2 Hz, 1H), 8.14 (s, 1H), 8.06 (br s, 1H), 7.87−7.75 (m, 4H), 7.58 (t, J = 8.0 Hz, 2H), 7.00−6.50 (br m, 2H), 4.77−4.57 (br m, 1H), 3.87−3.62 (br m, 1H), 3.65−3.06 (br m, 2H), 2.95−2.78 (br m, 1H), 1.91−1.71 (br m, 3H), 1.74−1.58 (br m, 1H). MS (ES+): calcd for $C_{24}H_{22}F_3N_5O_3$ + H$^+$ 486.17, found 486.17.

*N-{4-[1-(3-Oxazol-5-yl-benzoyl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**29**).* Using the general procedure for HATU amide bond coupling with 3-(1,3-oxazol-5-yl)benzoic acid (46.2 mg, 0.244 mmol) in DMA (2 mL) and **52a** (92 mg, 0.222 mmol) followed by the general BOC deprotection procedure afforded the title compound as the formate salt after preparative HPLC purification (70 mg, 63% over two steps). ^{1}H NMR (DMSO-d_6): δ 12.77 (br s, 1H), 8.50 (s, 1H), 8.36 (d, J = 1.3 Hz, 1H), 8.26 (d, J = 8.2 Hz, 1H), 8.14 (s, 1H), 8.06 (br s, 1H), 7.87−7.75 (m, 4H), 7.58 (t, J = 8.0 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.00−6.50 (br m, 3H), 4.79−4.61 (br m, 1H), 3.80−3.62 (br m, 1H), 3.40−3.10 (br m, 2H), 2.95−2.78 (br m, 1H), 1.94−1.72 (br m, 3H), 1.73−1.57 (br m, 1H). MS (ES+): calcd for $C_{24}H_{22}F_3N_5O_3$ + H$^+$ 486.17, found 485.98.

*N-[4-(1-Cyclopentanecarbonyl-piperidin-4-yl)-3-trifluoromethyl-benzoyl]-guanidine (**52**).* Using the general procedure for HATU amide bond coupling with cyclopentanecarboxylic acid (27.9 mg, 0.244 mmol) in DMA (2 mL) and **63a** (92 mg, 0.222 mmol) followed by the general BOC deprotection procedure afforded the title compound as the formate salt after preparative HPLC purification (52 mg, 55%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.64 (br s, 1H), 8.29 (s, 1H), 8.20 (d, J = 8.2 Hz, 1H), 8.20−7.80 (m, 1H), 8.13 (s, 1H), 7.77 (br s, 1H), 6.91 (s, 1H), 4.60 (d, J = 12.1 Hz, 1H), 4.13 (d, J = 12.9 Hz, 1H), 3.19−3.06 (m, 2H), 3.07−2.97 (m, 1H), 2.60 (t, J = 12.6 Hz, 1H), 1.85−1.47 (m, 12H). MS (ES+): calcd for $C_{20}H_{25}F_3N_4O_2$ + H$^+$ 411.2, found 410.84.

*N-{4-[1-(3-Methyl-butyryl)-piperidin-4-yl]-3-trifluoromethyl-benzoyl}-guanidine (**53**).* Using the general procedure for HATU amide bond coupling with isovaleric acid (24.9 mg, 0.244 mmol) in DMA (2 mL) and **63a** (92 mg, 0.222 mmol) followed by the general BOC deprotection procedure afforded the title compound as the formate salt after preparative HPLC purification (69 mg, 76%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.70 (br s, 1H), 8.32 (s, 1H), 8.22 (d, J = 8.1 Hz, 1H), 7.98 (br s, 1H), 8.13 (s, 1H), 7.73−7.65 (m, 1H), 6.91 (s, 1H), 4.61 (d, J = 12.6 Hz, 1H), 4.03 (d, J = 13.6 Hz, 5H), 3.16−3.05 (m, 2H), 2.66−2.51 (m, 1H), 2.23 (d, J = 6.9 Hz, 2H), 2.07−1.97 (m, 1H), 1.76−1.64 (m, 3H), 1.64−1.52 (m, 1H), 0.97−0.89 (m, 6H). MS (ES+): calcd for $C_{19}H_{25}F_3N_4O_2$ + H$^+$ 399.20, found 398.92.

*N-[4-(1-Butyryl-piperidin-4-yl)-3-trifluoromethyl-benzoyl]-guanidine (**54**).* Using the general procedure for HATU amide bond coupling with butyric acid (21.5 mg, 0.244 mmol) in DMA (2 mL) and **63a** (92 mg, 0.222 mmol) followed by the general BOC deprotection procedure afforded the title compound as the formate salt after preparative HPLC purification (59 mg, 67%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.45 (br s, 1H), 8.20 (br s, 1H), 8.26 (s, 1H), 8.18 (d, J = 8.3 Hz, 1H), 8.13 (s, 1H), 7.85−7.75 (m, 1H), 6.91 (s, 1H), 4.60 (d, J = 12.2 Hz, 1H), 4.02 (d, J = 13.2 Hz, 1H), 3.18−3.04 (m, 2H), 2.66−2.54 (m, 1H), 2.33 (t, J = 7.4 Hz, 2H), 1.78−1.63 (m, 3H), 1.65−1.50 (m, 3H), 0.91 (t, J = 7.4 Hz, 3H). MS (ES+): calcd for $C_{18}H_{23}F_3N_4O_2$ + H$^+$ 385.18, found 384.93

*4-Benzyloxy-5-iodo-2-methyl-benzoic Acid Methyl Ester (**66**).* To a solution 4-hydroxy-5-iodo-2-methyl-benzoic acid methyl ester **65** (9.70 g, 33.2 mmol) in DMF (130 mL) was added benzyl bromide (4.46 mL, 36.5 mmol) and K_2CO_3 (13.8 g, 99.6 mmol). The mixture was stirred at 80 °C for 2 h and was cooled to rt. The mixture was partitioned between ethyl acetate and H_2O. The layers were separated, and the aqueous layer was further extracted with ethyl acetate. The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The crude material was purified via flash chromatography using a gradient elution of 0−8% ethyl acetate/hexanes to afford the desired product (8.32 g, 66%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.23 (s, 1H), 7.55−7.47 (m, 2H), 7.46−7.39 (m, 2H), 7.38−7.31 (m, 1H), 7.08 (s, 1H), 5.27 (s, 2H), 3.79 (s, 3H), 2.51 (s, 3H). MS (ES+): calcd for $C_{16}H_{15}IO_3$ + H$^+$ 383.19, found 383.24.

*4-Benzyloxy-2-methyl-5-trifluoromethyl-benzoic Acid Methyl Ester (**67**).* To a solution of **66** (8.32 g, 21.8 mmol) in 1-methyl-2-pyrrolidone (100 mL) was added potassium trifluoroacetate (16.6 g, 108.8 mmol) and copper(I) iodide (20.7 g, 108.8 mmol). The reaction was stirred at 150 °C for 5 h and then was cooled to rt. The mixture was partitioned between ethyl acetate and saturated aqueous NH_4Cl and was filtered through a pad of Celite, rinsing with ethyl acetate. The layers of the filtrate were separated, and the aqueous phase was further extracted with ethyl acetate. The combined organic phases were washed with brine and were dried (Na_2SO_4) and concentrated in vacuo. The crude material was purified via flash chromatography using a gradient elution of 0−8% ethyl acetate/hexanes to afford the desired product (6.30 g, 89%) as an off-white solid. ^{1}H NMR (DMSO-d_6): δ 8.07 (s, 1H), 7.51−7.31 (m, 6H), 5.35 (s, 2H), 3.82 (s, 3H), 2.61 (s, 3H). MS (ES+): calcd for $C_{17}H_{15}F_3O_3$ + H$^+$ 325.29, found 325.20.

*4-Hydroxy-2-methyl-5-trifluoromethyl-benzoic Acid Methyl Ester (**68**).* To a solution of **67** (6.30 g, 19.4 mmol) in EtOH (150 mL) purged with Ar was added 10 wt % Pd/C (400 mg). The mixture was stirred under an H_2 atmosphere overnight. The mixture was filtered through a pad of Celite and was rinsed with EtOH. The solvent was evaporated under reduced pressure to afford the desired product (4.34 g, 95%). ^{1}H NMR (DMSO-d_6): δ 11.39 (s, 1H), 8.00 (s, 1H), 6.93 (s, 1H), 3.79 (s, 3H), 2.52 (s, 3H). MS (ES+): calcd for $C_{10}H_9F_3O_3$ + H$^+$ 235.17, found 235.20.

*4-(4-Methoxycarbonyl-5-methyl-2-trifluoromethyl-phenyl)-3,6-dihydro-2H-pyridine-1-carboxylic Acid tert-Butyl Ester) (**69a**).* To a solution of **68** (4.34 g, 18.5 mmol) in DMF was added DIEA (3.55 mL, 20.4 mmol) followed by *N*-phenyltrifluoromethanesulfonimide. The reaction was stirred at room temperature overnight. The mixture was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$. The layers were separated, and the aqueous layer was further extracted with ethyl acetate. The combined organic phases were washed with brine and were dried (Na_2SO_4) and concentrated in vacuo to afford the desired crude intermediate. The crude intermediate was divided into four equal portions and was placed into four 20 mL microwave reaction vials: To a solution of intermediate in THF (48 mL) was added **16** (Digital, 5.61 g, 18.1 mmol) and 2 M aqueous K_2CO_3 (18.1 mL, 36.3 mmol). After the mixture was degassed, tetrakis(triphenylphosphine)palladium(0) (Strem, 3.78 g, 3.27 mmol) was added. Each vial was sealed with a Teflon lined septa cap and was irradiated in a microwave reactor at 110 °C for 30 min. After cooling to room temperature, the mixtures from all of the vials were pooled together and were partitioned between ethyl acetate and H_2O. The mixture was filtered to remove the remaining undissolved solid, rinsing with ethyl acetate and water. The layers of the filtrate were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo. The crude material was purified via flash chromatography using a gradient elution of 0−8% ethyl acetate/hexanes to afford the desired product (5.68 g, 78%) as a light-yellow oil. ^{1}H NMR (DMSO-d_6): δ 8.09 (s, 1H), 7.41 (s, 1H), 5.64 (s, 1H), 3.99−3.91 (m, 2H), 3.87 (s, 3H), 3.57−3.49 (m, 2H), 2.58 (s, 3H), 2.34−2.26 (m, 2H), 1.44 (s, 9H).

MS (ES+): calcd for $C_{20}H_{24}F_3NO_4$ + H^+ 400.40, found 422.20 (M + Na^+).

*4-(4-Methoxycarbonyl-5-methyl-2-trifluoromethyl-phenyl)-piperidine-1-carboxylic Acid tert-Butyl Ester (**84**).* To a solution of **69** (5.68 g, 14.2 mmol) in EtOH (70 mL) purged with Ar was added 10 wt % Pd/C (200 mg). The mixture was stirred under an H_2 atmosphere. Additional catalyst was added, and the mixture continued to stir under an H_2 atmosphere until the reaction was complete. The mixture was filtered through a pad of Celite and was rinsed with DCM. The solvent was evaporated under reduced pressure to afford the desired product (5.15 g, 90%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.06 (s, 1H), 7.67 (s, 1H), 4.20−4.02 (m, 2H), 3.85 (s, 3H), 2.96 (br s, 1H), 2.90−2.67 (m, 2H), 2.59 (s, 3H), 1.75−1.61 (m, 4H), 1.43 (s, 9H). MS (ES+): calcd for $C_{20}H_{26}F_3NO_4$ + H^+ 402.42, found 387.36 (M − CH_3 H^+).

*4-(4-Carboxy-5-methyl-2-trifluoromethyl-phenyl)-piperidine-1-carboxylic Acid tert-Butyl Ester (**85**).* To a solution of **84** (5.15 g, 12.8 mmol) in MeOH (40 mL) and H_2O (20 mL) was added LiOH monohydrate (807 mg, 19.2 mmol). The reaction was stirred at 40 °C overnight and then was cooled to rt. The MeOH was removed in vacuo, and the aqueous mixture was acidified (pH 5) with 1N aqueous HCl. The resulting solid was collected by filtration and dried in vacuo at 50 °C to afford the desired product (5.00 g, 100%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 7.79 (s, 1H), 7.26 (s, 1H), 4.18−4.02 (m, 2H), 2.97−2.66 (m, 3H), 2.48 (s, 3H), 1.68−1.56 (m, 4H), 1.43 (s, 9H). MS (ES+): calcd for $C_{19}H_{24}F_3NO_4$ + H^+ 388.39, found 388.40

*4-[5-Methyl-4-(N-(carbobenzyloxy)-guanidinocarbonyl)-2-trifluoromethyl-phenyl]-piperidine-1-carboxylic Acid tert-Butyl Ester (**86**).* To a solution of **85** (5.00 g, 12.9 mmol) in 1-methyl-2-pyrrolidone (50 mL) was added 2-chloro-1-methylpyridinium iodide (3.63 g, 14.2 mmol). The mixture was stirred at room temperature for 30 min. *N*-Carbobenzyloxy-guanidine (2.99 g, 15.5 mmol) and DIEA were added to the reaction, and the mixture was allowed to stir at room temperature overnight. The mixture was partitioned between ethyl acetate and H_2O, and the layers were separated. The aqueous layer was further extracted with ethyl acetate, and the combined organic phases were washed with H_2O, brine, and were dried (Na_2SO_4) and concentrated in vacuo. The crude material was purified via flash chromatography using a gradient elution of 10−20% ethyl acetate/hexanes to afford the desired product (5.68 g, 78%). ^{1}H NMR (DMSO-d_6): δ 11.22 (s, 1H), 9.53 (br s, 1H), 8.79 (s, 1H), 8.05 (s, 1H), 7.53 (s, 1H), 7.44−7.29 (m, 5H), 5.16 (s, 2H), 4.22−4.01 (m, 2H), 3.02−2.65 (m, 3H), 2.52 (s, 3H), 1.73−1.60 (m, 4H), 1.42 (s, 9H). MS (ES+): calcd for $C_{28}H_{33}F_3N_4O_5$ + H^+ 563.58, found 563.20.

*N-(2-Methyl-4-piperidin-4-yl-5-trifluoromethyl-benzoyl)-N′-(carbobenzyloxy)-guanidine (**87**).* To a solution of **86** (5.68 g, 10.1 mmol) in MeOH (20 mL) was added 4 M HCl in 1,4-dioxane (10 mL). The mixture was stirred at room temperature for 6 h. The mixture was concentrated under reduced pressure to afford the desired product (5.0 g, 100%) as the hydrochloride salt. ^{1}H NMR (DMSO-d_6): δ 10.03 (s, 1H), 9.48 (br s, 1H), 9.31−9.17 (m, 1H), 9.16−9.06 (m, 1H), 8.11 (s, 1H), 7.48 (s, 1H), 7.46−7.32 (m, 5H), 5.27 (s, 2H), 3.41−3.30 (m, 2H), 3.22−2.97 (m, 3H), 2.55 (s, 3H), 2.21−2.03 (m, 2H), 1.87−1.74 (m, 2H). MS (ES+): calcd for $C_{23}H_{25}F_3N_4O_3$ + H^+ 463.48, found 463.20.

*N-[4-(1-Acetyl-piperidin-4-yl)-2-methyl-5-trifluoromethyl-benzoyl]-guanidine (**78**).* To a solution of **87** (250 mg, 0.50 mmol) in DCM (2.5 mL) was added DIEA (0.26 mL, 1.50 mmol) followed by acetic anhydride (0.048 mL, 0.50 mmol). The mixture was stirred at room temperature for 45 min. The mixture was diluted with DCM and was washed with saturated aqueous $NaHCO_3$. The aqueous layer was back-extracted with DCM, and the combined organic phases were washed with brine and were dried (Na_2SO_4) and concentrated in vacuo. To a solution of the residue in DMF (1 mL) and EtOH (10 mL) purged with Ar was added 10 wt % Pd/C (50 mg). The mixture was stirred under an H_2 atmosphere overnight. The mixture was filtered through glass filter paper rinsing with ethyl acetate. The solvent was evaporated, and the residue was purified via preparative HPLC using a gradient elution from 10% to 75% acetonitrile/water with 0.1% TFA to obtain the desired product as the trifluoroacetate salt (85 mg, 35% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.87 (s, 1H), 8.86−8.25 (br m, 4H), 7.91 (s, 1H), 7.70 (s, 1H), 4.63−4.50 (m, 1H), 4.00−3.88 (m, 1H), 3.21−3.00 (m, 2H), 2.65−2.53 (m, 1H), 2.47 (s, 3H), 2.04 (s, 3H), 1.87−1.73 (m, 1H), 1.72−1.57 (m, 3H). MS (ES+): calcd for $C_{17}H_{21}F_3N_4O_2$ + H^+ 371.38, found 371.20.

*4-Bromo-5-methanesulfonyl-2-methyl-benzoic Acid (**71**).* To chlorosulfonic acid (15.0 mL, 226 mmol) cooled to 0 °C was added 4-bromo-2-methyl-benzoic acid **70** (5.00 g, 23.3 mmol) portionwise over 2 min. The reaction was allowed to warm to room temperature and was heated at 100 °C for 2 h. The reaction was cooled to room temperature and was added dropwise (very slowly) to ice (750 g). The resulting solid was collected by filtration and was dried in vacuo to afford the desired intermediate as a white solid. To a stirred solution of sodium sulfite (3.58 g, 28.4 mmol) and $NaHCO_3$ (8.52 g, 101.4 mmol) in H_2O (75 mL) heated to 70 °C was added a solution of the intermediate in THF (25 mL), dropwise over 20 min. After the addition was complete, the mixture was stirred at 70 °C for 1 h and then was cooled to rt. Iodomethane (6.31 mL, 101.4 mmol) was added, and the reaction was allowed to stir at 50 °C overnight. The mixture was cooled to room temperature, and the THF was removed in vacuo. The aqueous mixture was acidified (pH 5) with 4N aqueous HCl, and the resulting precipitate was collected by filtration and dried in vacuo at 50 °C to afford the desired product (4.88 g, 72% for two steps) as a white solid. ^{1}H NMR (DMSO-d_6): δ 13.52 (s, 1H), 8.45 (s, 1H), 7.95 (s, 1H), 3.37 (s, 3H), 2.60 (s, 3H). MS (ES+): calcd for $C_9H_9BrO_4S$ + H^+ 293.14, found 293.00.

*4-Bromo-5-methanesulfonyl-2-methyl-benzoic Acid Methyl Ester (**72**).* To a solution of **71** (4.88 g, 16.6 mmol) in MeOH (85 mL) was added acetyl chloride (12.9 mL, 166 mmol), and the mixture was stirred at 50 °C overnight. The reaction was concentrated to dryness, and the residue was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$. The layers were separated, and the aqueous layer was further extracted with ethyl acetate. The combined organic phases were washed with saturated aqueous $NaHCO_3$ and brine and were dried (Na_2SO_4) and concentrated in vacuo to afford the desired product (4.47 g, 87%) as an off-white solid. ^{1}H NMR (DMSO-d_6): δ 8.44 (s, 1H), 7.99 (s, 1H), 3.87 (s, 3H), 3.38 (s, 3H), 2.60 (s, 3H). MS (ES+): calcd for $C_{10}H_{11}BrO_4S$ + H^+ 307.16, found 307.00.

*4-(2-Methanesulfonyl-4-methoxycarbonyl-5-methyl-phenyl)-3,6-dihydro-2H-pyridine-1-carboxylic Acid tert-Butyl Ester (**69b**).* Each reactant was divided into three equal portions and was placed into three 20 mL microwave reaction vials: To a solution of **72** (3.50 g, 11.4 mmol) in 1,4-dioxane (8.5 mL) was added 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-3,6-dihydro-2*H*-pyridine-1-carboxylic acid *tert*-butyl ester (Digital, 3.52 g, 11.4 mmol) and 2 M aqueous K_2CO_3 (11.4 mL, 22.8 mmol). After the mixture was degassed, tetrakis(triphenylphosphine)palladium(0) (Strem, 1.32 g, 1.14 mmol) was added. Each vial was sealed with a Teflon lined septa cap and was irradiated in a microwave reactor at 170 °C for 30 min. After cooling, the mixtures from all of the vials were pooled together and were partitioned between ethyl acetate and water. The mixture was filtered to remove the remaining undissolved solid, rinsing with ethyl acetate and water. The layers of the filtrate were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo. The crude material was purified via flash chromatography using a gradient elution of 10−40% ethyl acetate/hexanes to afford the desired product (4.8 g, 100%) as a light-yellow oil. ^{1}H NMR (DMSO-d_6): δ 8.37 (s, 1H), 7.37 (s, 1H), 5.74 (s, 1H), 4.00−3.94 (br m, 2H), 3.87 (s, 3H), 3.56−3.49 (m, 2H), 3.20 (s, 3H), 2.59 (s, 3H), 2.38−2.30 (m, 2H), 1.43 (s, 9H). MS (ES+): calcd for $C_{20}H_{27}NO_6S$ + H^+ 410.50, found 432.20 (M + Na^+).

*4-(2-Methanesulfonyl-4-methoxycarbonyl-5-methyl-phenyl)-piperidine-1-carboxylic Acid tert-Butyl Ester (**88**).* To a solution of **69b** (2.70 g, 6.59 mmol) in acetic acid (40 mL) purged with Ar was added platinum(IV) oxide (750 mg). The mixture was stirred under an H_2 atmosphere overnight. The mixture was filtered through a pad of Celite rinsing with ethyl acetate. The solvent was evaporated under reduced pressure, and the residue was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$. The layers were separated, and the aqueous layer was further extracted with ethyl acetate. The

combined organic phases were washed with brine and were dried (Na_2SO_4) and concentrated in vacuo to afford the desired product (2.24 g, 83%) as an off-white solid. ^{1}H NMR (DMSO-d_6): δ 8.35 (s, 1H), 7.67 (s, 1H), 4.18−4.01 (br m, 2H), 3.85 (s, 3H), 3.59−3.47 (m, 1H), 3.28 (s, 3H), 2.93−2.69 (br m, 2H), 2.60 (s, 3H), 1.81−1.56 (m, 4H), 1.42 (s, 9H). MS (ES+): calcd for $C_{20}H_{29}NO_6S$ + H^+ 412.51, found 434.34 (M + Na^+).

*4-(4-Carboxy-2-methanesulfonyl-5-methyl-phenyl)-piperidine-1-carboxylic Acid tert-Butyl Ester (**89**).* The compound was prepared according to the procedure for compound **85** using as the starting material **88** (2.24 g, 5.44 mmol) to afford the desired product (1.85 g, 86%). ^{1}H NMR (DMSO-d_6): δ 13.23 (s, 1H), 8.36 (s, 1H), 7.62 (s, 1H), 4.19−4.01 (m, 2H), 3.58−3.46 (m, 1H), 3.27 (s, 3H), 2.95−2.69 (br m, 2H), 2.60 (s, 3H), 1.80−1.55 (m, 4H), 1.43 (s, 9H).

*4-{2-Methanesulfonyl-5-methyl-4-[N-(carbobenzyloxy)-guanidinocarbonyl]-phenyl}-piperidine-1-carboxylic Acid tert-Butyl Ester (**90**).* The compound was prepared and purified via flash chromatography using a gradient elution of 25−50% ethyl acetate in hexanes according to the procedure for compound **7** using as the starting material **90** (1.85 g, 4.65 mmol) to afford the desired product (2.15 g, 81%). MS (ES+): calcd for $C_{28}H_{36}N_4O_7S$ + H^+ 573.67, found 573.20.

*4-[5-Methanesulfonyl-2-methyl-4-piperidine-4-yl-benzoyl)-N-(carbobenzyloxy)-guanidine (**91**).* The compound was prepared according to the procedure for compound **87** using as the starting material **90** (2.15 g, 3.75 mmol) to afford the desired product (1.91 g, quantitative) as the hydrochloride salt. ^{1}H NMR (DMSO-d_6): δ 9.98 (br s, 1H), 9.50 (br s, 1H), 9.30−9.08 (m, 2H), 8.76−8.51 (m, 1H), 8.27 (s, 1H), 7.54−7.31 (m, 5H), 5.26 (s, 2H), 3.78−3.62 (m, 1H), 3.39−3.30 (m, 5H), 3.10−2.93 (m, 2H), 2.55 (s, 3H), 2.16−2.01 (m, 2H), 1.97−1.85 (m, 2H). MS (ES+): calcd for $C_{23}H_{28}N_4O_5S$ + H^+ 473.57, found 473.20.

*N-[5-Methanesulfonyl-4-(1-propionyl-piperidin-4-yl)-2-methyl-benzoyl]-guanidine (**82**).* To a solution of propionic acid (31 mg, 0.41 mmol) in DMF (3 mL) was added 1,1′-carbonyldiimidazole (70 mg, 0.43 mmol). The mixture was stirred at room temperature for 90 min, after which **91** (200 mg, 0.39 mmol) and DIEA (0.21 mL, 1.18 mmol) were added. The mixture was stirred at room temperature overnight. The mixture was poured over H_2O, and the resulting solid was collected by filtration. To a solution of the solid in ethanol (12 mL) and DMF (2 mL) purged with Ar was added 10 wt % Pd/C (35 mg). The mixture was stirred under an H_2 atmosphere overnight. The mixture was filtered through glass filter paper rinsing with ethyl acetate. The solvent was evaporated, and the residue was purified via preparative HPLC using a gradient elution from 5% to 60% acetonitrile/water with 0.1% TFA to obtain the desired product as the trifluoroacetate salt (84 mg, 42% over two steps). ^{1}H NMR (DMSO-d_6): δ 11.76 (s, 1H), 8.74−8.25 (br m, 4H), 8.10 (s, 1H), 7.71 (s, 1H), 4.64−4.53 (m, 1H), 4.05−3.94 (m, 1H), 3.71−3.59 (m, 1H), 3.31 (s, 1H), 3.18−3.06 (m, 1H), 2.68−2.54 (m, 1H), 2.48 (s, 3H), 2.36 (q, J = 7.3 Hz, 2H), 1.84−1.52 (m, 4H), 1.02 (t, J = 7.5 Hz, 3H). MS (ES+): calcd for $C_{18}H_{26}N_4O_4S$ + H^+ 395.50, found 395.20.

*N-[4-(1-Acetyl-piperidin-4-yl)-5-methanesulfonyl-2-methyl-benzoyl]-guanidine (**83**).* The compound was prepared and purified by preparative HPLC using a gradient elution from 1% to 50% acetonitrile/water with 0.1% TFA as the eluent according to the procedure for compound **78** using the starting material **92** (200 mg, 0.39 mmol) to give the desired intermediate which was deprotected using 10 wt % Pd/C (25 mg) to afford the desired product (50 mg, 26% over two steps) as the trifluoroacetate salt. ^{1}H NMR (DMSO-d_6): δ 11.77 (s, 1H), 8.72−8.25 (br m, 4H), 8.10 (s, 1H), 7.71 (s, 1H), 4.62−4.50 (m, 1H), 4.00−3.89 (m, 1H), 3.71−3.59 (m, 1H), 3.30 (s, 3H), 3.23−3.09 (m, 1H), 2.68−2.54 (m, 1H), 2.48 (s, 3H), 2.05 (s, 3H), 1.84−1.69 (m, 3H), 1.68−1.54 (m, 1H). MS (ES+): calcd for $C_{17}H_{24}N_4O_4S$ + H^+ 381.47, found 381.20.

*4-(2-Methanesulfonyl-4-methoxycarbonyl-phenyl)-3,6-dihydro-2H-pyridine-1-carboxylic Acid tert-Butyl Ester (**92**).* The compound was prepared and purified via flash chromatography using a gradient elution of 10−40% ethyl acetate/hexanes according to the procedure for compound **88**, starting from 4-bromo-3-methanesulfonyl-benzoic acid methyl ester (3.00 g, 10.2 mmol), to afford the desired product (2.73 g, 68%). ^{1}H NMR (DMSO-d_6): δ 8.48 (d, J = 1.7 Hz, 1H), 8.21 (dd, J = 1.9 Hz, 8.0 Hz, 1H), 7.53 (d, J = 7.6 Hz, 1H), 5.75 (s, 1H), 4.01−3.95 (br m, 2H), 3.91 (s, 3H), 3.58−3.50 (m, 2H), 3.25 (s, 3H), 2.39−2.30 (m, 2H), 1.43 (s, 9H). MS (ES+): calcd for $C_{19}H_{25}NO_6S$ + H^+ 396.47, found 396.30.

*4-(2-Methanesulfonyl-4-methoxycarbonyl-phenyl)-piperidine-1-carboxylic Acid tert-Butyl Ester (**93**).* The compound was prepared according to the procedure for compound **89**, using the starting material **92** (2.73 g, 6.90 mmol) to afford the desired product (2.00 g, 73%). ^{1}H NMR (DMSO-d_6): δ 8.46 (d, J = 1.9 Hz, 1H), 8.18 (dd, J = 1.8 Hz, 8.2 Hz, 1H), 7.83 (d, J = 8.5 Hz, 1H), 4.17−4.01 (br m, 2H), 3.89 (s, 3H), 3.65−3.54 (m, 1H), 3.33 (s, 3H), 2.95−2.71 (m, 2H), 1.82−1.70 (m, 2H), 1.69−1.55 (m, 2H), 1.42 (s, 9H). MS (ES+): calcd for $C_{19}H_{27}NO_6S$ + H^+ 398.49, found 342.20 (M − tBu^+).

*4-(4-Carboxy-2-methanesulfonyl-phenyl)-piperidine-1-carboxylic Acid tert-Butyl Ester (**94**).* The compound was prepared according to the procedure for compound **85** using starting material **93** (2.00 g, 5.03 mmol) to afford the desired product (1.90 g, 99%). ^{1}H NMR (DMSO-d_6): δ 13.31 (br s, 1H), 8.45 (d, J = 1.8 Hz, 1H), 8.16 (dd, J = 1.8 Hz, 8.2 Hz, 1H), 7.80 (d, J = 8.3 Hz, 1H), 4.17−4.01 (br m, 2H), 3.66−3.53 (m, 1H), 3.32 (s, 3H), 2.96−2.70 (m, 2H), 1.82−1.70 (m, 2H), 1.69−1.55 (m, 2H), 1.43 (s, 9H). MS (ES+): calcd for $C_{18}H_{25}NO_6S$ + H^+ 384.46, found 384.20.

*4-{2-Methanesulfonyl-4-[N-(carbobenzyloxy)-guanidinocarbonyl]-phenyl}-piperidine-1-carboxylic Acid tert-Butyl Ester (**95**).* The compound was prepared and purified via flash chromatography using a gradient elution of 25−50% ethyl acetate in hexanes as the eluent according to the procedure for compound **86**, using starting material **94** (1.90 g, 4.96 mmol) to afford the desired product (2.80 g, 100%). ^{1}H NMR (DMSO-d_6): δ 11.28 (s, 1H), 9.83 (br s, 1H), 8.75 (s, 1H), 8.63 (s, 1H), 8.30 (dd, J = 1.3 Hz, 8.1 Hz, 1H), 7.74 (d, J = 8.3 Hz, 1H), 7.48−7.26 (m, 5H), 5.22 (s, 2H), 4.22−4.01 (br m, 2H), 3.66−3.52 (m, 1H), 3.29 (s, 3H), 2.97−2.67 (m, 2H), 1.82−1.70 (m, 2H), 1.69−1.54 (m, 2H), 1.42 (s, 9H). MS (ES+): calcd for $C_{27}H_{34}N_4O_7S$ + H^+ 559.65, found 559.20.

*N-(3-Methanesulfonyl-4-piperidine-4-yl-benzoyl)-N′-(carbobenzyloxy)-guanidine (**96**).* The compound was prepared according to the procedure for compound **83**, using the starting material **95** (2.80 g, 5.01 mmol) and heating at 50 °C to afford the desired product (2.00 g, 81%) as the hydrochloride salt. ^{1}H NMR (DMSO-d_6): δ 10.04 (br s, 1H), 9.33−9.02 (br m, 2H), 8.64−8.59 (m, 1H), 8.49−8.41 (m, 1H), 7.72−7.66 (m, 1H), 7.47−7.34 (m, 5H), 5.27 (s, 2H), 3.79−3.63 (m, 1H), 3.52−3.31 (m, 5H), 3.12−2.96 (m, 2H), 2.18−2.00 (m, 2H), 1.97−1.85 (m, 2H). MS (ES+): calcd for $C_{22}H_{26}N_4O_5S$ + H^+ 459.54, found 459.20.

*N-[3-Methanesulfonyl-4-(1-propionyl-piperidin-4-yl)-benzoyl]-guanidine (**80**).* The compound was prepared and purified by preparative HPLC using a gradient elution from 5 to 60% acetonitrile/water with 0.1% TFA as the eluent according to the procedure for compound **82**, using the starting material **97** (200 mg, 0.40 mmol) to give the desired intermediate which was deprotected using 10 wt % palladium on carbon (50 mg) to afford the desired product (63 mg, 41% over two steps) as the trifluoroacetate salt. ^{1}H NMR (DMSO-d_6): δ 11.66 (s, 1H), 8.85−8.32 (br m, 4H), 8.45 (d, J = 1.9 Hz, 1H), 8.18 (dd, J = 1.8 Hz, 8.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 1H), 4.66−4.53 (m, 1H), 4.08−3.94 (m, 1H), 3.76−3.63 (m, 1H), 3.37 (s, 3H), 3.21−3.05 (m, 1H), 2.69−2.57 (m, 1H), 2.37 (q, J = 7.4 Hz, 2H), 1.88−1.53 (m, 4H), 1.02 (t, J = 7.4 Hz, 3H). MS (ES+): calcd for $C_{17}H_{24}N_4O_4S$ + H^+ 381.47, found 381.20.

*N-[4-(1-Acetyl-piperidin-4-yl)-3-methanesulfonyl-benzoyl]-guanidine (**81**).* The compound was prepared and purified by preparative HPLC using a gradient elution from 1% to 50% acetonitrile/water with 0.1% TFA as the eluent according to the procedure for compound **78**, using the starting material **97** (200 mg, 0.40 mmol) to give the desired intermediate which was deprotected using 10 wt % palladium on carbon (40 mg) to afford the desired product (91 mg, 47% over two steps) as the trifluoroacetate salt. ^{1}H NMR (DMSO-d_6): δ 11.62 (s, 1H), 8.79−8.29 (br m, 4H), 8.45 (d, J = 2.0 Hz, 1H), 8.18 (dd, J = 2.0 Hz, 8.2 Hz, 1H), 7.94 (d, J = 8.2 Hz, 1H), 4.62−4.51 (m,

1H), 4.01−3.90 (m, 1H), 3.76−3.64 (m, 1H), 3.37 (s, 3H), 3.23−3.11 (m, 1H), 2.69−2.57 (m, 1H), 2.05 (s, 3H), 1.86−1.72 (m, 3H), 1.68−1.53 (m, 1H). MS (ES+): calcd for $C_{16}H_{22}N_4O_4S$ + H^+ 367.44, found 367.20.

*4-Benzyloxy-2-hydroxy-benzoic Acid Methyl Ester (**74**).* 2,4-Dihydroxy-benzoic acid methyl ester **73** (25.0 g, 148.7 mmol) was dissolved in acetone (600 mL) and treated with K_2CO_3 (22.6 g, 163.5 mmol) and stirred at room temperature for 1 h. Benzyl bromide (19.43 mL, 163.5 mmol) was then added, and the reaction was heated to reflux for 3 h. The reaction was then cooled to room temperature and filtered. The filtrate was concentrated and triturated with water. The resulting solid was collected by filtration, resuspended in methanol, filtered, and dried under vacuum. A second crop of product was isolated by filtration of the filtrate after standing to give the desired product as a white solid (29.5 g, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 1H), 7.72 (d, J = 9.5 Hz, 1H), 7.46−7.34 (m, 5H), 6.62−6.59 (m, 2H), 5.16 (s, 2H), 3.86 (s, 3H).

*5-Bromo-2,4-dihydroxy-benzoic Acid Methyl Ester (**75**).* To a solution of **74** (29.04 g, 112.441 mmol) in chloroform (300 mL) at 0 °C was added a solution of bromine (6.34 mL, 123.7 mmol) in chloroform (80 mL). The resulting mixture was warmed to room temperature and stirred for 30 min, at which time it was washed with water. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The resulting solids were triturated with methanol to give the desired product as a solid (30.75 g, 81.1%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.73 (s, 1H), 7.91 (s, 1H), 7.48−7.33 (m, 5H), 6.82 (s, 1H), 5.27 (s, 2H), 3.86 (s, 3H).

*4-Benzyloxy-5-bromo-2-methoxy-benzoic Acid Methyl Ester (**76**).* To a solution of **75** (10.0 g, 26.7 mmol) in THF (250 mL) at 0 °C was added potassium *tert*-butoxide solution (1 M in THF, 35.6 mL, 35.6 mmol), and the mixture was stirred for 30 min. At this time, methyl iodide (2.40 mL, 38.6 mmol) was added and the reaction stirred 72 h at room temperature. The reaction mixture was concentrated and the residue taken up in water and neutralized by slow addition of 1N HCl. The resulting precipitate was collected by filtration and washed with water until the solids were white. The resulting solid was dissolved in ethyl acetate and washed with water, 1 M NaOH, and brine. The organic phase was collected, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was triturated with hexanes, and the solids were collected by filtration to give the desired product as a solid (8.10 g, 77.8%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.88 (s, 1H), 7.52−7.34 (m, 5H), 6.94 (s, 1H), 5.34 (s, 2H), 3.86 (s, 3H), 3.74 (s, 3H).

*4-Benzyloxy-2-methoxy-5-trifluoromethyl-benzoic Acid Methyl Ester (**77**).* **76** (7.50 g, 21.4 mmol), CuI (24.4 g, 128 mmol), and potassium trifluoroacetate (19.5 g, 128 mmol) were combined in a round-bottom flask, and the vessel was evacuated and backfilled with argon three times. To the mixture was then added NMP (150 mL), and the reaction was heated at 150 °C overnight. The reaction was cooled to room temperature and poured into water (1.2 L), and the resulting precipitate was collected by filtration. The filter cake was then rinsed with ethyl acetate (750 mL) into a separate filtration flask, and the filtrate was dried over $MgSO_4$, filtered, and concentrated to afford a paste. The paste was treated with water, and the resulting solid was collected by filtration to give the desired product as a brown solid (6.14 g, 84.5%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.95 (s, 1H), 7.48−7.34 (m, 5H), 7.02 (s, 1H), 5.40 (s, 2H), 3.93 (s, 3H), 3.76 (s, 3H).

*4-Hydroxy-2-methoxy-5-trifluoromethyl-benzoic Acid Methyl Ester (**97**).* Synthesized according to the procedure for phenol **68** to give the desired product as a brown solid (3.36 g, 74.4%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.42 (s, 1H), 7.88 (s, 1H), 6.67 (s, 1H), 3.82 (s, 3H), 3.74 (s, 3H).

*4-(4-Carboxy-5-methoxy-2-trifluoromethyl-phenyl)-3,6-dihydro-2H-pyridine-1-carboxylic Acid tert-Butyl Ester (**69c**).* To a solution of **97** (3.36 g, 13.4 mmol) and DIEA (2.57 mL, 14.8 mmol) in DMF (75.0 mL) was added *N*-phenyltrifluoromethanesulfonimide (5.04 g, 14.1 mmol), and the resulting mixture was stirred overnight. The reaction was then poured into a stirred saturated solution of $NaHCO_3$ in water. The mixture was then extracted with ethyl acetate (3×), and the combined extracts were washed with water (4×), saturated aqueous $NaHCO_3$, and brine. The organic phase was collected, dried over Na_2SO_4, filtered, and concentrated to give the desired intermediate as a brown oil. A solution of this intermediate in 1,4-dioxane (56.0 mL) was added to a mixture of $Pd(Ph_3P)_4$ (1.58 g, 1.37 mmol), **16** (4.22 g, 13.6 mmol), and K_2CO_3 (3.53 g, 27.3 mmol) in a pressure flask. To this mixture was then added water (14.0 mL), and the flask was sealed and heated at 140 °C overnight. The reaction was cooled to room temperature, poured over ice, and extracted with ethyl acetate. The extracts were dried over Na_2SO_4, filtered, and concentrated to afford a brown oil, which was purified via flash chromatography using a gradient elution of 0−100% ethyl acetate/hexanes to afford the desired product as a solid (3.60 g, 66.7% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.02 (s, 1H), 7.92 (s, 1H), 7.09 (s, 1H), 5.63 (bs, 1H), 3.94 (bs, 1H), 3.89 (s, 3H), 3.52 (t, J = 5.4 Hz, 2H), 2.32 (bs, 2H), 1.42 (s, 9H).

*4-(4-Carboxy-5-methoxy-2-trifluoromethyl-phenyl)-piperidine-1-carboxylic Acid tert-Butyl Ester (**99**).* Synthesized according to the procedure for **85** to give the desired product as a solid (3.10 g, 85.7%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.93 (bs, 1H), 7.89 (s, 1H), 7.24, (s, 1H), 4.11 (bd, J = 12.8 Hz, 2H), 3.92, (s, 3H), 2.97−2.91 (m, 1H), 2.78 (bs, 2H), 1.82−1.72 (m, 2H), 1.648 (bd, J = 11.8 Hz, 2H), 1.42 (s, 9H).

*4-[5-Methoxy-4-(N-(carbobenzyloxy)-guanidinocarbonyl)-2-trifluoromethyl-phenyl]-piperidine-1-carboxylic Acid tert-Butyl Ester (**100**).* Synthesized according to the procedure for **86** to give the desired product which was used crude without purification (4.08 g, 91.8%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.00 (bs, 1H), 8.92 (bs, 1H), 8.72 (bs, 1H), 8.07 (s, 1H), 7.38−7.30 (m, 6H), 5.08 (s, 2H), 4.12−4.05 (m, 5H), 2.99−2.93 (m, 1H), 2.79 (bs, 2H), 1.85−1.77 (m, 2H), 1.66 (bd, J = 12.4, 2H), 1.42 (s, 9H).

*N-(2-Methoxy-4-piperidin-4-yl-5-trifluoromethyl-benzoyl)-N′-(carbobenzyloxy)-guanidine (**101**).* Synthesized according to the procedure for **87** to give the desired product hydrochloride salt as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.24 (bs, 1H), 9.67 (bs, 1H), 9.37 (bs, 1H), 8.98−8.91 (m, 2H), 8.14 (s, 1H), 7.44−7.36 (m, 6H), 5.22 (s, 2H), 4.10 (s, 3H), 3.40−3.35 (m, 2H), 3.25−3.19 (m, 1H), 3.13−3.04 (m, 2H), 2.28−2.19 (m, 2H), 1.84 (bd, J = 12.6 Hz, 2H). MS (ES+): calcd for $C_{23}H_{25}F_3N_4O_4$ + H^+ 479.48, found 479.74.

*N-[4-(1-Acetyl-piperidin-4-yl)-2-methoxy-5-trifluoromethyl-benzoyl]-guanidine (**102**).* The CBZ-protected amide intermediate was obtained using the CDI coupling general procedure from acetic acid (12.2 μL, 0.214 mmol) and **101** (100 mg, 0.165 mmol). The intermediate obtained was used crude in the following step. The CBZ protecting group was removed according to the general procedure using $Pd(OH)_2$ on carbon (20 wt % Pd, 50% water wet, 10.0 mg, 0.007 mmol). The residue was purified via preparative HPLC using a gradient elution from 10% to 100% acetonitrile/water with 0.1% TFA to obtain the desired product (26.0 mg, 32% over two steps) as the trifluoroacetate salt. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.82 (s, 1H), 8.6−8.35 (br m, 4H), 8.02 (s, 1H), 7.40 (s, 1H), 4.59 (d, J = 14.0 Hz, 1H), 4.05 (br s, 3H), 3.96 (br d, J = 12.9, 1H), 3.17−3.04 (br m, 2H), 2.61−2.55 (m, 1H), 2.05 (br s, 2H), 1.94−1.85 (br m, 1H), 1.77−1.64 (br m, 2H). MS (ES+): calcd for $C_{17}H_{21}F_3N_4O_3$ + H^+ 387.38, found 387.20.

Biological Assays. *Evaluation of NHE1 Inhibition (pH_i).* HT-29 cells (purchased from American Type Culture Collection (ATCC), Manassas, VA) resuspended in DMEM supplemented with 10% FBS, 1% NEAA, and 1% Penn−Strep were seeded at 10000 cells/well in collagen-coated 384-well plates, which were then incubated for 24 h at 37C. The next day, the medium was removed and the cells were dye (Invitrogen's BCECF) loaded for 30′ at 37C, washed three times with an acid loading buffer (10 mM NH_4Cl, 1.8 mM $CaCl_2$, 90 mM choline Cl, 5 mM glucose, 15 mM Hepes, 5 mM KCl, 1 mM $MgCl_2$, adjusted to pH 7.5 with KOH), and further incubated at RT for 30 min. At the end of the incubation, the buffer was removed and 5 μL of fresh acid loading buffer was added to each well to prevent the desiccation of the cells. The plate was placed in a Hamamatsu FDSS6000 instrument, and candidate compounds were added to the cells in buffer lacking the

NH_4Cl. The plates were read, and the compound IC_{50}s were calculated as measurements of the 50% inhibition of the intracellular pH recovery.

Evaluation of NHE2 and -3 Inhibition (pH_i). PS120 cells stably transfected with NHE-2 or 3 were resuspended in DMEM supplemented with 10% FBS, 1% NEAA, and 1% Penn−Strep are seeded at 10000 cells/well in collagen-coated 384-well plates which were then incubated for 24 h at 37 °C under 5% CO_2. The next day, the medium were removed and the cells were dye (Invitrogen's BCECF) loaded for 30 min at 37 °C, washed three times with an acid loading buffer (10 mM NH_4Cl, 1.8 mM $CaCl_2$, 90 mM choline Cl, 5 mM glucose, 15 mM Hepes, 5 mM KCl, 1 mM $MgCl_2$, adjusted to pH 7.5 with KOH), and further incubated at RT for 30 min. At the end of the incubation, the buffer was removed and 5 μL of fresh acid loading buffer was added to each well to prevent the desiccation of the cells. The plate was placed in a Hamamatsu FDSS6000 instrument, and candidate compounds were added to the cells in buffer lacking the NH_4Cl. The plates were read, and the compound IC_{50}s were calculated as measurements of the 50% inhibition of the intracellular pH recovery.

Evaluation of Functional Potency in Human Platelets (hPSA). Human blood was collected into 10 mL K_2 EDTA tubes (BD, no. 366643) at room temperature and centrifuged at 150*g* for 10 min at room temperature, and platelet rich plasma (PRP) that comprised the upper two-thirds of the plasma layer was used for the assessment of platelet swelling. The remaining plasma was further centrifuged at 1400*g* to obtain platelet poor plasma (PPP). First, 22 μL test compounds (10×) and vehicle controls were added to 96-well plates to which 28 μL/well PRP was then added. Then 172 μL/well of propionate medium (PM) (sodium propionate 140 mM, HEPES 20 mM, glucose 10 mM, KCL 5 mM, $MgCl_2$ 1 mM and $CaCl_2$ 1 mM; pH 6.7) was placed into 96-well plates to initiate platelet swelling. Platelet swelling was measured as a decrease in optical density at 680 mM measured over 6 s over 5 min using a microplate reader (Molecular Devices VMAX). Slope values were calculated, and POC was calculated using the changes in slope from control values.

Inhibition of Cytochrome P-450s 2C19, 2C9, 2D6, and 3A4. Global lot human liver microsomes (HLM, Gentest) were used in all CYP IC_{50} assessments with drug probes. CYP selective probe substrates (see Table 11) and HLM were mixed with 0−12.5 μM test compound, followed by 2 mM NADPH, and incubated at 37 °C for a defined time period depending on the probe reactions. Reaction was quenched with acetonitrile containing an internal standard (IS). The levels of respective metabolites generated in the CYP-selective probe reactions were quantified against the IS using LC/MS/MS. The IC_{50} values were generated by fitting data using the GraFit 5 program.

Table 11

CYP isoform	probe substrate	incubation time (min)
2C19	7-ethoxy-3-cyanocoumarin	45
2C9	7-methoxy-4-(trifluoromethyl)-coumarin	45
2D6	3-[2-(*N*,*N*-diethyl-*N*-methylamino)ethyl]-7-methoxy-4-methylcoumarin	45
3A4	7-benzyloxy-4-(trifluoromethyl)-coumarin	30

Assessment of Stability in HLM and RLM. Stability of the test compounds was assessed in commercially obtained human and rat liver microsomes (BD Sciences). Incubations in duplicate consisted of 1 μM test compound, 1 mg/mL microsomal protein, 2.5 mM NADPH, and 50 mM potassium phosphate buffer, pH 7.4. The compound was preincubated at 37 °C in the assay buffer for 5 min, and then reaction was initiated with NADPH addition. Aliquots were removed at eight time points up to 60 min, reaction quenched with acetonitrile, and analyzed by LC/MS/MS for depletion of parent compound relative to initial concentration.

Assessment of Membrane Permeability Using the PAMPA Assay.[33] The parallel artificial membrane permeability assay (PAMPA) was carried out using a PAMPA Evolution permeability analyzer form pION, Inc., A "sandwich" was formed using 96-well microtiter plate and a 96-well filter plate. The pH values of the buffer solutions in donor wells were adjusted to 5.0, 6.2, and 7.4 with 0.5 M NaOH, the upper acceptor plate was filled with a buffer solution ASB-7.4 (pION Inc.), and the filters (pore size 0.45, thickness 125 μm) were coated with 4 μL of lipid (pION Inc.). The stock solution of the test compound was dissolved in DMSO at 10 mM concentration, of which the donor solution of 41.7 μM was prepared in diluted buffer solution. The PAMPA sandwich was incubated for 4 h without stirring. The concentration of the test compound was measured in duplicate for the reference, donor, and acceptor plates with a UV plate reader. The effective permeability of the test compound was calculated using the PAMPA Evolution permeability analyzer software. Result reporting (sum of Pe at 3 pHs): Low permeability: $<2 \times 10^{-6}$ cm/s. High permeability: $\geq 2 \times 10^{-6}$ cm/s.

Determination of hERG Pharmacology Using the PatchXpress Platform. The hERG pharmacology of test agents was assessed on the PatchXpress automated electrophysiology platform (Molecular Devices, CA) as previously described[34] with some minor modifications. A HEK cell line stably expressing hERG potassium channels was loaded onto PatchXpress at a final concentration of 1×10^6 cells/mL. Voltage-dependent hERG currents were recorded from single cells using the whole-cell configuration of the patch clamp technique. Peak hERG tail current was monitored during application of successive drug concentrations until a new steady state was achieved. Multiple test article concentrations were applied cumulatively in ascending order without washout between applications. Data points were collected at a frequency of 0.1 Hz. Drugs were applied once a stable baseline had been recorded for 5 min. For PatchXpress experiments, 0.1% DMSO (v/v) in extracellular solution was added as a negative control before drug application. The steady-state hERG current amplitude before and after test article application was used to calculate the percentage of current inhibited at each concentration and to derive an IC_{50} value. Valid whole-cell recordings met the following criteria: Membrane resistance (Rm) ≥ 200 MW, access resistance (Ra) ≤ 8 MW (after series resistance compensation), peak hERG tail current ≥200 pA, and leak current ≤25% peak current.

Assessment of Membrane Permeability and Transport Using Caco-2 Cells. Caco-2 cells (American Type Culture Collection, Rockville, MD) from passage number 36 were grown for 21 days in 12-transwell plates, and cells with TEER value greater than 350 Ω·cm^2 were used to determine compound permeability and efflux ratio. Incubations with 10 μM compound were conducted on Tecan Genesis 150. Aliquots of the compound from apical (A−B) and basolateral (B−A) sides were transferred at various time points up to 150 min for analysis by LC/MS/MS. Apparent permeability coefficient (cm/s) was determined using the following relationship:

$$P_{app} = \frac{\Delta C \cdot V}{\Delta t \cdot A \cdot S} \quad \text{(cm/s)}$$

where P_{app} is apparent permeability coefficient (cm/s), C is concentration in receiver compartment (μmol/cm^3), A is filter area (cm^2), t is time (s), S is concentration in donor compartment (μmol/cm^3), and V is volume in receiver compartment (cm^3).

Evaluation of Rat Pharmacokinetics. With approval from Institutional Animal Care and Use Committee, jugular and femoral vein precannulated male Sprague−Dawley rats (Charles River Laboratories) were used to assess the pharmacokinetic profile of compounds. The compound was dissolved in 20% hydroxypropyl β-cyclodextrin (HPBCD) at 1 mg/mL for intravenous (iv) dosing and suspended in 0.015% Tween 80 + 0.5% aqueous methylcellulose for oral (PO) dosing to overnight fasted rats (N = 3 per group) at 1 and 5 mg/kg, respectively. Serial blood samples were collected up to 24 h in K_2EDTA tubes for plasma collection. Plasma samples were frozen at −20 °C until analysis. The samples were processed by protein precipitation with acetonitrile, and supernatants were analyzed by LC/MS/MS. The instrumentation consisted of Hewlett-Packard series 1200 pump, Leap CTC PAL autosampler, and Applied Biosystems API4000 mass spectrometer, operating in MRM mode.

Evaluation of Dog Pharmacokinetics. Single dose iv and po PK studies were performed in male Beagle dog (N = 3 per group) using the dosing vehicles as described for rat PK studies. After 1 mg/kg iv or 5 mg/kg po doses to overnight fasted animals, blood sampling was done up to 72 h post dose, followed by plasma sample analysis as described for rat PK.

Assessment of CYP 3A4 Induction. DPX cells (stably transformed with human PXR and a reporter gene vector containing the enhancer regions of CYP3A4) were used for assessment of potential PXR activation and CYP3A4 induction by the test compound at concentrations of 0.1, 1, and 10 μM. Rifampicin, mifepristone, and androstanol were used as positive controls for strong, moderate, and weak CYP3A4 induction. The induction potentials at each of the concentrations were read as percent of induction at 10 μM of the positive controls.

Assessment of CYP3A4 Inactivation. Time- and concentration-dependent inhibition of CYP3A4 by test compound was tested in hLM and was characterized by determining residual enzyme activity using midazolam as the probe substrate. Preincubations were done (37 °C) from 0 to 20 min with 0.1 mg/mL hLM, at 5 concentrations of test compound ranging from 0 to 50 μM with NADPH generating system. Secondary incubation with midazolam was performed at 37 °C for 8 min, followed by analysis of midazolam metabolites.

AMES Mutagenicity Evaluation.[35] The mutagenic potential was assessed using *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 uvrA (pKM101) in a standard plate incorporation format. Compounds were dissolved in DMSO and added to bacterial cultures at concentrations up to 5000 μg/plate in the presence or absence of an Aroclor 1254-induced rat liver S9 metabolic activating system. Each dose level, vehicle, and positive control was plated in triplicate.

Cytotoxicity Potential Determination. The cytotoxicity potential of the compound was assessed using HeLa cells in a 96-well format. Cells were seeded in 96-well plates overnight. Compounds were dissolved in DMSO and added to cell plates at concentrations up to 100 μM in triplicates. After a 24 h dosing period, plates were stained with Cytotoxicity 1 HitKit and imaged on Thermo Scientific Cellomics ArrayScan VTI. Nuclear morphology and size,[36] membrane permeability status,[37] lysosome mass/pH changes,[38] and cell density changes[39] were assessed against vehicle control to generate a cytotoxicity index for each compound.

Phospholipidosis Potential Determination. Phospholipidosis was measured in a Nile Red flow cytometry assay[40,41] using U937 cells. Cells were treated with compound at concentrations up to 500 μM. After 48 h incubation, the cells were fixed and labeled with Nile Red and propidium iodine dyes. Nile Red fluorescence intensity was assessed against vehicle control, and values were corrected to cell viability.

Ex Vivo Ischemia-Reperfusion Study. Experiments were performed on isolated hearts obtained from male Sprague–Dawley rats weighing 275–300 g (Charles River, St. Constant, Quebec, Canada). The animals were maintained in the Health Sciences Animal Care Facility of the University of Western Ontario in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, Ontario, Canada). For heart perfusion, the rats were euthanized by decapitation and the hearts were immediately removed, placed in cold Krebs–Henseleit buffer (see composition below) to inhibit any further contractions, and then mounted by the aorta on a stainless steel cannula and arranged for retrograde perfusion at a flow rate of 10 mL/min according to the Langendorff method. Flow was maintained at a constant rate with the use of a peristaltic pump. The perfusion fluid was Krebs–Henseleit buffer composed of the following (in mmol/L): 120 NaCl, 4.63 KCl, 1.17 KH_2PO_4, 1.25 $CaCl_2$, 1.2 $MgCl_2$, 20 $NaHCO_3$, and 8 glucose. The buffer was initially equilibrated and then continuously gassed with a 95% O_2–5% CO_2 mixture. The pH of the buffer was 7.4, and temperature was maintained at 37 °C by enclosing the entire system in a series of water-jacketed coils. Coronary pressure was measured via a side arm of the perfusion cannula, which was connected to a pressure transducer (model P23 XL, Spectramed). A latex water-filled balloon fixed to a pressure transducer was inserted through the mitral valve into the left ventricle (LV) for the determination of LV developed pressure (LVDP). LV end-diastolic pressure (LVEDP) was adjusted to 5 mmHg before the start of the experiment by adjusting the volume in the intraventricular balloon with the aid of a micrometer syringe. Hearts were electrically paced at a rate of 5 Hz throughout the entire perfusion period. All determinations of ventricular performance were obtained online with a Biopac (Goleta, CA) data analysis system. After a 30 min equilibration period, zero flow ischemia was induced by shutting of the peristaltic pump for 45 min after which flow was rapidly reintroduced for a further 30 min. When studied, **60** was administered 10 min before initiating ischemia and then throughout the ischemia and reperfusion period. Data were analyzed using a repeated measures ANOVA followed by Tukey's posthoc test. N = 6 for all groups. *P < 0.05 from **60**-free group.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

APD, action potential duration; CDI, carbonyl diimidazole; DIEA, diisopropylethylamine; hERG, human ether-a-go-go channel; hLM, human liver microsome; hPSA, human platelet swelling assay; KOtBu, potassium *tert*-butoxide; LV, left ventrical; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; MRT, mean residence time; NCX, sodium calcium exchanger; NHE, sodium hydrogen exchanger; NMM, *N*-methyl morpholine; rLM, rat liver microsome; Pd/C, palladium on carbon; pHi, pH internal; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; Vdss, volume of distribution at steady-state; HATU, 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

REFERENCES

(1) Karmazyn, M.; Gan, X. T.; Humphreys, R. A.; Yoshida, H.; Kusumoto, K. The myocardial Na^+–H^+ exchanger: Structure, regulation, and its role in heart disease. *Circ. Res.* **1999**, *85* (9), 777–786.

(2) Orlowski, J; Kandasamy, R. A.; Shull, G. E. Molecular cloning of putative members of the Na/H exchanger gene family: cDNA cloning, deduced amino acid sequence, and mRNA tissue expression of the rat Na/H exchanger NHE-1 and two structurally related proteins. *J. Biol. Chem.* **1992**, *267*, 9331–9339.

(3) (a) Fliegel, L.; Dyck, J. R. B. Molecular biology of the cardiac sodium/hydrogen exchanger. *Cardiovasc. Res.* **1995**, *29* (2), 155−159. (b) Tani, M.; Neely, J. R. Role of intracellular Na^+ in Ca2$^+$ overload and depressed recovery of ventricular function of reperfused ischemic rat hearts. Possible involvement of H−Na and Na−Ca exchange. *Circ. Res.* **1989**, *65*, 1045−1056. (c) Haigney, M. C.; Miyata, H.; Lakatta, E. G.; Stern, M. D.; Silverman, H. S. Dependence of hypoxic cellular calcium loading on Na^+−Ca^{2+} exchange. *Circ. Res.* **1992**, *71*, 547−557. (d) Allen, D. G.; Xiao, X.-H. Role of the cardiac Na^+/H^+ exchanger during ischemia and reperfusion. *Cardiovasc. Res.* **2003**, *57* (4), 934−941. (e) Baartscheer, A.; Schumacher, C. A.; Belterman, C. N. W.; Coronel, R.; Fiolet, J. W. T. $[Na^+]_i$ and the driving force of the Na^+/Ca^{2+}-exchanger in heart failure. *Cardiovasc. Res.* **2003**, *57*, 986−995. (f) Baartscheer, A.; Borren, M. M. C.; Schumacher, C. A.; Belterman, C. N. W.; Coronel, R.; Fiolet, J. W. T. Increased Na^+/H^+-exchange activity is the cause of increased $[Na^+]_i$ in heart failure and underlies disturbed calcium handling. *Cardiovasc. Res.* **2003**, *57* (4), 1015−1024.

(4) Avkiran, M. Rational basis for use of sodium−hydrogen exchange inhibitors in myocardial ischemia. *Am. J. Cardiol* **1999**, *83* (10), 10G−18G.

(5) Avkiran, M. Protection of the myocardium during ischemia and reperfusion: Na^+/H^+ exchange inhibition versus ischemic preconditioning. *Circulation* **1999**, *100*, 2469−2472.

(6) Avkiran, M.; Marber, M. S. Na^+/H^+ exchange inhibitors for cardioprotective therapy: progress, problems and prospects. *J. Am. Coll. Cardiol.* **2002**, *39*, 747−753.

(7) Engelhardt, S.; Hein, L.; Keller, U.; Klambt, K.; Lohse, M. J. Inhibition of Na^+−H^+ exchange prevents hypertrophy, fibrosis, and heart failure in beta(1)-adrenergic receptor transgenic mice. *Circ. Res.* **2001**, *19*, 814−819.

(8) Gumina, R. J.; Buerger, E.; Eickmeier, C.; Moore, J.; Daemmgen, J.; Gross, G. J. Inhibition of the Na^-/H^- exchanger confers greater cardioprotection against 90 minutes of myocardial ischemia than ischemic preconditioning in dogs. *Circulation* **1999**, *100*, 2519−2526.

(9) Camillión de Hurtado, M. C.; Portiansky, E. L.; Perez, N. G.; Rebolledo, O. R.; Cingolani, H. E. Regression of cardiomyocyte hypertrophy in SHR following chronic inhibition of the Na^+/H^+-exchanger. *Cardiovasc. Res.* **2002**, *53* (4), 862−868.

(10) Chen, L.; Chen, C. X.; Can, X. T.; Beier, N.; Scholz, W.; Karmazyn, M. Inhibition and reversal of myocardial infarction-induced hypertrophy and heart failure by NHE-1 inhibition. *Am. J. Physiol.* **2004**, *286* (1), H381−H387.

(11) Baartscheer, A.; Schumacher, C. A.; Belterman, C. N. W.; Coronel, R.; Fiolet, J. W. T. Chronic inhibition of Na^+/H^+-exchanger attenuates cardiac hypertrophy and prevents cellular remodelling in heart failure. *Cardiovasc. Res.* **2005**, *65* (1), 83−92.

(12) Chahine, M.; Bkaily, G.; Nader, M.; Al-Khoury, J.; Jacques, D.; Beier, N.; Scholz, W.. NHE-1-dependent intracellular sodium overload in hypertrophic hereditary cardiomyopathy: prevention by NHE-1 inhibitor. *J. Mol. Cell. Cardiol.* **2005**, *38* (4), 571−582.

(13) Baartscheer, A. Chronic inhibition of the Na^+/H^+-exchanger in the heart. *Curr. Vasc. Pharmacol.* **2006**, *4* (1), 23−29.

(14) Baartscheer, A.; Hardziyenka, M.; Schumacher, C. A.; Belterman, C. N. W.; van Borren, M. M. G. J.; Verkerk, A. O.; Coronel, R.; Fiolet, J. W. T. Chronic inhibition of the Na^+/H^+ exchanger causes regression of hypertrophy, heart failure, and ionic and electrophysiological remodelling. *Br. J. Pharmacol.* **2008**, *154* (6), 1266−1275.

(15) Mraiche, F.; Oka, T.; Gan, X. T.; Karmazyn, M.; Fliegel, L. Activated NHE1 is required to induce early cardiac hypertrophy in mice. *Basic Res. Cardiol.* **2011**, *106*, 603−616.

(16) Touret, N.; Tanneur, V.; Godart, H.; Seidler, R.; Taki, N.; Burger, E.; Dammgen, J.; Counillon, L. Characterization of sabiporide, a new specific NHE-1 inhibitor exhibiting slow dissociation kinetics and cardioprotective effects. *Eur. J. Pharmacol.* **2003**, *459* (2−3), 151−158.

(17) Knight, D. R.; Smith, A. H.; Flynn, D. M.; MacAndrew, J. T.; Ellery, S. S.; Kong, J. X.; Marala, R. B.; Wester, R.t.; Guzman-Perez, A.; Hill, R. J.; Magee, W. P.; Tracey, W. R. A novel sodium-hydrogen exchanger isoform-1 inhibitor, zoniporide, reduces ischemic myocardial injury in vitro and in vivo. *J. Pharmacol. Exp. Ther.* **2001**, *297* (1), 254−259.

(18) (a) Scholz, W.; Jessel, A.; Albus, U. Development of the Na^+/H^+ exchange inhibitor cariporide as a cardioprotective drug: from the laboratory to the GUARDIAN trial. *J. Thromb. Thrombolysis* **1999**, *8* (1), 61−69. (b) Karmazyn, M. Pharmacology and clinical assessment of cariporide for the treatment of coronary artery diseases. *Expert Opin. Invest. Drugs* **2000**, *9* (5), 1099−1108. (c) Yeshmina, T.; Akao, M.; Jones, S. P.; Marban, E. Cariporide (HOE642), a selective Na^+−H^+ exchange inhibitor, inhibits the mitochondrial death pathway. *Circulation* **2003**, *108*, 2275−2281.

(19) Zeymer, U.; Suryapranata, H.; Monassier, J. P.; Opolski, G.; Davies, J.; Rasmanis, G.; Linssen, G.; Tebbe, U.; Schroder, R.; Tiemann, R.; Machnig, T.; Neuhaus, K. L. The Na^+/H^+ exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction. *J. Am. Coll. Cardiol.* **2001**, *38*, 1644−1650.

(20) Gumina, R. J.; Auchampach, J.; Wang, R.; Buerger, E.; Eickmeier, C.; Moore, J.; Daemmgen, J.; Gross, G. J. Na^+/H^+ exchange inhibition-induced cardioprotection in dogs: effects on neutrophils versus cardiomyocytes. *Am. J. Physiol.: Heart Circ. Physiol.* **2000**, *279* (4), 1563−1570.

(21) Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, J. P. Biotransformation reactions of five-membered aromatic heterocyclic rings. *Chem. Res. Toxicol.* **2002**, *15* (3), 269−299.

(22) Kalgutkar, A. S.; Gardner, I.; Obach, R. S.; Shaffer, C. L.; Callegari, E.; Henne, K. R.; Mutlib, A. E.; Dalvie, D. K.; Lee, J. S.; Nakai, Y.; O'Donnell, J. P.; Boer, J.; Harriman, S. P. A comprehensive listing of bioactivation pathways of organic functional groups. *Curr. Drug Metab.* **2005**, *6*, 161−225.

(23) (a) Mukaiyama, T.; Usui, M.; Shimada, E.; Saigo, K. A convenient method for the synthesis of carboxylic esters. *Chem. Lett.* **1975**, 1045−1048. (b) Saigo, K.; Usui, M.; Kikuchi, K.; Shimada, E.; Mukaiyama, T. New method for the preparation of carboxylic esters. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1863−1866. (c) Bald, E.; Saigo, K.; Mukaiyama., T. A facile synthesis of carboxamides by using 1-methyl-2-halo pyridinium iodides as coupling reagents. *Chem. Lett.* **1975**, 1163−1166.

(24) Baumgarth, M.; Beier, N.; Gericke, R. (2-Methyl-5-(methylsulfonyl)benzoyl)guanidine Na^+/H^+ antiporter Inhibitors. *J. Med. Chem.* **1997**, *40*, 2017−2034.

(25) (a) Ahmad, S.; Doweyko, L. M.; Dugar, S.; Grazier, N.; Ngu, K.; Wu, S. C.; Yost, K. J.; Chen, B.-C.; Gougoutas, J. Z.; DiMarco, J. D.; Lan, S.-J.; Gavin, B. J.; Chen, A. J.; Dorso, C. R.; Serafino, R.; Kirby, M.; Atwal, K. S. Arylcyclopropanecarboxyl guanidines as novel, potent, and selective inhibitors of the sodium hydrogen exchanger isoform-1. *J. Med. Chem.* **2001**, *44*, 3302−3310. (b) Ahmad, S.; Ngu, K.; Combs, D. W.; Wu, S. C.; Weinstein, S. D.; Liu, W.; Chen., B.-C.; Chandrasena, G.; Dorso, C.; Kirby, M.; Atwal, K. S. Aminoimidazoles as bioisosteres of acylguanidines: novel, potent, selective, and orally bioavailable inhibitors of the sodium hydrogen exchanger isoform-1. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 177−180. (c) Atwal, K. S.; O'Neil, S. V.; Ahmad, S.; Doweyko, L.; Kirby, M.; Dorso, C. R.; Chandrasena, G.; Chen, B.-C.; Zhao, R.; Zahler, R. Synthesis and biological activity of 5-aryl-4-(4-(5-methyl-1*H*-imidazol-4-yl)piperidin-1-yl)pyrimidine analogues as potent, highly selective, and orally bioavailable NHE-1 inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4796−4799. (d) Lee, S.; Yang, K.; Lee, B. H.; Yoon, B. S. Synthesis and biological evaluation of 4-heteroaryl-2-amino-5-methylimidazole analogs as NHE-1 inhibitors. *Bull. Korean Chem. Soc.* **2009**, *30* (11), 2621−2624.

(26) Wang, R.; Gao, Y.; Lai, L. Calculating partition coefficient by atom-additive method. *Perspect. Drug Discovery Des.* **2000**, *19*, 47−66.

(27) Riley, R. J.; Parker, A. J.; Trigg, S.; Manners, C. N. Development of a generalized, quantitative physicochemical model of CYP3A4 inhibition for use in early drug discovery. *Pharm. Res.* **2001**, *18* (5), 652−655.

(28) (a) Langendorff, O. Geschichtliche Betrachtungen zur Methodedes überlebenden Warmblüterherzens. Muench. *Med. Wochenschr.*

1903, *50*, 508−509. (b) Zimmer, H.-G. The isolated perfused heart and its pioneers. *News Physiol. Sci.* **1998**, *13*, 203−210.
(29) Schmuck, C.; Bickert, V.; Merschky, M.; Geiger, L.; Rupprecht, D.; Dudaczek, J.; Wich, P.; Rehm, T.; Machon, U. A Facile and Efficient Multi-Gram Synthesis of *N*-Protected 5-(Guanidinocarbonyl)-1*H*-pyrrole-2-carboxylic Acids. *Eur. J. Org. Chem.* **2008**, 324−329.
(30) Pérez, A.; Gimeno, N.; Vera, F.; Ros, M. B.; Serrano, J. L.; De la Fuente, M. R. New H-bonded complexes and their supramolecular liquid-crystalline organizations. *Eur. J. Org. Chem.* **2008**, 826−833.
(31) Garneau-Tsodikova, S.; Stapon, A.; Kahne, D.; Walsh, C. T. Installation of the pyrrolyl-2-carboxyl pharmacophore by CouN1 and CouN7 in the late biosynthetic steps of the aminocoumarin antibiotics clorobiocin and coumermycin A1. *Biochemistry* **2006**, *45*, 8568−8578.
(32) Kleeman, H.-W; Substituted benzoylguanidines, method for production and use thereof as medicament or diagnostic and medicament comprising the same. World Patent Application WO 2006/050830, 2006.
(33) (a) Kansy, M.; Senner, F.; Gubernator, K. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. *J. Med. Chem.* **1998**, *41* (7), 1007−1010. (b) Wohnsland, F.; Faller, B. High-throughput permeability pH profile and high-throughput alkane/water log *P* with artificial membranes. *J. Med. Chem.* **2001**, *44* (6), 923−930.
(34) Guo, L.; Guthrie, H. Automated electrophysiology in the preclinical evaluation of drugs for potential QT prolongation. *J. Pharmacol. Toxicol. Methods* **2005**, *52* (1), 123−135.
(35) Ames, B. N.; Durston, W. E.; Yamasaki, E.; Lee, F. D. Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70* (8), 2281−2285.
(36) Ohno, Y.; Miyajima, A.; Sunouchi, M. Alternative methods for mechanistic studies in toxicology.: screening of hepatotoxicity of pesticides using freshly isolated and primary cultured hepatocytes and non-liver-derived cells, SIRC cells. *Toxicol. Lett.* **1998**, *102−103*, 569−573.
(37) Ulrich, R. G.; Bacon, J. A.; Cramer, C. T.; Peng, G. W.; Petrella, D. K.; Stryd, R. P.; Sun, E. L. Cultured hepatocytes as investigational models for hepatic toxicity: practical applications in drug discovery and development. *Toxicol. Lett.* **1995**, *82−83*, 107−115.
(38) Horakova, K. The use of cell culture systems for the assessment of general cellular toxicity and to detect the nature and location of free radical damage. *Gen. Physiol. Biophys.* **1999**, *18*, 63−69.
(39) Klaassen, C. D. *The Basic Science of Poisons*,; 3rd ed.; Klaassen, C. D., Amdur, M. O.; Doull, J., Eds.; MacMillan Publishing Co.: New York, 1986; p 16.
(40) Casartelli, A.; Bonato, M.; Cristofori, P.; Crivellente, F.; Dal Negro, G.; Masotto, I.; Mutinelli, C.; Valko, K.; Bonfante, V A cell-based approach for the early assessment of the phospholipidogenic potential in pharmaceutical research and drug development. *Cell Biol. Toxicol.* **2003**, *19* (3), 161−176.
(41) Perico, M. E.; Crivellente, F.; Faustinelli, I.; Suozzi, A.; Cristofori, P Flow cytometry, with double staining with Nile red and anti-CD3 antibody, to detect phospholipidosis in peripheral blood lymphocytes of rats treated with amiodarone. *Cell Biol. Toxicol.* **2009**, *25* (6), 587−598.