(2-Methyl-5-(methylsulfonyl)benzoyl)guanidine Na^+/H^+ Antiporter Inhibitors†

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The inhibition of the Na^+/H^+ exchanger during cardiac ischemia and reperfusion has been shown to be beneficial for the preservation of the cellular integrity and functional performance. The aim of the present investigation was to come up with potent and selective benzoylguanidines as NHE inhibitors for their use as an adjunctive therapy in the treatment of acute myocardial infarction. During the course of our investigations it became clear that the substitution ortho to the acylguanidine was of crucial importance for the potency of the compounds. 4-Chloro- and 4-fluoro-2-methylbenzoic acids **6** and **7** were prepared using the directed ortho metalation technique with the carboxylic acid as the directing group. With the LDA/methyl iodide system the 2-methyl group could be extended to an ethyl group. 4-Alkyl groups were inserted by the palladium-catalyzed cross-coupling reaction into the 4-bromo-2-methylbenzoic acid methyl ester (**20**). Starting with benzoic acids **6**−**19**, the methylsulfonyl group was introduced by a sequence of standard reactions (sulfochlorination, reduction, and methylation). 4-Aryl derivatives **68**−**75** were synthesized by the palladium-catalyzed Suzuki reaction. A large number of nucleophilic displacement reactions in the 4-position were carried out with *S*-, *O*-, and *N*-nucleophiles as well as with the cyano and trifluoromethyl group. Using the ester method, acid chlorides, or Mukaiyama's procedure, the 5-(methylsulfonyl)benzoic acid derivatives were finally converted to the (5-(methylsulfonyl)benzoyl)guanidines **165**−**267** with excessive guanidine. In some cases nucleophilic substitutions with pyridinols and piperidine derivatives were carried out at the end of the reaction sequence with the 4-halo-*N*-(diaminomethylene)-5-(methylsulfonyl)-benzamides. Variations in the 4-position were most reasonable, but the volume of the substituents was of crucial importance. Substitution in the 3- and particularly in the 6-position led to considerable worsening of the inhibitory effects of the Na^+/H^+ exchanger. The 2-methyl compounds, however, showed without exception higher in vitro activities than their respective demethyl counterparts as they are exemplified by the reference compounds **266** and **267**, obviously caused by a conformational restriction of the acylguanidine chain. The development compound (2-methyl-5-(methylsulfonyl)-4-pyrrolobenzoyl)guanidine, methanesulfonate (**246**) is a NHE-1 subtype specific NHE inhibitor, being 27-fold more potent toward the NHE-1 than the NHE-2 isoform. **246** was found to act cardioprotectively not only when given before an experimentally induced ischemia, but also curatively after the onset of symptoms of acute myocardial infarction when given prior to the induction of reperfusion.

Introduction

Na^+/H^+ antiporters or exchangers are integral membrane proteins which belong to a family of at least four different protein isoforms, termed NHE-1 to NHE-4.[1] They are capable of exchanging intracellular H^+ for extracellular Na^+ ions. Metabolic acid is actively extruded from the cells in order to maintain the cytosolic pH within the physiological range. Biological functions of this antiport mechanism include the regulation of intracellular pH and cell volume as well as the transcellular transport of Na^+ and HCO_3^-.[2]

Alterations in Na^+/H^+ exchange have been implicated in pathophysiological processes such as essential hypertension, postischemic dysfunction, and cellular death. The regulation of internal myocardial pH is of special importance to the function of the heart. The resting intracellular pH, which is typically near 7.2, can drop dramatically during ischemia. Excessive activation of the Na^+/H^+ exchange leads to a significant elevation of Na^+ influx into the endangered tissue. Since an increase in cytosolic Na^+ in turn activates the Na^+/K^+ ATPase, ATP consumption is increased. Eventually, because of decreased energy stores and increased Na^+ influx, the intracellular Na^+ concentration is markedly increased. Cellular Na^+ overload finally causes cellular Ca^{2+} overload due to a coupling of the Na^+ and Ca^{2+} concentrations via the Na^+/Ca^{2+} exchanger. Especially Ca^{2+} overload is deleterious, since it causes serious contractile dysfunction and arrhythmias and may contribute to cellular death.

Protocols for reperfusion of ischemic myocardium with acidic media or agents that inhibit the Na^+/H^+ exchange mechanism have shown to protect myocardial function and structure.[3] Commonly applied inhibitors of the exchanger are amiloride and its 5-*N*-substituted derivatives (Chart 1), e.g. 5-*N*-(ethylisopropyl)-amiloride (EIPA). While all these compounds showed protective effects in different models of cardiac ischemia and reperfusion, their specificity and tolerability has been questioned in recent studies. Nevertheless, it is encouraging that novel, more specific inhibitors of the NHE-1 isoform have been discovered. These are the lead compound **266** (HOE 694)[4] and its more potent and more NHE-1 specific follow up compound **267** (cariporide, HOE 642).[5] They seem to be well tolerated according to reports from preclinical studies. All compounds have in common an

† Dedicated to Professor Ekkehard Winterfeldt on the occasion of his 65th birthday.

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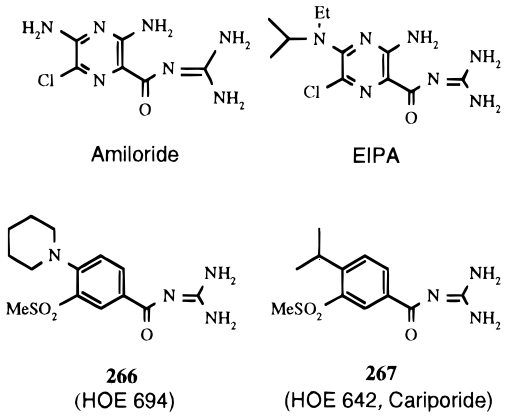
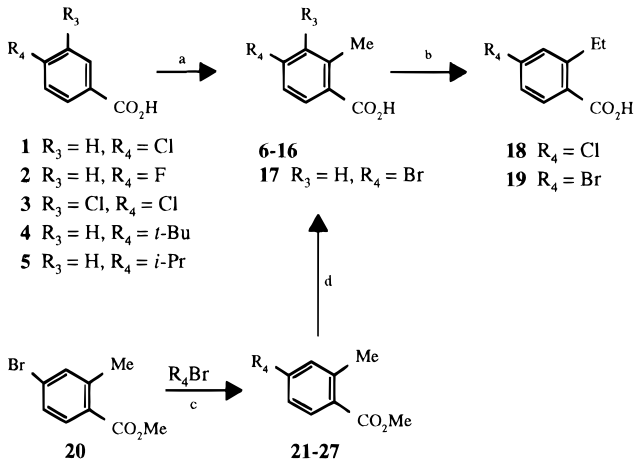
Chart 1. Na^+/H^+ Antiporter Inhibitors



Scheme 1[a]



[a] (a) s-BuLi/TMEDA, THF, MeI; (b) LDA, THF, MeI; (c) Mg, THF, $ZnBr_2$, $PdCl_2$(dppf); (d) NaOH, MeOH.

acylguanidine group attached to an aromatic ring. In the present study we have investigated the influence of substituents on the activity of the compounds, in particular of those in the 2- and 4-positions of the aromatic ring, which were found to have the greatest effects.

Chemistry

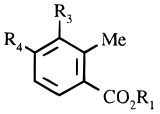
Higher substituted 2-alkylbenzoic acids are known, but their availability leaves much to be desired. In most cases the benzoic acid is generated in several steps from the corresponding aniline via the benzonitrile derivative. However, Cu salts resulting from the Sandmeyer reaction are unwelcome waste products in the chemical industry. The use of directed ortho metalation[6] opened up a modern approach to higher substituted benzoic acid derivatives, particularly when Mortier et al.[7] recently found the carboxylic acid as a suitable group for this technique. To suppress ketone formation on quenching with methyl iodide as electrophile, the metalation was carried out according to the conditions given with 2.2 equiv of *sec*-butyllithium (*s*-BuLi)/*N*,*N*,*N*′,*N*′-tetramethyl-1,2-ethylenediamine (TMEDA) at −90 °C in THF. By this method, which was also found to be feasible for large-scale preparation, 4-chloro-2-methylbenzoic acid[8] (**6**) could be prepared in 73% isolated yield from 4-chlorobenzoic acid (**1**, Scheme 1, Table 1). While 4-fluorobenzoic acid (**2**) and 3,4-dichlorobenzoic acid (**3**) could be handled analogously (→**7**[9] and **8**), the preparation of 4-bromo-2-methylbenzoic acid[10] (**17**) failed due to halogen−metal exchange. As expected, **3** was lithiated regioselectively in the 2-fold activated 2-position. Despite numerous variations of the standard conditions, no complete conversion could be achieved with the 4-alkylbenzoic acids **4** and **5** and there was no useful way to separate the mixture of the methylated and nonmethylated acids. The raw products **6** and **7** obtained were only contaminated slightly with the parent acids (<5%) and suitable for all further reactions.

In no case did methylation occur adjacent to R_4; nevertheless, the substituent considerably influenced the reaction. Taking into account the results of Mortier et al.,[7] increasing yields could be observed in the following sequence: *t*-Bu < *i*-Pr < H < OMe < Cl < F. These results seem to cohere with the strength of the inductive effect of R_4. Thus, the halogens and OMe exhibit electron-withdrawing (F > Cl > OMe) and the alkyl compounds (*t*-Bu > *i*-Pr) electron-donating inductive effects, which should be responsible for stabilization or destabilization of the anion in the meta position, respectively. The strong inductive effects of R_4 in the halogen compounds enable a higher temperature of metalation as well. In particular, the fluoro compound **7** could already be prepared at −70 °C without any losses of yield or quality.

Deprotonation of 2-methylbenzoic acids occurs at the methyl group, leading to an extension of the group by a C1 unit on quenching the dianion with MeI. 4-Chloro-2-ethylbenzoic acid (**18**) was thus obtained using 2.3 equiv of LDA at −70 °C.[13] The 4-bromo-2-ethylbenzoic acid (**19**) could be prepared from **17** employing the same protocol. Starting with 4-bromo-2-methylbenzoic acid methyl ester[14] (**20**), 4-alkyl-2-methylbenzoic acid methyl esters **21**−**27** were produced by the palladium-catalyzed cross-coupling reaction.[15] Due to their compatibility with the ester function, alkylzinc reactants were used, prepared by transmetalation of the corresponding Grignard compounds with $ZnCl_2$. The dichloro[1,1′-bis-(diphenylphosphino)ferrocene]palladium(II) [$PdCl_2$(dppf)] was a suitable catalyst in this reaction, and the esters **21**−**27** formed were then hydrolyzed to give the acids **10**−**16**. Recently the Hoechst group has found that the Negishi−Kumada type coupling can also be achieved in the presence of an *o*-methylsulfonyl substituent, which we introduced afterward, when running the reaction under copper(I) cocatalysis.[16]

Starting with benzoic acids **6**−**19**, the methylsulfonyl group was introduced by a sequence of standard reactions (Scheme 2).[17] Due to potential instabilities and reduced possibilities for purification, intermediates **28** and **29** were not characterized. Sulfochlorination with excessive chlorosulfonic acid which required reaction temperatures of about 140 °C, but 85 °C in the case of the activated 4-alkylbenzoic acids **10**−**16**, exclusively took place in the 5-position. 4-*tert*-Butyl-2-methylbenzoic acid (**9**) already decomposed at this temperature. Sulfinic acids **29** were prepared by Na_2SO_3 reduction of the sulfonyl chlorides **28**. Using basic reaction conditions, the following alkylations could be performed with or without isolation of the more stable sodium sulfinate intermediates. The methylsulfonyl compounds could be obtained as acids **30**−**47** on alkylation under aqueous/methanolic conditions (method D, Table 2), or alternatively in the form of the methyl esters **48**−**60**

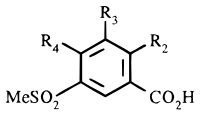
Table 1. Physical Constants for Benzoic Acid Derivatives **6**−**16** and **21**−**27**



compd	R_1	R_3	R_4	method	yield, %	mp, °C	recryst solvent	formula[a]
6	H	H	Cl	A	73	169−170.5	PhMe	$C_8H_7ClO_2$[b]
7	H	H	F	A	80	168−169	$(i\text{-Pr})_2O$	$C_8H_7FO_2$[c]
8	H	Cl	Cl	A	40	186	PhMe	$C_8H_6Cl_2O_2$
9	H	H	*t*-Bu	A	18	138−139	Et_2O	$C_{12}H_{16}O_2$[d]
10	H	H	*i*-Pr	A	49[e]	ND[f]	ND	$C_{11}H_{14}O_2$[g]
10	H	H	*i*-Pr	B	84	89−90	petroleum ether	$C_{11}H_{14}O_2$[g]
11	H	H	Et	B	98	81−83	petroleum ether/Et_2O	$C_{10}H_{12}O_2$
12	H	H	Pr	B	83	93−95	petroleum ether	$C_{11}H_{14}O_2$
13	H	H	c-C_6H_{11}	B	82	132−135	petroleum ether/Et_2O	$C_{14}H_{18}O_2$
14	H	H	$-(CH_2)_4Me$	B	94	59−60	petroleum ether	$C_{13}H_{18}O_2 \cdot 0.6H_2O$
15	H	H	c-C_4H_7	B	99	131−134	Et_2O	$C_{12}H_{14}O_2$
16	H	H	*s*-Bu	B	99	66−68	petroleum ether	$C_{12}H_{16}O_2$
21	Me	H	*i*-Pr	C	92	oil		$C_{12}H_{16}O_2$
22	Me	H	Et	C	98	oil		$C_{11}H_{14}O_2$
23	Me	H	Pr	C	94	oil		$C_{12}H_{16}O_2$
24	Me	H	c-C_6H_{11}	C	88	oil		$C_{15}H_{20}O_2$
25	Me	H	$-(CH_2)_4Me$	C	97	oil		$C_{14}H_{20}O_2$
26	Me	H	c-C_4H_7	C	95	oil		$C_{13}H_{16}O_2$
27	Me	H	*s*-Bu	C	72	oil		$C_{13}H_{18}O_2$

[a] Analyses for C, H, and Cl are within ±0.4% of the expected value for the formula. [b] Lit.[8] [c] Lit.[9] [d] Lit.[11] [e] The rate of conversion was determined by HPLC. [f] Not done. [g] Lit.[12]

Table 2. Physical Constants for 5-Methylsulfonylbenzoic Acids **30**−**47**



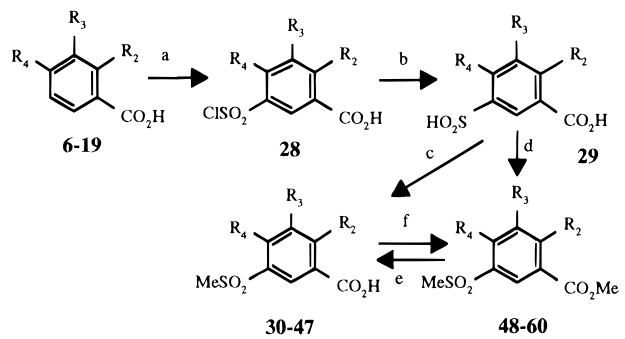
compd	R_2	R_3	R_4	method	reaction temperature,[a] °C	overall yield, %	mp, °C	recryst solvent	formula[b]
30	Me	H	Cl	D	135−140	68	217−218	MeOH	$C_9H_9ClO_4S$
31	Me	H	F	B		90	190−193	EtOAc	$C_9H_9FO_4S$
32	Me	Cl	Cl	B		51	180−182	MeCN	$C_9H_8Cl_2O_4S$
33	H	H	F	D	140	26	207−209	*i*-PrOH	$C_8H_7FO_4S$
34	Me	H	*i*-Pr	D	85	64	179−182	H_2O	$C_{12}H_{16}O_4S$
35	Me	H	Et	D	85	51	194−196	H_2O	$C_{11}H_{14}O_4S \cdot 0.25H_2O$
36	Me	H	Pr	D	85	42	146−149	H_2O	$C_{12}H_{16}O_4S$
37	Me	H	c-C_6H_{11}	D	85	30	219−222	H_2O	$C_{15}H_{20}O_4S$
38	Me	H	$-(CH_2)_4Me$	D	85	33	152−154	H_2O	$C_{14}H_{20}O_4S$
39	Me	H	c-C_4H_7	D	85	62	176−179	H_2O	$C_{13}H_{16}O_4S \cdot 0.25H_2O$
40	Me	H	*s*-Bu	D	85	39	149−151	EtOAc	$C_{13}H_{18}O_4S \cdot 0.25H_2O$
41	Et	H	Br	D	120−130	38	204−206	*i*-PrOH	$C_{10}H_{11}BrO_4S$
42	Me	H	Br	D	140	82	218−220	MeOH	$C_9H_9BrO_4S$
43	Et	H	Cl	B		89	187−188	H_2O	$C_{10}H_{11}ClO_4S$
44	F	H	F	B		81	193−196	H_2O	$C_8H_6F_2O_4S \cdot 0.75H_2O$
45	Cl	H	F	D	140	25	214−216	EtOAc	$C_8H_6ClFO_4S$
46	OMe	H	Cl	D	70	18	236−237	MeOH	$C_9H_9ClO_5S$
47[c]	Me	Me	Cl	D	140	41	188−189	EtOAc	$C_{10}H_{11}ClO_4S$

[a] Bath temperature of chlorosulfonation. [b] Analyses for C, H, Cl, and S are within ±0.4% of the expected value for the formula. [c] The starting material was 4-chloro-2,3-dimethylbenzoic acid.[18]

(method E, Table 3) using DMF as the solvent. In no case was the formation of isomeric methyl sulfinates observed. While the latter technique offered the advantage of a simpler way of working, the former usually provided the higher yields. Frequently small percentages of methyl benzoates were formed in addition to the acids in method D. Alkaline hydrolyses of methyl benzoates (method B) as well as esterifications of benzoic acids (method F) were performed in some cases.

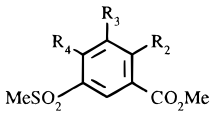
4-Chloro-2-methyl-3-(methylsulfonyl)benzoic acid, which is a positional isomer of **30**, could not be straightforwardly prepared via sulfochlorination of **6**, but nitration led to a mixture of the acids **61** and **62** (Scheme 3). The wanted benzoic acid **62** was separated as its ester **64** from the mixture with poor yield. After catalytic

Scheme 2[a]



[a] (a) $ClSO_3H$; (b) Na_2SO_3; (c) MeI, H_2O/MeOH, NaOH; (d) MeI, K_2CO_3, DMF; (e) NaOH, MeOH; (f) HCl/MeOH.

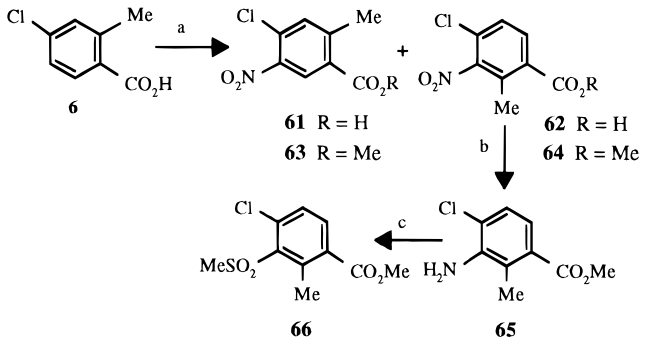
Table 3. Physical Constants for 5-(Methylsulfonyl)benzoic Acid Methyl Esters **48**−**60**



compd	R_2	R_3	R_4	method	reaction temperature,[a] °C	overall yield, %	mp, °C	recryst solvent	formula[b]
48	Me	H	Cl	E	140	39	152−153	MeOH	$C_{10}H_{11}ClO_4S$
49	Me	H	F	E	125	54	137−139	*i*-PrOH	$C_{10}H_{11}FO_4S{\cdot}0.25H_2O$
50	Me	Cl	Cl	E	140	69	124−125	Et_2O	$C_{10}H_{10}Cl_2O_4S$
51	H	H	F	E	140	24	108	*i*-PrOH	$C_9H_9FO_4S$
52	Et	H	Br	E	120−130	45	102−104	$(i\text{-Pr})_2O$	$C_{11}H_{13}BrO_4S$
53	Me	H	Br	E	140	55	146−148	EtOAc	$C_{10}H_{11}BrO_4S$
54	Et	H	Cl	E	140	59	98−100	*i*-PrOH	$C_{11}H_{13}ClO_4S$
55	F	H	F	E	155	11	140	EtOAc	$C_9H_8F_2O_4S$
56	Me	Me	Cl	F		82	137	EtOAc	$C_{11}H_{13}ClO_4S$
57	Me	H	Me	E	140	31	141−142	MeOH	$C_{11}H_{14}O_4S$
58	Cl	H	Cl	E	140	43	154−155	EtOH	$C_9H_8Cl_2O_4S$
59[c]	Me	Me	Me	E	140	20	130	EtOAc	$C_{12}H_{16}O_4S$
60	Cl	H	F	F		62	137	EtOAc	$C_9H_8ClFO_4S$

[a] Bath temperature of chlorosulfonation. [b] Analyses for C, H, Br, and S are within ±0.4% of the expected value for the formula. [c] The starting material was 2,3,4-trimethylbenzoic acid.[19]

Scheme 3[a]

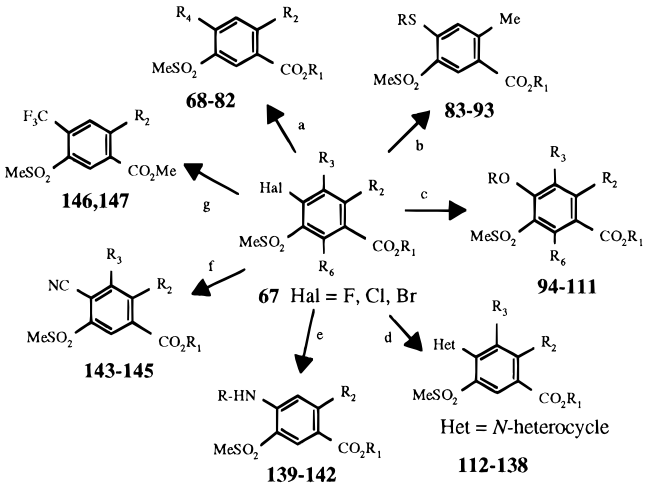


[a] (a) HNO_3, HCl/MeOH; (b) H_2, RaNi, MeOH; (c) $NaNO_2$, SO_2, $FeSO_4$, Cu, MeI, DMF.

hydrogenation (→**65**) the sulfinic acid could be introduced using Wittig and Hoffmann's method[20] by treatment of the diazonium salt with sulfur dioxide in the presence of $FeSO_4$ and copper metal followed by conversion to the sulfone with methyl iodide.

Further variations by cross-coupling reactions and by nucleophilic displacements of 4-halo-5-(methylsulfonyl)-benzoic acid derivatives of the general formula **67**, included in Tables 2 and 3 and the preparation of which is described above, are shown in Scheme 4. Starting with appropriately substituted arylboronic acids, 4-aryl derivatives **68**−**75** were synthesized by the Pd-catalyzed Suzuki reaction[15d,21] from methyl 4-bromobenzoates, and **76** with 2-(tributylstannyl)furan under Stille conditions[22] (Table 4). Substitutions were carried out with *S*-nucleophiles (alkyl, aryl, hetaryl thiols, →**83**−**93**), *O*-nucleophiles (alcohols and phenols, →**94**−**111**), and *N*-nucleophiles (such as piperidines, imidazoles, pyrazoles, and anilines, →**112**−**142**). The substitution conditions could be widely varied: In most cases the nucleophile was offered in excess and a base was added (NaH, NaOR, K_2CO_3). The reactions were run with or without additional solvent, usually with free benzoic acids as starting materials; sometimes methyl benzoates were used (see the Experimental Section). The reactivity of halogen in **67** is increased by the presence of activating groups (SO_2Me and CO_2R_1) and is highest for fluorine compounds. In some cases the esters thus obtained were hydrolyzed after cross-coupling or, vice

Scheme 4[a]

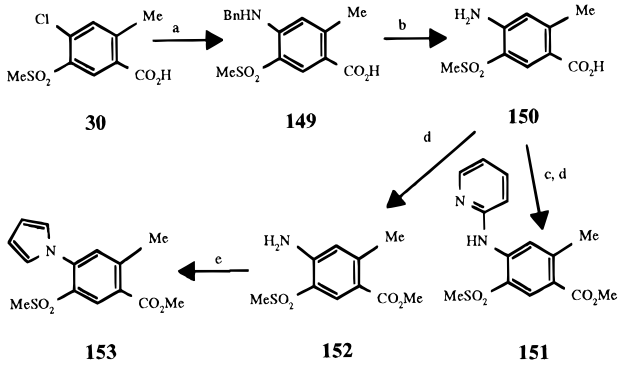


[a] (a) $R_4B(OH)_2$ or R_4SnBu_3; (b) RSH; (c) ROH; (d) HetH; (e) RNH_2; (f) CuCN; (g) CF_3CO_2K.

versa, the benzoic acids were esterified after substitution, and such raw intermediates were not always characterized. The cyano group was introduced with CuCN (→**143**−**145**), and the trifluoromethylation (→**146** and **147**) was performed by reacting a bromo derivative with CF_3CO_2K in the presence of CuI using the phase-transfer technique.[23]

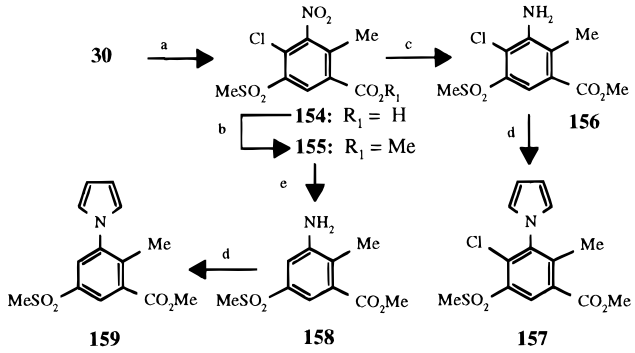
The important 4-amino intermediate **150** was advantageously prepared in two stages (Scheme 5): Substitution with benzylamine was followed by hydrogenation. With the use of **150**, the preparation of the amino-bridged pyridyl compound **151** was possible in the inverse way as it was carried out in the case of **139**−**142**. The 4-pyrrolyl compound **153** was built up with 2,5-dimethoxytetrahydrofuran starting with the methyl 4-aminobenzoate **152**. This synthesis was also applied to the 3-pyrrolyl compounds **157** and **159** (Scheme 6). The 3-amino compounds **156** and **158** required for that were prepared by nitration of **30**, esterification (**154**→**155**), and catalytic hydrogenation. Using Raney nickel (RaNi) the 4-chloro substituent was preserved (→**156**), but under Pd/C catalysis and triethylamine addition chlorine was simultaneously removed (→**158**). Side-chain bromination with *N*-bromosuccinimide (NBS)

Scheme 5[a]



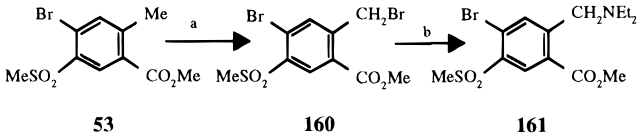
[a] (a) $BnNH_2$; (b) H_2, Pd/C, MeOH; (c) 2-fluoropyridine, NaH, NMP; (d) MeOH/HCl; (e) 2,5-dimethoxytetrahydrofuran, 4-chloropyridine, HCl, dioxane.

Scheme 6[a]



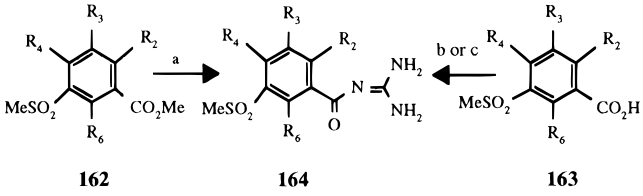
[a] (a) H_2SO_4/HNO_3; (b) HCl/MeOH; (c) H_2, RaNi, MeOH; (d) 2,5-dimethoxytetrahydrofuran, 4-chloropyridine, HCl, dioxane; (e) H_2, Pd/C, MeOH, THF, Et_3N.

Scheme 7[a]



[a] (a) NBS, CH_2Cl_2, *hν*; (b) Et_2NH, Na_2CO_3.

Scheme 8[a]

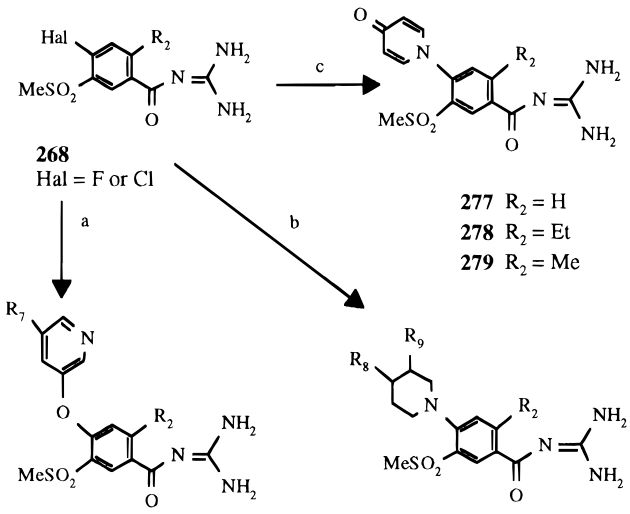


[a] (a) Guanidine, HCl, Na, MeOH; (b) guanidine, HCl, Na, MeOH, $SOCl_2$, 1,2-dimethoxyethane; (c) 2-chloro-1-methylpyridinium iodide, guanidine, HCl, *N*-ethyldiisopropylamine, NMP.

produced **160**, which was subsequently reacted with diethylamine (→**161**, Scheme 7).

In the last synthetic step the 5-(methylsulfonyl)-benzoic acid derivatives, most of which are listed in Table 4, were converted to benzoylguanidines of the general formula **164** (Scheme 8, Table 5). Compounds **164** were synthesized by simple heating of the methyl benzoates **162** with excessive guanidine in methanol (method N) or from the benzoic acids **163** by reaction of the acid chlorides with guanidine at room temperature in good yield (method O). Guanidine base was always freshly prepared from the hydrochloride using sodium methoxide in methanol. The mild reaction conditions

Scheme 9[a]



269 R_2 = Et, R_7 = H
270 R_2 = Me, R_7 = H
271 R_2 = Me, R_7 = Cl
272 R_2 = H, R_7 = Cl
273 R_2 = H, R_8 = NHBoc, R_9 = H
274 R_2 = H, R_8 = NH_2, R_9 = H
275 R_2 = Et, R_8 = H, R_9 = H
276 R_2 = Et, R_8 = H, R_9 = OH

[a] (a) 3-Hydroxypyridine, 5-chloro-3-hydroxypyridine, or 3-[(trimethylsilyl)oxy]pyridine; (b) 4-[(*tert*-butoxycarbonyl)amino]piperidine, HCl, piperidine, or 3-hydroxypiperidine; (c) 4-[(trimethylsilyl)oxy]pyridine.

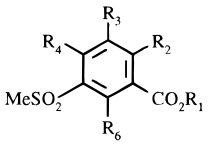
of Mukaiyama's procedure[25] were successful in cases where the former methods failed. 4-Amino-1-piperidinyl compounds **232**−**234** were prepared from the corresponding Boc substances **119**−**121** by ester synthesis followed by deprotection with HCl (method R). Compounds **165**−**267** of Table 5 were characterized in the form of the free bases, hydrochlorides, or methanesulfonates, respectively.

Nucleophilic substitutions, performed in great numbers with the 4-halo-3-(methylsulfonyl)benzoic acid derivatives **67** (Scheme 4), could also be carried out as the final step of the reaction sequence with (4-halo-5-(methylsulfonyl)benzoyl)guanidines **268** (Scheme 9). Pyridinols and piperidine derivatives were used as nucleophiles (→**269**−**279**). Analogous reaction conditions were employed, but due to the basic nature of the acylguanidines, alkaline workup was required (Table 6). Mostly the pyridinols were reacted in the form of their trimethylsilyl derivatives[30] without additional solvents in the presence of potassium carbonate at high temperature (method T). 4-[(Trimethylsilyl)oxy]pyridine only gave *N*-substitution.

Results and Discussion

The Na^+/H^+ antiport activity was assessed by observing the uptake of $^{22}Na^+$ ions into acidified rabbit erythrocytes; rabbit erythrocytes have been widely used[31] in investigations into the Na^+/H^+ exchange activity. The EIPA-sensitive portion of the $^{22}Na^+$ uptake into acidified erythrocytes was taken as the Na^+/H^+ dependent $^{22}Na^+$ uptake.[32] All compounds were tested as hydrochloride or methanesulfonate salts. On the basis of the lead compounds **266** and **267** (Chart 1), we evaluated variations of R_2−R_6 in the benzoylguanidine (Tables 5 and 6). So as not to exceed the scope of the paper, this was restricted to the 5-methylsulfonyl compounds. Extensive patent literature to date as well

Table 4. Physical Constants for 4-Substituted 5-(Methylsulfonyl)benzoic Acid Derivatives **68**−**148**

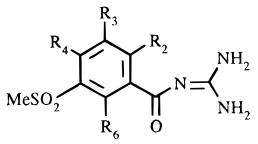


compd	R_1	R_2	$R_{3/6}$[a]	R_4	sm[b]	method	rt[c], °C	time, h	yield, %	mp, °C	recryst solvent	formula[d]
68	Me	H		*p*-tolyl	*e*	G	120	5	47	110	Et_2O	$C_{16}H_{16}O_4S$
69	Me	Me		*p*-tolyl	**53**	G	120	5	48	104−106	Et_2O	$C_{17}H_{18}O_4S·0.25H_2O$
70	Me	Me		4-chlorophenyl	**53**	G	130	6	71	130−132	$(i\text{-Pr})_2O$	$C_{16}H_{15}ClO_4S$
71	Me	Me		2,4-dichlorophenyl	**53**	G	130	6	22	110−112	Et_2O/PE[f]	$C_{16}H_{14}Cl_2O_4S$
72	Me	Me		4-fluorophenyl	**53**	G	130	12	34	128−130	Et_2O	$C_{16}H_{15}FO_4S·0.25H_2O$
73	Me	Me		3,5-bis(trifluoromethyl)	**53**	G	130	6	70	184−186	EtOAc	$C_{18}H_{14}F_6O_4S$
74	Me	Me		3,5-dichlorophenyl	**53**	G	130	6	31	202	EtOAc	$C_{16}H_{14}Cl_2O_4S$
75	Me	Me		Ph	**53**	G	130	6	58	110−112	$(i\text{-Pr})_2O$	$C_{16}H_{16}O_4S·0.25H_2O$
76	Me	Me		2-furyl	**53**	*g*	RF[h]	2	91	142−143	*t*-BuOMe/CH_2Cl_2	$C_{14}H_{14}O_5S$
77	H	Me		4-chlorophenyl	**70**	B	90	0.3	98	210−212	H_2O	$C_{15}H_{13}ClO_4S·0.25H_2O$
78	H	Me		2,4-dichlorophenyl	**71**	B	90	0.3	87	183−184	Et_2O	$C_{15}H_{12}Cl_2O_4S$
79	H	Me		4-fluorophenyl	**72**	B	90	0.5	97	192−194	Et_2O	$C_{15}H_{13}FO_4S$
80	H	Me		3,5-bis(trifluoromethyl)	**73**	B	RF	4	93	243	MeOH	$C_{17}H_{12}F_6O_4S$
81	H	Me		3,5-dichlorophenyl	**74**	B	RF	6	91	216−218	MeOH	$C_{15}H_{12}Cl_2O_4S$
82	H	Me		Ph	**75**	B	90	0.3	96	193	MeOH	$C_{15}H_{14}O_4S$
83	H	Me		SMe	**30**	H	140	9	86	250−252	*i*-PrOH	$C_{10}H_{12}O_4S_2·0.25H_2O$
84	H	Me		SEt	**30**	H	140	2	80	215−217	MeCN	$C_{11}H_{14}O_4S_2·0.25H_2O$
85	H	Me		S-*i*-Pr	**30**	H	130	2	32	208−210	MeCN	$C_{12}H_{16}O_4S_2·0.5H_2O$
86	H	Me		SPr	**31**	H	80	1	60	177−178	*i*-PrOH	$C_{12}H_{16}O_4S_2·0.25H_2O$
87	H	Me		S-*t*-Bu	**31**	H	130	20	82	125	H_2O	$C_{13}H_{18}O_4S_2$
88	H	Me		S-(3-chlorophenyl)	**30**	H	130	2.5	68	233−235	EtOAc/Et_2O	$C_{15}H_{13}ClO_4S_2·0.5H_2O$
89	H	Me		S-(2-chlorophenyl)	**30**	H	140	5	62	289−292	MeOH	$C_{15}H_{13}ClO_4S_2$
90	H	Me		S-(4-chlorophenyl)	**30**	H	140	5	23	221−223	MeOH	$C_{15}H_{13}ClO_4S_2·0.25H_2O$
91	H	Me		S-Ph	**30**	H	180	1.5	22	274−275	MeCN	$C_{15}H_{14}O_4S_2$
92	H	Me		S-(4-pyridyl)	**30**	H	140	5	28	238−240	*i*-PrOH	$C_{14}H_{13}NO_4S_2·2.5H_2O$
93	Me	Me		S-*t*-Bu	**87**	F	60	3	16	124−126	$(i\text{-Pr})_2O$	$C_{14}H_{20}O_4S_2·0.25H_2O$
94	H	Me		OEt	**30**	I	RF	72	75	221−223	EtOAc/Et_2O	$C_{11}H_{14}O_5S·0.25H_2O$
95	H	Me		O-*i*-Pr	**30**	I	RF	24	72	168−171	Et_2O/PE	$C_{12}H_{16}O_5S·0.25H_2O$
96	H	Me		O-cyclopentyl	**30**	I	RF	24	75	180−182[i]	Et_2O/PE	$C_{14}H_{18}O_5S·0.5H_2O$
97	H	Me		O-cyclohexyl	**30**	I	RF	9	31	195−197	Et_2O	$C_{15}H_{20}O_5S·0.25H_2O$
98	Me	Me		O-*t*-Bu	**31**	I	RF	18	24	152−154	Et_2O	$C_{14}H_{20}O_5S$
99	H	Cl		OMe	**45**	I	RF	30	92	233−235	EtOAc	$C_9H_9ClO_5S$
100	H	Et		OMe	**43**	I	135[j]	6	75	224−226	H_2O	$C_{11}H_{14}O_5S·0.25H_2O$
101	Me	Me		OMe	**49**	I	RF	24	73	161−162	MeOH	$C_{11}H_{14}O_5S$
102	Me	Cl		O-(3-pyridyl)	**60**	J	130	2	34	97−99	MeOH	$C_{14}H_{12}ClNO_5S$
103	Me	Me	R_3 = Me	O-(3-pyridyl)	**56**	J	130	20	41	144	EtOAc	$C_{16}H_{17}NO_5S$
104	Me	H	R_6 = Me	O-(3-pyridyl)	**66**	J	130	2	78	89−90	EtOAc/Et_2O	$C_{15}H_{15}NO_5S$
105	H	Me		OPh	**30**	J	180	6	24	175−176	MeCN	$C_{15}H_{14}O_5S$
106	H	Me		O-(2-chlorophenyl)	**30**	J	180	30	27	199−201	$(i\text{-Pr})_2O$	$C_{15}H_{13}ClO_5S$
107	H	Me		O-(3-chlorophenyl)	**30**	J	180	7	24	224−226	EtOAc/*i*-PrOH	$C_{15}H_{13}ClO_5S$
108	H	Me		O-(4-chlorophenyl)	**30**	J	180	6.5	56	155−158	$(i\text{-Pr})_2O$	$C_{15}H_{13}ClO_5S$

109	H	Me		OBn	**49**	J	150	7	16	236−238	H_2O	$C_{16}H_{16}O_5S$
110	H	Me		OH	**109**	*k*	25	7.5	77	228−230	MeOH/Et_2O	$C_9H_{10}O_5S$
111	Me	Cl		OMe	**99**	F	RF	48	76	140	MeOH	$C_{10}H_{11}ClO_5S$
112	H	Me		1-pyrrolidinyl	**30**	K	RF	24	77	198−199	H_2O	$C_{13}H_{17}NO_4S$
113	H	Me		1-piperidinyl	**42**	K	RF	16	91	244−246	MeOH	$C_{14}H_{19}NO_4S$
114	H	Me		3-hydroxy-1-piperidinyl	**42**	K	170	4	32	243	EtOAc/PE	$C_{14}H_{19}NO_5S$
115	H	H		3-hydroxy-1-piperidinyl	*l*	K	170	2	56	181−183	MeOH	$C_{13}H_{17}NO_5S\cdot0.5H_2O$
116	H	NH_2		1-piperidinyl	*m*	K	RF	42	45	241−243	MeOH	$C_{13}H_{18}N_2O_4S\cdot0.25H_2O$
117	H	NH_2		3-hydroxy-1-piperidinyl	*m*	K	170	4	51	236−237	MeOH	$C_{13}H_{18}N_2O_5S\cdot0.5H_2O$
118	H	1-piperidinyl		1-piperidinyl	*n*	K	RF	11	73	220−221	MeOH	$C_{18}H_{26}N_2O_4S\cdot H_2O$
119	Me	Me		4-Boc-amino-1-piperidinyl[o]	**42**	K	160	2	45	151−154	PE/Et_2O	$C_{20}H_{30}N_2O_6S$
120	Me	OMe		4-Boc-amino-1-piperidinyl	**46**	K	RF	36	63	135−137	MeOH	$C_{20}H_{30}N_2O_7S$
121	Me	Cl		4-Boc-amino-1-piperidinyl	**45**	K	100	2	59	186−187	CH_2Cl_2/Et_2O	$C_{19}H_{27}ClN_2O_6S$
122	Me	Cl		3-hydroxy-1-piperidinyl	**45**	K	100	3	13	128−129	EtOAc	$C_{14}H_{18}ClNO_5S$
123	Me	Me		4-(2-pyrimidyl)piperazinyl	**30**	K	160	96	12	203−204	MeOH	$C_{18}H_{22}N_4O_4S$
124	Me	Me		1-imidazolyl	**30**	L	95	96	36	166	EtOAc/MeOH	$C_{13}H_{14}N_2O_4S$
125	Me	Me		1-benzimidazolyl	**30**	L	120	50	38	168−169	EtOAc	$C_{17}H_{16}N_2O_4S$
126	Me	Me		2-methyl-1-imidazolyl	**30**	L	120	50	30	191−192	EtOAc	$C_{14}H_{16}N_2O_4S$
127	Me	H		1-pyrazolyl	*l*	L	80	18	15	167−168	EtOAc	$C_{12}H_{12}N_2O_4S$
128	Me	Cl		1-imidazolyl	**45**	L	80	2	12	189−190	MeOH/EtOAc	$C_{12}H_{11}ClN_2O_4S$
129	Me	Me	$R_3 = Me$	1-imidazolyl	**47**	L	160	90	49	184	EtOAc	$C_{14}H_{16}N_2O_4S\cdot0.5H_2O$
130	H	Me		4-methyl-1-imidazolyl	**30**	L	90	96	53	280−282	H_2O/Me_2CO	$C_{13}H_{14}N_2O_4S$
131	H	Me		1-pyrazolyl	**30**	L	100	96	54	273−274	MeOH	$C_{12}H_{12}N_2O_4S$
132	H	H		1-imidazolyl	**51**	L	80	24	48	308−310	MeCN	$C_{11}H_{10}N_2O_4S$
133	Me	Me		1-piperidinyl	**113**	F	RF	16	88	119	MeOH	$C_{15}H_{21}NO_4S$
134	Me	Me		3-hydroxy-1-piperidinyl	**114**	F	RF	16	100	77	MeOH	$C_{15}H_{21}NO_5S\cdot H_2O$
135	Me	NH_2		1-piperidinyl	**116**	F	RF	6	88	207−208	MeOH	$C_{14}H_{20}N_2O_4S$
136	Me	NH_2		3-hydroxy-1-piperidinyl	**117**	F	RF	72	32	168−169	(*i*-Pr)$_2$O/CH_2Cl_2	$C_{14}H_{20}N_2O_5S$
137	Me	1-piperidinyl		1-piperidinyl	**118**	F	RF	72	88	143	MeOH	$C_{19}H_{28}N_2O_4S$
138	Me	H		3-hydroxy-1-piperidinyl	**115**	F	60	1	24	128−131	EtOAc/Et_2O	$C_{14}H_{19}NO_5S$
139	H	Me		NHPh	**31**	K	140	5	37	182−184	EtOAc/Et_2O	$C_{15}H_{15}NO_4S\cdot0.5H_2O$
140	H	Me		NH-(3-chlorophenyl)	**31**	K	170	8	16	201−202	EtOAc/Et_2O	$C_{15}H_{14}ClNO_4S$
141	Me	Cl		NH-2-pyrimidyl	**45**	L	120	48	24	222−225	MeOH	$C_{13}H_{12}ClN_3O_4S$
142	Me	Me		NH-2-pyrimidyl	**31**	L	120	24	20	212−213	MeOH	$C_{14}H_{15}N_3O_4S$
143	H	H		CN	*l*	M	180	48	51	234	EtOAc/MeOH	$C_9H_7NO_4S$
144	H	Me		CN	**30**	M	150	72	55	249−250	MeOH	$C_{10}H_9NO_4S$
145	Me	Me	$R_3 = Me$	CN	**56**	M	160	72	42	169−170	MeOH	$C_{12}H_{13}NO_4S$
146	Me	Me		CF_3	**53**	*g*	155	4	28	135−136	EtOAc/CH_2Cl_2	$C_{11}H_{11}F_3O_4S$
147	Me	H		CF_3	*e*	*g*	155	4	14	146−147	EtOAc	$C_{10}H_9F_3O_4S$
148	Me	NH_2		Cl	*m*	F	RF	72	91	195	MeOH	$C_9H_{10}ClNO_4S$

[a] Only given if R_3 or $R_6 \neq H$. [b] Starting material. [c] Reaction temperature. [d] The compounds were analyzed for C, H, S, and, if present, Cl and N, except for **122** (no Cl and S available) and **141** (no Cl). Analytical results are within ±0.4% of the theoretical values except for **92** (H: calcd, 4.93; found, 4.26). Compound **130** was isolated as the hydrochloride dihydrate. The exact composition is $C_{13}H_{14}N_2O_4S\cdot2H_2O\cdot HCl$. [e] 4-Bromo-3-(methylsulfonyl)benzoic acid methyl ester.[15d] [f] Petroleum ether. [g] See Experimental Section. [h] Reflux temperature. [i] Melts under decomposition. [j] In a glass bomb. [k] See preparation of compound **65**. The catalyst was Pd/C (5%). [l] 4-Chloro-3-(methylsulfonyl)benzoic acid.[17b,d,e] [m] 2-Amino-4-chloro-5-(methylsulfonyl)benzoic acid.[17d] [n] 2,4-Dichloro-5-(methylsulfonyl)benzoic acid.[17d] [o] The nucleophile was 4-[(*tert*-butoxycarbonyl)amino]piperidine.[24]

Table 5. (5-(Methylsulfonyl)benzoyl)guanidines **164** of Scheme 8



compd	R_2	$R_{3/6}$[a]	R_4	sm[b]	method	yield[c]	mp, °C	recryst solvent	formula	anal.	IC_{50},[d] nM
165	Me		F	**31**	O	69	208−210	MeOH	$C_{10}H_{12}FN_3O_3S$	C, H, N, S	44
166	Me		Cl	**48**	N	57	207−208	MeOH	$C_{10}H_{12}ClN_3O_3S$	C, H, Cl, N, S	25
167	Me		Br	**42**	O + Q	93	229	Me_2CO	$C_{10}H_{12}BrN_3O_3S$[e]	C, H, Br, N, S	12
168	Et		Cl	**54**	N	42	160−162	MeCN	$C_{11}H_{14}ClN_3O_3S$	C, H, Cl, N, S	63
169	Et		Br	**41**	O	23	170−172	EtOAc	$C_{11}H_{14}BrN_3O_3S$	C, H, Br, N, S	41
170	F		F	**44**	P + S	25	111−114	H_2O	$C_9H_9F_2N_3O_3S\cdot0.25H_2O$[f]	C, H, Cl, S; N[g]	630
171	Cl		Cl	**58**	N	15	189	MeOH	$C_9H_9Cl_2N_3O_3S$	C, H, Cl, N, S	140
172	Me	R_3 = Cl	Cl	**32**	O	37	228−230	Et_2O	$C_{10}H_{11}Cl_2N_3O_3S$	C, H, Cl, N, S	29
173	Me	R_3 = Me	Cl	**56**	N + S	39	253−255	MeOH	$C_{11}H_{14}ClN_3O_3S\cdot0.5H_2O$[f]	C, H, Cl, N, S	58
174	Me	R_3 = NO_2	Cl	**155**	N	7	231−232	EtOAc/MeOH	$C_{10}H_{11}ClN_4O_5S$	C, H, Cl, N, S	44
175	Me	1-pyrrolyl	Cl	**157**	N	35	250	EtOAc/MeOH	$C_{14}H_{15}ClN_4O_3S$	C, H, Cl, N, S	58
176	H	R_6 = Me	Cl	**66**	N	38	225−227	CH_2Cl_2/MeOH	$C_{10}H_{12}ClN_3O_3S$	C, H, Cl, N, S	1300
177	NH_2		Cl	**148**	N	62	245	MeOH	$C_9H_{11}ClN_4O_3S$	C, H, Cl, N, S	130
178	CH_2NEt_2		Br	**161**	N	28	147−148	EtOAc	$C_{14}H_{21}BrN_4O_3S$	C, H, N, S	9500
179	Me		Me	**57**	N	32	196−197	MeOH	$C_{11}H_{15}N_3O_3S$	C, H, N, S	11
180	Me		Et	**35**	O	53	195−197	H_2O	$C_{12}H_{17}N_3O_3S$	C, H, N, S	4.8
181	Me		Pr	**36**	O + Q	66	168−171	Me_2CO	$C_{13}H_{19}N_3O_3S$[e]	C, H, N, S	5.7
182	Me		*i*-Pr	**34**	O	79	220−223	EtOAc	$C_{13}H_{19}N_3O_3S\cdot H_2O$	C, H, N, S	2
183	Me		2-Bu	**40**	O	15	88−92	H_2O	$C_{14}H_{21}N_3O_3S$	C, H, N, S	8
184	Me		$-(CH_2)_4Me$	**38**	O + Q	77	150−155	Me_2CO	$C_{15}H_{23}N_3O_3S$[e]	C, H, N, S	27
185	Me		cyclobutyl	**39**	O	43	206−209	H_2O	$C_{14}H_{19}N_3O_3S\cdot0.25H_2O$	C, H, N, S	8.2
186	Me		cyclohexyl	**37**	O + Q	28	208−210	Et_2O	$C_{16}H_{23}N_3O_3S$[e]	C, H, N, S	22
187	Me	R_3 = Me	Me	**59**	N	71	243−244	EtOAc/MeOH	$C_{12}H_{17}N_3O_3S$	C, H, N, S	55
188	Br		Me	*h*	N	29	198−199	CH_2Cl_2/MeOH	$C_{10}H_{12}BrN_3O_3S$	C, H, N, S	70
189	H		*p*-tolyl	**68**	N	53	242−244	*i*-PrOH	$C_{16}H_{17}N_3O_3S$	C, H, S; N[i]	100
190	Me		*p*-tolyl	**69**	N	63	222−224	EtOH	$C_{17}H_{19}N_3O_3S\cdot0.25H_2O$	C, H, N, S	25
191	Me		4-chlorophenyl	**77**	O + S	47	248−250	EtOH	$C_{16}H_{16}ClN_3O_3S$[f]	C, H, Cl, N, S	45
192	Me		2,4-dichlorophenyl	**78**	O	24	209−211	EtOAc	$C_{16}H_{15}Cl_2N_3O_3S\cdot0.25H_2O$	C, H, Cl, N, S	42
193	Me		4-fluorophenyl	**79**	O + Q	86	236	MeOH	$C_{16}H_{16}FN_3O_3S$[e]	C, H, N, S	36
194	Me		3,5-bis(trifluoromethyl)	**80**	O	44	218−220	*i*-PrOH/Et_2O	$C_{18}H_{15}F_6N_3O_3S$	C, H, N, S	2800
195	Me		3,5-dichlorophenyl	**81**	O + Q	74	231−233	Me_2CO	$C_{16}H_{15}Cl_2N_3O_3S$[e]	C, H, Cl, N, S	130
196	Me		Ph	**82**	O + Q	71	249−252	Me_2CO	$C_{16}H_{17}N_3O_3S$[e]	C, H, N, S	19
197	Me		2-furyl	**76**	N + Q	26	280−281	MeOH	$C_{14}H_{15}N_3O_4S$[e]	C, H, N, S	16
198	Me		SMe	**83**	O	56	220−222	Et_2O	$C_{11}H_{15}N_3O_3S_2$	C, H, N, S	6
199	Me		SEt	**84**	O + Q	46	177−180	*i*-PrOH/H_2O	$C_{12}H_{17}N_3O_3S_2$[e]	C, H, N, S	5.9
200	Me		SPr	**86**	O + Q	48	195−197	Me_2CO	$C_{13}H_{19}N_3O_3S_2$[e]	C, H, N, S	6.8
201	Me		S-*i*-Pr	**85**	O + Q	41	192−194	*i*-PrOH	$C_{13}H_{19}N_3O_3S_2$[e]	C, H, N, S	12
202	Me		S-*t*-Bu	**87**	P + Q	34	200−202	Me_2CO/Et_2O	$C_{14}H_{21}N_3O_3S_2\cdot0.5H_2O$[e]	C, H, N, S	22
203	Me		S-(3-chlorophenyl)	**88**	O	39	198−202	MeOH	$C_{16}H_{16}ClN_3O_3S_2\cdot0.25H_2O$	C, H, Cl, N, S	37
204	Me		S-(2-chlorophenyl)	**89**	O + Q	84	254	MeOH	$C_{16}H_{16}ClN_3O_3S_2$[e]	C, H, Cl, N, S	14
205	Me		S-(4-chlorophenyl)	**90**	O + Q	71	256	MeOH	$C_{16}H_{16}ClN_3O_3S_2$[e]	C, H, Cl, N, S	19
206	Me		S-Ph	**91**	O	31	215−217	MeCN	$C_{16}H_{17}N_3O_3S_2$	C, H, N, S	14
207	Me		S-(4-pyridyl)	**92**	O	37	208−210	EtOH	$C_{15}H_{16}N_4O_3S_2\cdot0.5H_2O$	C, H, N, S	13
208	Me		OBn	**109**	O	42	235−237	CH_2Cl_2/MeOH	$C_{17}H_{19}N_3O_4S$	C, H, N, S	28
209	Me		OH	**110**	O	77	228−230	EtOH	$C_{10}H_{13}N_3O_4S\cdot0.25H_2O$	C, H, N, S	220
210	Me		OMe	**101**	N	14	189−190	MeOH	$C_{11}H_{15}N_3O_4S$	C, H, N, S	16
211	Et		OMe	**100**	O	45	192−195	*i*-PrOH	$C_{12}H_{17}N_3O_4S\cdot0.25H_2O$	C, H, N, S	30
212	Me		OEt	**94**	O + S	27	118−119	H_2O	$C_{12}H_{17}N_3O_4S$[f]	C, H, Cl, N, S	19
213	Me		O-*i*-Pr	**95**	O + S	32	216−220	H_2O	$C_{13}H_{19}N_3O_4S\cdot0.5H_2O$[f]	C, H, Cl, N, S	19

214	Me		O-cyclopentyl	**96**	O + S	15	262−266	H_2O	$C_{15}H_{21}N_3O_4S$[f]	C, H, Cl, N, S	27
215	Me		O-cyclohexyl	**97**	O + S	13	215−220	H_2O	$C_{16}H_{23}N_3O_4S$[f]	C, H, Cl, N, S	37
216	Me		O-*t*-Bu	**98**	N + Q	53	164−167	Me_2CO	$C_{14}H_{21}N_3O_4S$[e]	C, H, N, S	9.4
217	Me		OPh	**105**	O	60	260−262	MeCN/MeOH	$C_{16}H_{17}N_3O_4S$	C, H, N, S	10
218	Me		O-(2-chlorophenyl)	**106**	O + Q	11	217−219	EtOAc/MeOH	$C_{16}H_{16}ClN_3O_4S$[e]	C, H, Cl, N, S	14
219	Me		O-(3-chlorophenyl)	**107**	O	84	205−207	H_2O	$C_{16}H_{16}ClN_3O_4S$	C, H, Cl, N, S	21
220	Me		O-(4-chlorophenyl)	**108**	O + Q	37	274	MeOH	$C_{16}H_{16}ClN_3O_4S$[e]	C, H, Cl, N, S	13
221	Cl		OMe	**111**	N	50	210−211	MeOH	$C_{10}H_{12}ClN_3O_4S$	C, H, Cl, N, S	40
222	Cl		O-(3-pyridyl)	**102**	N	51	215−216	MeOH	$C_{14}H_{13}ClN_4O_4S$	C, H, Cl, N, S	52
223	Me	R_3 = Me	O-(3-pyridyl)	**103**	N + S	19	258−260	MeOH	$C_{16}H_{18}N_4O_4S \cdot H_2O$[f]	C, H, N, S	23
224	H	R_6 = Me	O-(3-pyridyl)	**104**	N	26	214−216	CH_2Cl_2/MeOH	$C_{15}H_{16}N_4O_4S$	C, H, N, S	2200
225	Me		1-pyrrolidinyl	**112**	O + Q	1	224−225	Me_2CO	$C_{14}H_{20}N_4O_3S$[e]	C, H, N, S	11
226	Me		1-piperidinyl	**133**	N + S	16	247	MeOH	$C_{15}H_{22}N_4O_3S$[j]	C, H, Cl, N, S	21
227	Me		3-hydroxy-1-piperidinyl	**134**	N	27	194−196	Me_2CO/MeOH	$C_{15}H_{22}N_4O_4S$	C, H, N, S	30
228	H		3-hydroxy-1-piperidinyl	**138**	N	87	248−250	MeOH	$C_{14}H_{20}N_4O_4S$	C, H, N, S	65
229	NH_2		1-piperidinyl	**135**	N	32	240−241	MeOH	$C_{14}H_{21}N_5O_3S$	C, H, N, S	190
230	NH_2		3-hydroxy-1-piperidinyl	**136**	N	18	232−233	MeOH	$C_{14}H_{21}N_5O_4S$	C, H, N, S	160
231	1-piperidinyl		1-piperidinyl	**137**	N + S	5	189−190	MeOH	$C_{19}H_{29}N_5O_3S \cdot H_2O$[f]	C, H, Cl, N, S	1000
232	Me		4-NH_2-1-piperidinyl	**119**	R	25	305−310 dec	MeOH	$C_{15}H_{23}N_5O_3S$[j]	C, H, Cl, N, S	9.8
233	OMe		4-NH_2-1-piperidinyl	**120**	R	51	270 dec	MeOH	$C_{15}H_{23}N_5O_4S \cdot 0.25H_2O$[j]	C, H, Cl, N, S	73
234	Cl		4-NH_2-1-piperidinyl	**121**	R	58	302−305 dec	MeOH	$C_{14}H_{20}ClN_5O_3S \cdot 0.25H_2O$[j]	C, H, Cl, N	20
235	Cl		3-hydroxy-1-piperidinyl	**122**	N	47	165−168 dec	EtOAc/MeOH	$C_{14}H_{19}ClN_4O_4S \cdot 0.25H_2O$	C, H, Cl, N, S	60
236	Me		4-(2-pyrimidyl)piperazinyl	**123**	N	15	240−242	EtOAc	$C_{18}H_{23}N_7O_3S$	C, H, S; N[k]	27
237	Me		1-imidazolyl	**124**	N + S	19	192 dec	MeOH	$C_{13}H_{15}N_5O_3S \cdot H_2O$[j]	C, H, Cl, N, S	23
238	Me		1-benzimidazolyl	**125**	N	15	257	EtOAc/MeOH	$C_{17}H_{17}N_5O_3S$	C, H, N, S	100
239	Me		2-methyl-1-imidazolyl	**126**	N	29	251	EtOAc/MeOH	$C_{14}H_{17}N_5O_3S$	C, H, N, S	29
240	H		1-pyrazolyl	**127**	N	41	209−211	MeOH	$C_{12}H_{13}N_5O_3S \cdot 0.25H_2O$	C, H, N, S	160
241	Cl		1-imidazolyl	**128**	N	19	219−220	MeCN/MeOH	$C_{12}H_{12}ClN_5O_3S$	C, H, Cl, N, S	130
242	Me	R_3 = Me	1-imidazolyl	**129**	N	26	249	EtOAc/MeOH	$C_{14}H_{17}N_5O_3S$	C, H, N, S	290
243	Me		4-methyl-1-imidazolyl	**130**	P	23	237 dec	Me_2CO	$C_{14}H_{17}N_5O_3S$	C, H, N, S	28
244	Me		1-pyrazolyl	**131**	P	18	227−229	EtOAc/MeOH	$C_{13}H_{15}N_5O_3S$	C, H, N, S	25
245	H		1-imidazolyl	**132**	P	11	242	MeOH	$C_{12}H_{13}N_5O_3S$	C, H, N, S	150
246	Me		1-pyrrolyl	**153**	N + Q	66	278−280	H_2O	$C_{14}H_{16}N_4O_3S$[e]	C, H, N, S	8
247	Me		NH_2	**152**	N + Q	32	240−241	MeOH	$C_{10}H_{14}N_4O_3S$[e]	C, H, N, S	16
248	Me		NHPh	**139**	O + S	30	260	H_2O	$C_{16}H_{18}N_4O_3S$[f]	C, H, Cl, N, S	21
249	Me		NH-2-pyridyl	**151**	N + Q	33	235−237	MeOH	$C_{15}H_{17}N_5O_3S$[e]	C, H, N, S	9.2
250	Me		NH-(3-chlorophenyl)	**140**	O + Q	30	234−237	Me_2CO	$C_{16}H_{17}ClN_4O_3S \cdot 0.5H_2O$[e]	C, H, Cl, N, S	33
251	Me		NH-2-pyrimidyl	**142**	N + Q	29	243−245	MeOH	$C_{14}H_{16}N_6O_3S$[e]	C, H, N, S	8.3
252	Cl		NH-2-pyrimidyl	**141**	N	10	257−258 dec	THF/MeOH	$C_{13}H_{13}ClN_6O_3S$	C, H, Cl, S; N[l]	49
253	Me	R_3 = 1-pyrrolyl	H	**159**	N	25	216	EtOAc/MeOH	$C_{14}H_{16}N_4O_3S$	C, H, N, S	100
254	H		CN	**143**	P + S	19	243−244	MeOH	$C_{10}H_{10}N_4O_3S$[f]	C, H, Cl, N, S	140
255	Me		CN	**144**	P + S	21	227−228	Me_2CO/MeOH	$C_{11}H_{12}N_4O_3S$[f]	C, H, Cl, N	70
256	Me	R_3 = Me	CN	**145**	N	38	242−243	MeOH	$C_{12}H_{14}N_4O_3S$	C, H, N, S	370
257	Me		CF_3	**146**	N	62	208−209	EtOAc	$C_{11}H_{12}F_3N_3O_3S$	C, H, N, S	37
258	H		CF_3	**147**	N	78	233−234	MeOH	$C_{10}H_{10}F_3N_3O_3S$	C, H, N	140
259[m]	H		OMe								270
260[n]	H		O-(3-pyridyl)								76
261[o]	H		cyclohexyl								340
262[m]	H		Me								210
263[p]	H		Cl								380
264[m]	H		Br								180
265[q]	Me		H								150
266[p]	H		1-piperidinyl	(HOE 694)							130
267[m]	H		*i*-Pr	(HOE 642)							26

[a] Only given if R_3 or $R_6 \neq$ H. [b] Starting material. [c] Overall yield (%). [d] Drug concentration to achieve half-maximal inhibition of the EIPA-sensitive $^{22}Na^+$ uptake into rabbit erythrocytes. The standard deviation of the IC_{50} values was 14% on the average. [e] Methanesulfonate. The formula must be extended by CH_4O_3S. Items of the list apply to the methanesulfonate. [f] Hydrochloride. The formula must be extended by HCl. Items of the list apply to the hydrochloride. [g] N: calcd, 13.21; found, 12.74. [h] 2-Bromo-4-methyl-5-(methylsulfonyl)benzoic acid methyl ester.[26] [i] N: calcd, 12.68; found, 11.79. [j] Dihydrochloride. The formula must be extended by 2HCl. Items of the list apply to the dihydrochloride. [k] N: calcd, 23.48; found, 23.05. [l] N: calcd, 22.79; found, 22.19. [m] Lit.[27] [n] Lit.[28] [o] Lit.[15d] [p] Lit.[17e] [q] Lit.[29]

Table 6. (5-(Methylsulfonyl)benzoyl)guanidines of Scheme 9

compd	sm[a]	method	rt,[b] °C	time, h	solvent	yield,[c] %	mp, °C	recryst solvent	formula[d]	IC_{50},[e] nM
269	**168**	T	140	3	WS[f]	23	257−258	MeCN	$C_{16}H_{18}N_4O_4S \cdot 0.25H_2O$	27
270	**166**	T + S	140	3	WS	19	273−274	H_2O	$C_{15}H_{16}N_4O_4S \cdot 2HCl \cdot 1.5H_2O$	8.8
271	**166**	J	RF[g]	2	DMF	12	208−210	EtOH	$C_{15}H_{15}ClN_4O_4S$	14
272	**263**	J	RF	2	DMF	26	233	CH_2Cl_2/MeOH	$C_{14}H_{13}ClN_4O_4S$	75
274	**263**	K + R[h]	150	2	sulfolane	19	239−241	H_2O	$C_{14}H_{21}N_5O_3S \cdot H_2O$	160
275	**168**	K[i]	RF	5	WS	24	218−220	Et_2O	$C_{16}H_{24}N_4O_3S \cdot 0.25H_2O$	25
276	**168**	K	100	3	WS	21	225	MeCN	$C_{16}H_{24}N_4O_4S$	41
277	**263**	T	160	12	WS	1	282	MeOH	$C_{14}H_{14}N_4O_4S$	710
278	**168**	T	130	5	WS	49	256−258	EtOH	$C_{16}H_{18}N_4O_4S \cdot 0.5H_2O$	930
279	**165**	T	135	4.5	WS	61	267−268	MeOH	$C_{15}H_{16}N_4O_4S \cdot 0.5H_2O$	75

[a] Starting material. [b] Reaction temperature. [c] Overall yield. [d] Analyses for C, H, Cl, N, and S are within ±0.4% of the expected value for the formula. [e] Drug concentration to achieve half-maximal inhibition of the EIPA-sensitive $^{22}Na^+$ uptake into rabbit erythrocytes. The standard deviation of the IC_{50} values was 14% on the average. [f] Without additional solvent. [g] Reflux temperature. [h] The nucleophile used was 4-[(*tert*-butoxycarbonyl)amino]piperidine,[24] and the Boc intermediate **273** was not characterized. The free base **274** was prepared by HCl treatment as described in the second part of method R followed by neutralization with NaOH. [i] Due to the basic nature of the acylguanidine, alkaline workup was required.

as our own research[33] has shown that 5-substituents such as acetyl, trifluoromethyl, and heterocycles can also be introduced without any substantial loss of activity.

Modification of Position 2 of 164. The most striking and surprising result of our investigation is the higher in vitro activity of the (2-methyl-5-(methylsulfonyl)benzoyl)guanidines in comparison with their respective demethyl counterparts. Without exception, all 17 cases tested show marked improvements upon introduction of a methyl group adjacent to the acylguanidine (**166** vs **263**, **167** vs **264**, **179** vs **262**, **182** vs **267**, **186** vs **261**, **190** vs **189**, **210** vs **259**, **226** vs **266**, **227** vs **228**, **232** vs **274**, **237** vs **245**, **244** vs **240**, **255** vs **254**, **257** vs **258**, **270** vs **260**, **271** vs **272**, **279** vs **277**). The 2-methyl compounds are more potent by a factor of at least 2, but mostly the difference is considerably greater, e.g. compounds **266** and **226** differ by a factor of 6.2; in the case of the Hoechst compound **267** and its methyl analogue **182**, the factor is 13.

With one exception (**278**) the insertion of a 2-ethyl group into the unsubstituted species also led to an improvement of activity. However, with respect to the 2-methyl analogues, all ethyl compounds prepared (**168**, **169**, **211**, **269**, **275**, **276**, **278**) show a reduced inhibitory effect on the Na^+/H^+ exchanger. The same is true with other substituents introduced in the 2-position; they do not show the inhibitory activity seen with the methyl compounds. With the (2-halobenzoyl)- (**170**, **171**, **188**, **221**, **222**, **234**, **235**, **241**, **252**), (2-aminobenzoyl)- (**177**, **229**, **230**), and (2-methoxybenzoyl)guanidines (**233**), similar results as with the 2-ethyl compounds were obtained: with few exceptions the substances are more effective than the nonmethylated counterparts. Larger substituents such as (diethylamino)methyl (**178**) and piperidinyl (**231**) give rise to a loss of potency.

Modifications of Positions 3 and 6. While introduction of methyl ortho to the acylguanidine residue in the 2-position leads to an improvement of in vitro activity, a methyl group in the other ortho position (position 6) leads to a clear worsening of this. The activity of the respective isomers differs by factors of 52 and 250 (**166** vs **176** and **270** vs **224**). Various 3-substituents were introduced in the (5-(methylsulfonyl)benzoyl)guanidine molecule in addition to the existing 2-methyl group: Cl (**172**), Me (**173**, **187**, **223**, **242**, **256**), NO_2 (**174**), and 1-pyrrolyl (**175**). This led to a drop in activity by on average a factor of 4. Compound **253** represents a special case: Here the 3-position is substituted with 1-pyrrolyl, but the 4-position is left free. It turned out that pyrrole in the 3-position is not as good as in the 4-position; however, the occupied 3-position is better than both left unsubstituted (**253** vs **246** and **265**).

Modification of Position 4. Compared to the other ring positions, the 4-position could be broadly varied. Only those (5-(methylsulfonyl)benzoyl)guanidines having an optimal substitution pattern (R_2 = Me, R_3 and R_6 = H) are discussed in this chapter. A relatively weak Na^+ uptake inhibition (IC_{50} = 150 nM) of the unsubstituted compound **265** indicates that there must be a substituent in the 4-position. Halogen substitution (**165**−**167**) improves this value up to 1 order of magnitude; the potency increases from 4-fluoro to 4-bromo.

Insertion of alkyl groups (**179**−**186**) produced further improvement of the in vitro activity. The inhibitory effect rises from methyl to ethyl and culminates in the isopropyl compound. To our knowledge, (4-isopropyl-2-methyl-5-(methylsulfonyl)benzoyl)guanidine (**182**) with an IC_{50} value of 2 nM is the most potent Na^+/H^+ antiporter inhibitor known to date. Larger alkyl groups lead to a reduction in activity again, which is most pronounced with the pentyl and cyclohexyl residues.

(4-Arylbenzoyl)guanidines **190**−**196** as well as the 4-(2-furyl) compound **197** are moderately active. Ring substitution always led to a reduction in potency. Nanomolar potencies were established with the sulfur compounds **198**−**207**, in particular in alkylthio subtypes with lower alkyl groups (Me, Et, and Pr), but the arylthio and hetarylthio compounds **203**−**207** were found to be somewhat weaker. In the alkoxy series (**210**, **212**−**215**, and **208**) the potency drops with growing alkyl residue, as was shown in the sulfur series. The *O-tert*-butyl compound **216** does not follow this trend: It is active in the nanomolar range whereas the hydroxy compound **209** is only weakly active, on the other hand. The activity of the aryloxy and hetaryloxy compounds (**217**−**220**, **270**, **271**) is in the same order as that of the alkoxy analogues, and again, the unsubstituted species are the best of these.

In the group of acylguanidines fitted with saturated nitrogen nucleophiles in the 4-position (**225**−**227**, **232**, **236**), enhanced activity of the 4-amino derivative **232** with respect to the parent **226** is noticeable while, as expected, a 3-hydroxy group (→**227**) leads to a reduction of activity. The relatively good activity of **236** is remarkable in view of the large 4-pyrimidin-2-yl-1-piperazinyl group as well. With the 4-(1,4-dihydro-4-

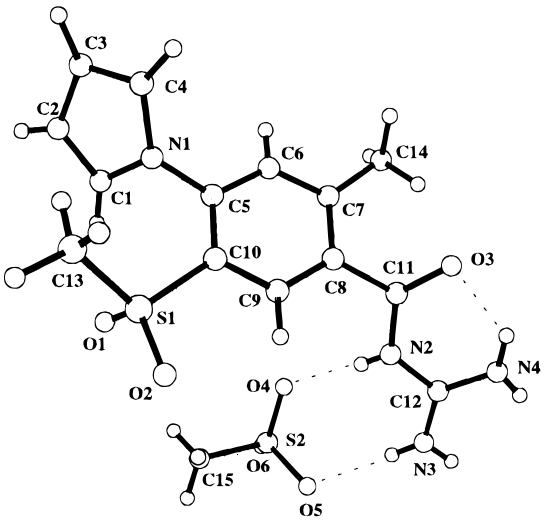


Figure 1. X-ray crystal structure of the methanesulfonate of compound **246**.

oxo-1-pyridyl) compound **279**, only modest activity was found. The outstanding compound of the unsaturated 5-membered *N*-heterocycles (**237**−**239**, **243**, **244**, **246**) is the pyrrole **246** having an IC_{50} of 8 nM. The change from the imidazole **237** to the benzimidazole **238** causes drastic reduction in potency. In the series of acylguanidines substituted with *N*-nucleophiles, last but not least those types have to be discussed in which the 4-amino function forms a bridge to an aryl or hetaryl ring (**248**−**251**). These substances can be regarded as *N*-equivalents to sulfur or oxygen compounds such as **207** and **270**, and again it turns out that with the attachment of a further ring system an enhancement in potency is possible even to the nanomolar region (**247** vs **249** and **251**). The cyano and trifluoromethyl compounds **255** and **257** were moderately active.

Constitutional and Conformational Requirements. The results given in Tables 5 and 6 clearly show, that only minor structural variations of the parent structures **266** and **267** are allowed. These variations seem to be most reasonable in the 4-position. Alkyl residues in this position show an increasing activity with respect to their size up to isopropyl (→**182**). For larger alkyl substituents a decrease can be observed. The space-filling nature of the alkyl group is of crucial importance. This relationship is inherent with other groups as well. Thus, substituents such as fluoro and hydroxy (→**165** and **209**) obviously are too small for optimal receptor binding. On the other hand, we have shown that residues in the 4-position with an extended spacial requirement led to decreased potency, for example when introducing additional substituents in phenyl or heterocyclic rings. As a measure of the size of the substituents, their molar refraction[34] (MR) was used and a MR range 10−26 was found to give the best results (→**180**−**183**, **185**, **198**−**200**, **246**, **251**, **270**).

An interesting result of this study lies in the completely different effects caused by methyl groups ortho to the acylguanidine. Whereas small groups in the 2-position—methyl being the optimum—always increase activity, 6-substitution led to considerable worsening. This gives rise to the supposition that the conformation of the acylguanidine chain required at the receptor site is influenced in a benefical way by the 2-methyl group. Figure 1 shows the X-ray crystal structure of the methanesulfonate salt of **246**. The benzene lies in the plane of the paper and the pyrrole ring is twisted by an angle of 40° by the vicinal methylsulfonyl group. The acylguanidine is protonated at the amide nitrogen, the anion of the methanesulfonic acid forming hydrogen bridges to N2 and N3 of the acylguanidine. Another hydrogen bridge forms a 6-membered ring between the amino (N4) and carbonyl group (O3). The planar acylguanidine group is also twisted by a torsion angle of 40° with respect to the benzene ring.

Figure 1 also shows the preferential conformation of the acylguanidine residue in the crystalline state. The carbonyl oxygen as the smaller group points upward in the direction of the methyl group whereas the guanidine is aligned downward, avoiding the sphere of the 2-substituent. In those compounds bearing a 6-methyl group (**176**, **224**) the acylguanidine residue should adopt the inverse orientation with the carbonyl group pointing downward. According to biological results, this must be an unfavorable arrangement for an approach to the receptor site. With respect to the biological activity, the dissolved drugs are of more relevance than the crystal structure. Methyl groups in the ortho position considerably restrict rotation of the acylguanidine, which leads more or less to orientations discussed for the solid state. In the compounds that are free of ortho methyl groups (e.g. **258**−**267**) the acylguanidine has freedom of rotation around the C8/C11 bond and both conformations are of similar probability. Consequently these compounds exhibit medium activities.

Clinical Relevance. It is well-known that the Na^+/H^+ exchanger constitutes a family of at least four different isoforms (NHE-1, -2, -3, and -4), all of which are cloned. While the NHE-1 subtype is distributed ubiquitously throughout the body in all tissues, the other subtypes are expressed selectively in specific organs like the kidney or the luminal and contraluminal wall of the small intestine. This distribution reflects the specific functions being served by the various isoforms, namely regulation of the intracellular pH and cell volume by the NHE-1 subtype and Na^+ uptake and re-uptake in the gut and kidney by the NHE-2 and -3 isoforms, respectively.

For an NHE inhibitor to be developed as a cardioprotective agent, it is mandatory for it to be a NHE-1 subtype specific one.[35] On the one hand, the NHE-1 isoform is the only one to be expressed in the heart; furthermore, it is this subtype which is activated during ischemic episodes. On the other hand, it is of interest to avoid gastrointestinal and renal complications. We therefore characterized the Na^+/H^+ antiport inhibitors with regard to their isoform selectivity by investigating a selected group of compounds toward the inhibition of the NHE isoforms 1−3. The various isoforms were stably expressed in mouse fibroblast cell lines. The inhibitory potency of the compounds were accessed by determining the EIPA-sensitive $^{22}Na^+$ uptake into the cells after intracellular acidosis.[1]

We observed for our developmental compound **246** (EMD 96785) half-maximal inhibitory concentrations (IC_{50}) of 10 nM for the NHE-1, 270 nM for the NHE-2, and 700 000 nM for the NHE-3 isoforms. Thus, compound **246** was about 27-fold more potent toward the NHE-1 than the NHE-2 isoform. Moreover, **246** was also more potent than the reference compound **267**, for

which Counillon et al. reported apparent K_i values of 50 nM for the NHE-1 and 3000 nM for the NHE-2 isoforms.[5] In our experiments we found respective IC_{50} values of 20 and 650 nM for this compound. In addition to that we could confirm the fact that amiloride does not differentiate between the NHE-1 and NHE-2 subtypes.[36] Therefore, amiloride-derived NHE inhibitors, and in particular amiloride itself, do not fulfill the prerequisite of being selective and specific NHE-1 inhibitors.

246 was able to reduce ischemia-induced arrhythmia, after both intravenous and oral administration. It was also found to reduce ischemia-induced myocardial damage in animal studies. Importantly the compound was not only effective when given prior to the experimentally induced ischemia but also when infused intravenously shortly before the re-initialization of the blood flow to the ischemic area.[37]

The therapeutic applicability of such a selective NHE-1 inhibitor appears to be manifold: Compound **246** thus seems to be useful not only preventively when given before the onset of ischemia, but also curatively after the onset of symptoms of acute myocardial infarction, adjunctively to percutaneous transluminal coronary angioplasty (PTCA) or thrombolysis. In addition, NHE inhibitors are expected to inhibit or diminish tissue damage and cell necrosis after pathophysiological hypoxic and ischemic episodes as might occur in the heart during angina pectoris attacks or in the central nervous system. Furthermore, NHE inhibitors are believed to be protective during short-term hypoperfusion of organs during open-chest angioplastic vessel or cardiac surgery as well as organ transplantation. The antiproliferative efficacy of NHE inhibitors may also indicate that the Na^+/H^+ exchanger plays a role in various pathologies like arteriosclerosis, pulmonary hypertension, insulin-dependent diabetes, tumor growth, fibrotic diseases, and organ/cell hypertrophy or hyperplasia. Furthermore, NHE inhibitors might turn out to be of diagnostic usefulness in diseases which are characterized by an exaggerated activity of the Na^+/H^+ exchanger in blood cells, e.g. erythrocytes, platelets, or leukocytes.

Conclusion

For the preparation of benzoylguanidine precursors, the ortho metalation technique was advantageously used with the carboxylic acid as the directing group. The 2-methyl species of this class of compounds were considerably more active in the Na^+/H^+ exchange inhibition in vitro assay than their respective demethyl analogues, and they were characterized as the most potent inhibitors known to date. The new compounds belong to the subtype-1 specific NHE inhibitors and are thus predestined for cardiac indications. The development of **246** as a cardioprotective agent has been initiated recently. The compound is designated for use in acute infarction as well as for preventive treatment under conditions of angina pectoris. The action of the Na^+/H^+ antiporter inhibitors in the treatment of cardiac ischemia is based on a novel therapeutic principle. Further applications of these drugs are within the realm of possibility.

Experimental Section

Melting points were determined with a Büchi 535 melting point apparatus and are uncorrected. IR and NMR spectra are in agreement with the structures cited and were recorded on a Bruker IFS 48 IR spectrophotometer and a Bruker AC 200 or WM 250 (TMS as internal standard), respectively. Crystal data were collected on a Enraf-Nonius CAD4 diffractometer with graphite-monochromated Mo Kα radiation. Microanalyses were obtained with an elementar Analysensysteme CHN-O-RAPID analyzer. Precoated silica gel 60 F_{254} plates from Merck KGaA, Darmstadt, Germany, were used for thin-layer chromatography.

Method A. 4-Chloro-2-methylbenzoic Acid[8] (6). A 2-L, three-necked, round-bottomed flask was equipped with a mechanical stirrer, a pressure-equalizing dropping funnel with drying tube, and a Claisen head fitted with a low-temperature thermometer and a nitrogen inlet. The flask was charged with TMEDA (99.6 mL, 660 mmol) and dry THF (600 mL), flushed with N_2, and cooled down to −90 °C using an external bath of EtOH/liquid N_2. Then consecutively *s*-BuLi (471 mL, 1.4 M cyclohexane solution, 660 mmol) and 4-chlorobenzoic acid (**1**; 47.0 g, 300 mmol) dissolved in THF (400 mL) were added dropwise, maintaining temperature and N_2 introduction, and the orange solution was stirred for an additional 1 h. MeI (80 mL, 1.28 mol) was dropped in at −80 °C, and the mixture was stirred for an additional 10 min at this temperature. After removal of the cold bath, H_2O (600 mL) was slowly added and the mixture warmed to room temperature. The layers were separated, the aqueous phase was washed with Et_2O (2 × 500 mL), and the organic phases were discarded. The aqueous phase was acidified with 25% aqueous HCl (600 mL), and the crystals were allowed to separate first with stirring in an ice bath for 2 h and then on standing in a refrigerator overnight. The precipitate was separated, washed with H_2O, dried in a vacuum oven at 75 °C, and recrystallized from toluene (350 mL) to give compound **6** (37.3 g, 73%). An analytical sample was prepared by repeated recrystallization from toluene: mp 169−170.5 °C.

Method B. 4-Isopropyl-2-methylbenzoic Acid[12] (10). A mixture of 4-isopropyl-2-methylbenzoic acid methyl ester (**21**; 30.8 g, 160 mmol), 20% aqueous NaOH (225 mL, 6.75 mol), and MeOH (150 mL) was stirred at 65 °C for 4 h. The MeOH portion was evaporated, and the resulting aqueous phase was acidified with HCl and extracted with EtOAc (2 × 300 mL). The combined organic phases were washed with H_2O (200 mL), dried, evaporated, and crystallized from Et_2O to give the desired product as white solid (24 g, 84%). An analytical sample was prepared by recrystallization from petroleum ether: mp 89−90 °C.

4-Bromo-2-ethylbenzoic Acid (19). A 2-L, three-necked flask equipped with a mechanical stirrer, dropping funnel, drying tube, N_2 inlet, and a thermometer was charged with dry THF (700 mL) and diisopropylamine (70 mL, 498 mmol). After the mixture was cooled to 0 °C, butyllithium (280 mL, 1.6 M hexane solution, 459 mmol) and 4-bromo-2-methylbenzoic acid[10] (**17**, 43 g, 200 mmol) dissolved in THF (300 mL) were slowly added, and the mixture was stirred for an additional 15 min at this temperature. MeI (52 mL, 832 mmol) was added dropwise at −70 °C, and the mixture was allowed to warm to 0 °C. After the reaction was quenched with H_2O (150 mL) the mixture was poured into H_2O (800 mL). The organic phase was separated and washed with H_2O (250 mL). The combined aqueous phases were acidified with HCl, extracted with EtOAc (3 × 200 mL), dried, and evaporated to dryness to give compound **19** (42.9 g, 94%). An analytical sample was prepared by recrystallization from $(i\text{-Pr})_2O$: mp 106−108 °C; ^1H NMR (DMSO-d_6) δ 1.16 (t, 7.5, 3H), 2.92 (q, 7.4, 2H), 7.48 (dd, 8.4, 2.0, 1H), 7.54 (d, 1.8, 1H), 7.71 (d, 8.5, 1H), 13.0 (s br, 1H). Anal. ($C_9H_9BrO_2$) C, H, Br.

Method C. 4-Isopropyl-2-methylbenzoic Acid Methyl Ester (21). A 250-mL, three-necked flask was charged with Mg (4.25 g, 175 mmol) and THF (10 mL). Under an N_2 atmosphere, the Grignard reaction was started by adding a first portion of *i*-PrBr (16.4 mL, 175 mmol) in THF (75 mL) and a few drops of CH_2Br_2 with stirring and gentle heating. The heating bath was removed, and the rest of *i*-PrBr was dropped in at such a rate that the internal temperature remained at 45 °C. The reaction mixture was stirred for an additional 1 h at room temperature. In a 500-mL three-necked flask was dissolved $ZnBr_2$ (39.1 g, 174 mmol) in dry THF (130

mL) with stirring and under N_2 protection. On the mixture cooling below 0 °C, the Grignard solution was added dropwise. A precipitate formed on exothermic reaction, and stirring was continued for an additional 15 min at 0 °C. Then the reaction mixture was cooled down to −78 °C, and $PdCl_2$(dppf) (584 mg, 0.8 mmol) and 4-bromo-2-methylbenzoic acid methyl ester[14] (20 g, 87.3 mmol) were added consecutively. The cooling bath was removed, and the reaction mixture was stirred overnight. After the reaction was quenched with 10% aqueous HCl (200 mL), the THF portion of the mixture was evaporated. The remaining aqueous phase was extracted with Et_2O (3 × 250 mL), and the combined organic phases were washed with H_2O (4 × 200 mL), dried, and evaporated. The residue was purified by means of a Kugelrohr distillation (2 mbar, 105 °C bath temperature) to give **21** (15.5 g, 92%) as a colorless oil: NMR (DMSO-d_6) δ 1.20 (d, 7.0, 3H), 2.51 (s, 3H), 2.90 (sept, 6.9, 1H), 3.80 (s, 3H), 7.17 (m, 1H), 7.19 (m, 1H), 7.77 (m, 1H). Anal. ($C_{12}H_{16}O_2$) C, H.

Method D. 4-Chloro-2-methyl-5-(methylsulfonyl)benzoic Acid (30). On cooling with an ice bath, **6** (165 g, 967 mmol) was added portionwise to chlorosulfonic acid (488 mL, 7.3 mol) at such a rate that the internal temperature remained at 20 °C. The resultant mixture was heated at 135−140 °C bath temperature for 6 h. After cooling, the reaction mixture was added dropwise to stirred ice water (3.5 L), and stirring was continued for an additional 30 min at 10 °C. The precipitate was collected by filtration and washed with ice water (100 mL) to give crude 4-chloro-5-(chlorosulfonyl)-2-methylbenzoic acid which was used directly in the next reaction.

The moist chlorosulfonyl compound was added in portions to a solution of Na_2SO_3 (308 g, 2.44 mol) in H_2O (600 mL) at 15−20 °C. By addition of 32% aqueous NaOH (∼230 mL) the pH was adjusted to 9.0. Stirring was continued for an additional 3 h, and then the mixture was left to stand overnight at room temperature. In an ice bath the mixture was acidified to pH 1 using 25% aqueous HCl. The 2-chloro-4-methyl-5-carboxybenzenesulfinic acid which precipitated was filtered, washed with ice water (100 mL), and used in the next step without further purification.

The moist sulfinic acid was placed in a 4-L, three-necked flask to which was added H_2O (600 mL), MeOH (800 mL), MeI (400 mL, 6.4 mol), and sufficient 32% aqueous NaOH to attain a pH of 9. The reaction mixture was refluxed for 30 h with occasional addition of NaOH to maintain pH 9. The MeOH was distilled at reduced pressure, and ice water (2 L) was added to give a precipitate of 4-chloro-2-methyl-5-(methylsulfonyl)benzoic acid methyl ester (**48**, 18 g, 7% overall): mp 152−153 °C (MeOH); NMR (DMSO-d_6) δ 2.61 (s, 3H), 3.37 (s, 3H), 3.88 (s, 3H), 7.79 (s, 1H), 8.42 (s, 1H). Anal. ($C_{10}H_{11}ClO_4S$) C, H, Cl, S.

The cold aqueous mother liquor was acidified with HCl (pH 1), and the product **30** was filtered, washed with H_2O (100 mL), and dried (163 g, 68% overall): mp 217−218 °C (MeOH); NMR (DMSO-d_6) δ 2.62 (s, 3H), 3.37 (s, 3H), 7.76 (s, 1H), 8.44 (s, 1H), 13.50 (s br, 1H); IR (KBr) 1686, 1316, 1295, 1273, 1155, 968, 769, 517, 500 cm^{-1}. Anal. ($C_9H_9ClO_4S$) C, H, Cl, S.

Method E. 4-Fluoro-2-methyl-5-(methylsulfonyl)benzoic Acid Methyl Ester (49). In analogy to method D, **7** (98 g, 636 mmol) was sulfochlorinated with $ClSO_3H$ (240 mL, 3.60 mol) at 125 °C bath temperature for 1.5 h and then reduced with Na_2SO_3 (78 g, 619 mmol) to give the crude sulfinic acid. This was dissolved in H_2O (600 mL), and sufficient 32% NaOH was added to attain pH 10. The H_2O was distilled off, and the resinous residue was triturated with Me_2CO (1.5 L), filtered, and dried to give 2-fluoro-4-methyl-5-carboxybenzenesulfinic acid sodium salt as a light brown amorphous powder.

A stirred mixture of the crude, air-dried sodium salt (160 g) and MeI (160 mL, 2.56 mol) in DMF was refluxed for 5 h. During this time K_2CO_3 (25 g, 181 mmol) was added in portions to maintain a pH >7. The MeI was removed, employing reduced pressure, the resultant mixture was poured in H_2O (1.7 L), and the crystals were allowed to separate with stirring in an ice bath. The product was collected, washed with H_2O (100 mL), and dried to obtain **49** (86 g, 54% overall) as a white solid: mp 137−139 °C; NMR (DMSO-d_6) δ 2.63 (s, 3H), 3.34 (s, 3H), 3.87 (s, 3H), 7.58 (d, 11.2, 1H), 8.28 (d, 7.3, 1H). Anal. ($C_{10}H_{11}FO_4S\cdot0.25H_2O$) C, H, S.

Method E. 4-Bromo-2-methyl-5-(methylsulfonyl)benzoic Acid Methyl Ester (53). Analogously to method D, 4-bromo-2-methylbenzoic acid[10] (**17**, 1.0 kg, 4.65 mol) was sulfochlorinated with $ClSO_3H$ (930 mL, 14.0 mol) at 140 °C bath temperature for 3 h, and then reduced with Na_2SO_3 (1.32 kg, 10.5 mol) to yield the crude 2-bromo-4-methyl-5-carboxybenzenesulfinic acid. On cooling, this was suspended in DMF (6 L), and K_2CO_3 (1.59 kg, 11.5 mol) was added within 15 min, followed by MeI (656 mL, 10.5 mol) over a period of 1 h with stirring, and stirring was continued overnight at room temperature. DMF was removed under reduced pressure, and the residue was treated with H_2O (10 L), filtered off, and washed with H_2O (2 L). After this procedure was repeated, the obtained crystals were air-dried and recrystallized from EtOAc (4 L) to give **53** (606 g, 55% overall): mp 146−148 °C; NMR (DMSO-d_6) δ 2.60 (s, 3H), 3.78 (s, 3H), 3.88 (s, 3H), 7.98 (s, 1H), 8.44 (s, 1H). Anal. ($C_{10}H_{11}BrO_4S$) C, H, Br, S.

Method F. 4-Chloro-2,3-dimethyl-5-(methylsulfonyl)benzoic Acid Methyl Ester (56). 4-Chloro-2,3-dimethyl-5-(methylsulfonyl)benzoic acid (**47**, 20 g, 76.1 mmol) was treated with saturated HCl/MeOH (150 mL) overnight at room temperature and heated under reflux for an additional 20 h. The solution was concentrated, and the residue was taken up in EtOAc (150 mL), washed with saturated $NaHCO_3$ solution (2 × 75 mL) and H_2O (75 mL), dried, filtered, and concentrated to a small volume. The desired product was collected by filtration as yellow crystals and dried at 90 °C (17.3 g, 82%): mp 137 °C; NMR (DMSO-d_6) δ 2.45 (s, 3H), 2.54 (s, 3H), 3.38 (s, 3H), 3.89 (s, 3H), 8.21 (s, 1H). Anal. ($C_{11}H_{13}ClO_4S$) C, H, Cl, S. Esterifications of compounds **93** and **138** were carried out with MeI and K_2CO_3 in DMF as described in method E.

4-Chloro-2-methyl-3-nitrobenzoic Acid Methyl Ester (64). To stirred HNO_3 (100%, 80 mL) was added 4-chloro-2-methylbenzoic acid (**6**, 20 g, 117 mmol) in portions at 5−10 °C. After an additional 1-h period of stirring at 0−5 °C, the mixture was poured onto ice, and the yellow precipitate was collected and washed with H_2O (100 mL). The moist substance was taken up with EtOAc (250 mL) and dried with Na_2SO_4. After concentration of the filtered solution to half of the volume, a precipitate (9 g) consisting mainly of **61** was separated and discarded. The evaporated mother liquor (15 g) consisting of a mixture of acids **61** and **62** was treated with saturated HCl/MeOH (150 mL) overnight at room temperature and heated under reflux for an additional 6 h. The solution was concentrated, mixed with $NaHCO_3$ solution (250 mL), extracted with EtOAc (2 × 150 mL), dried, and filtered, and the solvent was removed to yield a yellowish crystalline crop (15.2 g). This was chromatographed on silica gel (400 g, petroleum ether/*t*-BuOMe 97.5 : 2.5). The homogeneous nonpolar fractions were combined to give 4-chloro-2-methyl-3-nitrobenzoic acid methyl ester (**64**, 1.4 g, 5.2% overall): mp 76−77 °C; NMR (DMSO-d_6) δ 2.43 (s, 3H), 3.88 (s, 3H), 7.76 (dq, 8.5, 0.5, 1H), 8.01 (d, 8.5, 1H); IR (KBr) 1729, 1538, 1287, 1260, 1126 cm^{-1}. Anal. ($C_9H_8ClNO_4$) C, H, Cl, N.

After the mixed fractions (9.6 g) were separated, the homogeneous polar fractions yielded 4-chloro-2-methyl-5-nitrobenzoic acid methyl ester (**63**, 4.2 g, 16% overall): mp 75−76 °C; NMR (DMSO-d_6) δ 2.60 (s, 3H), 3.89 (s, 3H), 7.79 (s, 1H), 8.42 (s, 1H); IR (KBr) 1727, 1562, 1529, 1343, 1307, 1250, 1099 cm^{-1}. Anal. ($C_9H_8ClNO_4$) C, H, Cl, N.

3-Amino-4-chloro-2-methylbenzoic Acid Methyl Ester (65). A solution of compound **64** (44.1 g, 192 mmol) and RaNi (20 g) in MeOH was shaken in the presence of H_2 at atmospheric pressure for 3 h, while the mixture was allowed to warm to 45 °C. The solvent was removed, and the resulting dark oil was then purified by flash column chromatography on silica gel with *t*-BuOMe (10%) in PhMe as the eluent to give **65** (26.8 g, 70%) as white crystals: mp 53−56 °C (EtOH/H_2O); NMR (DMSO-d_6) δ 2.28 (s, 3H), 3.80 (s, 3H), 5.27 (s, 2H), 6.92 (d, 8.6, 1H), 7.18 (d, 8.3, 1H); IR (KBr) 3396, 1720, 1621, 1442, 1285, 1256 cm^{-1}. Anal. ($C_9H_{10}ClNO_2$) C, H, Cl, N.

4-Chloro-2-methyl-3-(methylsulfonyl)benzoic Acid Methyl Ester (66). Compound **65** (32 g, 160 mmol) was dissolved in a mixture of concentrated H_2SO_4 (120 mL), H_3PO_4 (89%, 160 mL), and H_2O (80 mL). A solution of $NaNO_2$ (13.44 g, 195 mmol) in H_2O (40 mL) was added to the well-stirred mixture at such a rate that the temperature was maintained at 5−10 °C, and stirring was continued overnight at this temperature. The mixture was cooled to −15 °C, and condensed SO_2 (128 mL) was poured into the reaction. This was immediately poured onto a cooled mixture of $FeSO_4 \cdot 7H_2O$ (89 g, 320 mmol) and Cu powder (1.6 g) with much foaming. After removal of the ice/salt bath, stirring was continued for 4 h. The obtained suspension was extracted with EtOAc (3 × 250 mL), and the organic layers were combined, extracted with 2 N NaOH (3 × 100 mL), and discarded. The combined aqueous phases were cooled, acidified with HCl, and extracted with EtOAc (200 mL), and the organic phase was dried, filtered, and evaporated to give the crude sulfinic acid (22 g). This was added to a cooled solution of dry DMF (92 mL), consecutively followed by K_2CO_3 (38.6 g, 279 mmol) and MeI (17.4 mL, 278 mmol), and the mixture was stirred for 24 h under an N_2 atmosphere at room temperature. H_2O was added, the reaction mixture was extracted with EtOAc (3 × 75 mL), and the combined organic layers were washed with H_2O (5 × 50 mL), dried, filtered, and concentrated. Silica gel chromatography (EtOAc/petroleum ether, 1:2, → EtOAc) gave crystalline **66** (12.7 g, 30% overall): mp 71 °C [CH_2Cl_2/(i-Pr)$_2$O]; NMR (DMSO-d_6) δ 2.70 (s, 3H), 3.43 (s, 3H), 3.87 (s, 3H), 7.67 (d, 8.3, 1H), 7.83 (d, 8.3, 1H); IR (KBr) 1726, 1310, 1281, 1156, 1097 cm^{-1}. Anal. ($C_{10}H_{11}ClO_4S$) C, H, Cl, S.

Method G. 2-Methyl-5-(methylsulfonyl)-4-phenylbenzoic Acid Methyl Ester (75). A suspension of powdered NaOH (400 mg, 10.0 mmol) in diglyme (20 mL) was heated to 90 °C with stirring. After addition of benzeneboronic acid (1.02 g, 8.37 mmol), $Pd(PPh_3)_4$ [99%; 150 mg, 0.13 mmol], and 4-bromo-2-methyl-5-(methylsulfonyl)benzoic acid methyl ester (**53**; 2.25 g, 7.32 mmol), the reaction mixture was stirred for 6 h at 130 °C. The solution was then diluted with H_2O (100 mL) at room temperature and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried and concentrated with the aid of an oil pump to give a viscous residue, which was triturated with Et_2O/petroleum ether. Recrystallization from (i-Pr)$_2$O yielded **75** (1.3 g, 58%): mp 110−112 °C; NMR (DMSO-d_6) δ 2.63 (s, 3H), 2.80 (s, 3H), 3.91 (s, 3H), 7.46 (m, 6H), 8.51 (s, 1H); IR (KBr) 1719, 1308, 1246, 1143, 1100, 770, 525 cm^{-1}. Anal. ($C_{16}H_{16}O_4S \cdot 0.25H_2O$) C, H, S.

4-(2-Furyl)-2-methyl-5-(methylsulfonyl)benzoic Acid Methyl Ester (76). $Pd(PPh_3)_4$ [99%; 7.27 g, 6.28 mmol] was added to a stirred solution of 2-(tributylstannyl)furan (20.5 mL, 62.8 mmol) and 4-bromo-2-methyl-5-(methylsulfonyl)benzoic acid methyl ester (**53**, 19.3 g, 62.8 mmol) in dry dioxane (500 mL), and the mixture was heated at reflux temperature under an argon atmosphere for 3 h. The reaction mixture was filtered through Celite, the filtrate was evaporated in vacuo, and the residue was extracted with boiling t-BuOMe. Evaporation of the solution and recrystallization of the residue from t-BuOMe/CH_2Cl_2 yielded **76** (16.9 g, 91%): mp 142−143 °C; NMR (DMSO-d_6) δ 2.66 (s, 3H), 3.31 (s, 3H), 3.90 (s, 3H), 6.71 (dd, 3.5, 1.8, 1H), 7.17 (d, 3.4, 1H), 7.80 (s, 1H), 7.96 (d, 1.9, 1H), 8.54 (s, 1H); IR (KBr) 1721, 1603, 1541, 1499, 1436, 1304, 1141 cm^{-1}. Anal. ($C_{14}H_{14}O_5S$) C, H, S.

Method H. 2-Methyl-5-(methylsulfonyl)-4-propylthiobenzoic Acid (86). To a solution of DMF (50 mL) under N_2 was added PrSH (3.6 g, 47.3 mmol) followed by NaH (60% in mineral oil, 1.5 g, 37.5 mmol), and the temperature was allowed to rise to 40 °C. After 30 min of stirring, 4-fluoro-2-methyl-5-(methylsulfonyl)benzoic acid (**31**, 2 g, 8.6 mmol) was added, and the mixture was heated for 1 h at 80 °C. The solution was poured into ice water (300 mL), washed with EtOAc (3 × 100 mL), acidified, and extracted with EtOAc (2 × 50 mL). The combined organic phases were dried, concentrated, and triturated with Et_2O to obtain the desired product (1.5 g, 60%). Recrystallization of a sample from i-PrOH gave **86** as white crystals: mp 177−178 °C; NMR (DMSO-d_6) δ 1.03 (t, 7.3, 3H), 1.70 (sext, 7.3, 2H), 2.64 (s, 3H), 3.15 (t, 7.2, 2H), 3.29 (s, 3H), 7.50 (s, 1H), 8.37 (s, 1H), 13.12 (s br, 1H); IR (KBr) 1693, 1307, 1264, 1150 cm^{-1}. Anal. ($C_{12}H_{16}O_4S_2 \cdot 0.25H_2O$) C, H, S. For **83**, **84**, and **87**, commercially available Na thiolates were used instead of the NaH addition. Compound **91** was prepared without DMF, and K_2CO_3 was used as the base. The analytical sample of **87** was prepared by esterification (→**93**), silica gel chromatography, and saponification.

Method I. 4-Ethoxy-2-methyl-5-(methylsulfonyl)benzoic Acid (94). Under an N_2 atmosphere Na (420 mg, 18.3 mmol) was dissolved in EtOH (50 mL). 4-Chloro-2-methyl-5-(methylsulfonyl)benzoic acid (**30**, 600 mg, 2.41 mmol) was added, and the mixture was heated under reflux for 72 h. The EtOH was evaporated, and the residue was taken up in H_2O (75 mL), acidified with HCl, and extracted twice with EtOAc (75 mL). The combined organic layers were dried, concentrated, and triturated with Et_2O/EtOAc to give crystalline **94** (475 mg, 75%): mp 221−223 °C; NMR (DMSO-d_6) δ 1.41 (t, 7.0, 3H), 2.63 (s, 3H), 3.24 (s, 3H), 4.30 (q, 7.0, 2H), 7.21 (s, 1H), 8.31 (s, 1H); IR (KBr) 1691, 1599, 1381, 1323, 1252, 1147, 1045 cm^{-1}. Anal. ($C_{11}H_{14}O_5S \cdot 0.25H_2O$) C, H, S.

Method J. 2,3-Dimethyl-5-(methylsulfonyl)-4-(3-pyridyloxy)benzoic Acid Methyl Ester (103). A mixture of 4-chloro-2,3-dimethyl-5-(methylsulfonyl)benzoic acid methyl ester (**56**, 6.2 g, 22.4 mmol), 3-hydroxypyridine (2.13 g, 22.4 mmol), and K_2CO_3 (9.3 g, 67.3 mmol) in dry DMF (125 mL) was stirred at 130 °C for 20 h. Saturated $NaHCO_3$ solution (300 mL) was added, and the mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with saturated NaCl solution (4 × 100 mL) and dried, and the solvent was removed to leave brownish crystals of **103** (3.1 g, 41%). An analytical sample was prepared by recrystallization from EtOAc: mp 144 °C; NMR (DMSO-d_6) δ 2.04 (s, 3H), 2.53 (s, 3H), 3.31 (s, 3H), 3.90 (s, 3H), 7.14 (ddd, 8.4, 2.9, 1.2, 1H), 7.34 (dd, 8.6, 4.6, 1H), 8.18 (s, 1H), 8.28 (m, 2H); IR (KBr) 1728, 1578, 1480, 1430, 1312, 1254 cm^{-1}. Anal. ($C_{16}H_{17}NO_5S$) C, H, N, S. Preparation of **105**−**109** was carried out without DMF but with a large excess (4 equiv) of the appropriate OH reagent instead. The raw ester was not characterized in the case of **109** but directly hydrolyzed using method B. The yield given is the overall yield.

Method K. 2-Methyl-5-(methylsulfonyl)-4-(phenylamino)benzoic Acid (139). A mixture of 4-fluoro-2-methyl-5-(methylsulfonyl)benzoic acid (**31**, 1.0 g, 4.30 mmol) and $PhNH_2$ (5 mL, 475 mmol) was heated at 140 °C for 5 h. The mixture was dissolved in 1 N NaOH (30 mL) and washed with EtOAc (2 × 50 mL). After acidification with HCl the aqueous phase was extracted with EtOAc (2 × 50 mL) and the combined organic layers were dried, evaporated, and triturated with EtOAc/Et_2O to give a white solid of **139** (500 mg, 37%): mp 182−184 °C; NMR ($CDCl_3$) δ 2.57 (s, 3H), 3.14 (s, 3H), 7.07 (s, 1H), 7.23 (m, 3H), 7.43 (t, 7.6, 2H), 8.26 (s, 1H), 8.61 (s, 1H). Anal. ($C_{15}H_{15}NO_4S \cdot 0.5H_2O$) C, H, N, S. Experiments **120**−**122** were run with an excess of 3 equiv of the nucleophile only but with additional solvent instead. In the case of **120** this was PhMe, in **121** and **122** sulfolane, and in **123** N-methylpyrrolidin-2-one (NMP). The formed benzoic acids were not characterized for **119**−**123** but in the following ester stage. Esterifications were carried out in the case of compounds **122** and **123** according to method F, in **119** and **120** with MeI/K_2CO_3 in DMF, and in **121** with CH_2N_2. Yields given in Table 4 are overall yields.

Method L. 2,3-Dimethyl-4-(1-imidazolyl)-5-(methylsulfonyl)benzoic Acid Methyl Ester (129). Under N_2 4-chloro-2,3-dimethyl-5-(methylsulfonyl)benzoic acid (**47**, 10.0 g, 38.1 mmol) was added in portions within 30 min at 0 °C to a well-stirred suspension of NaH (60% in mineral oil, 1.52 g, 38.1 mmol) in NMP (100 mL), and stirring was continued for 1 h at room temperature. A solution of imidazole sodium in NMP (100 mL) was analogously prepared from imidazole (2.6 g, 38.1 mmol) and NaH (60% in mineral oil, 1.52 g, 38.1 mmol). Both solutions were combined and heated at 160 °C for 90 h. NMP was removed under reduced pressure, and the residue of the crude benzoic acid was esterified with saturated HCl/MeOH (150 mL) according to method F to give **129** (5.8 g, 49% overall) upon recrystallization from EtOAc: mp 184 °C; NMR (DMSO-d_6) δ 1.82 (s, 3H), 2.52 (s, 3H), 3.30 (s, 3H), 3.92 (s, 3H), 7.17 (s, 1H), 7.36 (s, 1H), 7.77 (s, 1H), 8.23 (s, 1H); IR

(KBr) 1723, 1499, 1440, 1309, 1203, 1140 cm^{-1}. Anal. ($C_{14}H_{16}N_2O_4S \cdot 0.5H_2O$) C, H, N, S. For **130**−**132** esterifications were omitted and the compounds were isolated in the form of the benzoic acids.

Method M. 4-Cyano-2-methyl-5-(methylsulfonyl)benzoic Acid (144). A mixture of 4-chloro-2-methyl-5-(methylsulfonyl)benzoic acid (**30**, 50 g, 200 mmol), CuCN (44.6 g, 500 mmol), and NMP (400 mL) was stirred under an N_2 atmosphere at 150 °C for 72 h. Afterward this was poured into H_2O (2 L), EtOAc (2 L) was added, and the mixture was stirred for an additional 30 min. After filtration through Celite, the aqueous layer was separated and extracted with EtOAc (3 × 500 mL). The combined organic layers were washed with H_2O (6 × 750 mL), dried, and evaporated to give compound **144** (22.5 g, 55%): mp 249−250 °C (MeOH); NMR (DMSO-d_6) δ 2.65 (s, 3H), 3.39 (s, 3H), 8.21 (s, 1H), 8.39 (s, 1H); IR (KBr) 2235, 1703, 1681, 1597, 1443, 1416, 1312, 1265, 1149 cm^{-1}. Anal. ($C_{10}H_9NO_4S$) C, H, N, S.

2-Methyl-5-(methylsulfonyl)-4-(trifluoromethyl)benzoic Acid Methyl Ester (146). A mixture of 4-bromo-2-methyl-5-(methylsulfonyl)benzoic acid methyl ester (**53**, 10.0 g, 33 mmol), CF_3CO_2K (7.53 g, 49.5 mmol), CuI (13.0 g, 69.3 mmol), and tetramethylammonium bromide (660 mg, 3.3 mmol) was stirred into PhMe (300 mL), after which part of the PhMe (∼100 mL) was stripped. NMP (100 mL) was added, and PhMe and NMP were stripped until the temperature reached about 155 °C. The reaction mixture was maintained at this temperature for 4 h. After cooling, the mixture was diluted with EtOAc (1 L) and H_2O (1 L) and filtered through Celite. The organic phase was separated, washed with brine (3 × 500 mL), dried, and evaporated. The resulting dark residue was purified by column chromatography on silica gel with EtOAc/petroleum ether (1:1) as the solvent to give **146** (3.7 g, 28%) as white crystals: mp 135−136 °C (CH_2Cl_2/EtOAc); NMR (DMSO-d_6) δ 2.69 (s, 3H), 3.31 (s, 3H), 3.92 (s, 3H), 8.06 (s, 1H), 8.56 (s, 1H); IR (KBr) 1724, 1599, 1452, 1434, 1303, 1251, 1151, 1106, 1078 cm^{-1}. Anal. ($C_{11}H_{11}F_3O_4S$) C, H, S. Analogous treatment of 4-bromo-3-(methylsulfonyl)-benzoic acid methyl ester[15d] yielded compound **147**.

4-(Benzylamino)-2-methyl-5-(methylsulfonyl)benzoic Acid (149). The title compound was prepared according to method K by treatment of compound **30** with $BnNH_2$ at 160 °C for 4 h in an 88% yield: mp 225−226 °C (H_2O); NMR (DMSO-d_6) δ 2.46 (s, 3H), 3.19 (s, 3H), 4.55 (d, 5.8, 2H), 6.67 (s, 1H), 7.06 (t, 5.8, 1H), 7.24−7.40 (m, 5H), 8.22 (s, 1H), 12.41 (s br, 1H); IR (KBr) 3369, 1684, 1603, 1561, 1307, 1258 cm^{-1}. Anal. ($C_{16}H_{17}NO_4S$) C, H, N, S.

4-Amino-2-methyl-5-(methylsulfonyl)benzoic Acid (150). In an analogous manner as described for **65** the title compound was prepared by hydrogenation of **149** using a Pd/C (10%) catalyst as an off-white solid in a 92% yield: mp 268−269 °C (CH_2Cl_2/MeOH); NMR (DMSO-d_6) δ 2.48 (s, 3H), 3.12 (s, 3H), 6.54 (s, 2H), 6.72 (s, 1H), 8.16 (s, 1H), 12.39 (s br, 1H); IR (KBr) 3453, 3357, 1686, 1301, 1288 cm^{-1}. Anal. ($C_9H_{11}NO_4S$) C, H, N, S.

2-Methyl-5-(methylsulfonyl)-4-(2-pyridylamino)benzoic Acid Methyl Ester (151). Compound **150** (10.0 g, 43.6 mmol) was added in portions to a cooled suspension of NaH (60% in mineral oil, 4.2 g, 105 mmol) in NMP (130 mL). Under N_2 protection the suspension was stirred at room temperature for 1 h. 2-Fluoropyridine (12.5 mL, 145 mmol) was added, and the mixture was heated at 100 °C for 48 h. The solution was cooled down, mixed with some drops of H_2O, and evaporated with the help of an oil pump, leaving a dark gum. The crude benzoic acid was refluxed with saturated MeOH/HCl (150 mL) for 6 h and the MeOH removed to a great extent under reduced pressure. The residue was taken up in EtOAc (250 mL), washed with 2 N NaOH (100 mL) and H_2O (100 mL), dried, and evaporated to give **151** (4.6 g, 32%) on crystallization from MeOH: mp 141−142 °C; NMR (DMSO-d_6) δ 2.59 (s, 3H), 3.27 (s, 3H), 3.84 (s, 3H), 7.05 (ddd, 7.2, 5, 0.8, 1H), 7.14 (d, 8.2, 1H), 7.76 (ddd, 8.2, 7.3, 1.8, 1H), 8.31 (s, 1H), 8.34 (s, 1H), 8.89 (s, 1H); IR (KBr) 3353, 1703, 1478, 1420, 1296, 1127 cm^{-1}. Anal. ($C_{15}H_{16}N_2O_4S \cdot 0.25H_2O$) C, H, N, S.

4-Amino-2-methyl-5-(methylsulfonyl)benzoic Acid Methyl Ester (152). Starting with **150** the title compound was prepared according to method F in a 93% yield: mp 150−151 °C (EtOAc); NMR (DMSO-d_6) δ 2.47 (s, 3H), 3.11 (s, 3H), 3.76 (s, 3H), 6.62 (s br, 2H), 6.73 (s, 1H), 8.15 (s, 1H); IR (KBr) 3466, 3366, 1717, 1300, 1284, 1133 cm^{-1}. Anal. ($C_{10}H_{13}NO_4S$) C, H, N, S.

2-Methyl-5-(methylsulfonyl)-4-(1-pyrrolyl)benzoic Acid Methyl Ester (153). Compound **152** (89.6 g, 368 mmol), 2,5-dimethoxytetrahydrofuran (60.45 mL, 467 mmol), and 4-chloropyridine hydrochloride (5.53 g, 36.9 mmol) were heated under reflux in 1,4-dioxane (2.2 L) for 2.5 h. The mixture was concentrated to a small volume, and the residue was taken up in EtOAc (2 L), washed with H_2O (3 × 500 mL), dried, and filtered. After addition of charcoal (5 g) the solution was refluxed for 45 min, filtered, evaporated, and recrystallized from MeOH to give **153** (94 g, 87%): mp 161−162 °C; NMR (DMSO-d_6) δ 2.64 (s, 3H), 2.67 (s, 3H), 3.91 (s, 3H), 6.33 (t, 2.1, 2H), 7.01 (t, 2.2, 2H), 7.57 (s, 1H), 8.49 (s, 1H); IR (KBr) 1726, 1298, 1259, 1147, 1101 cm^{-1}. Anal. ($C_{14}H_{15}NO_4S$) C, H, N, S.

4-Chloro-2-methyl-5-(methylsulfonyl)-3-nitrobenzoic Acid (154). The title compound was similarly prepared as described for **61** and **62** by treatment of compound **30** with a 5:1 H_2SO_4 (98%)/HNO_3 (100%) mixture at 70−80 °C for 6 h as a white powder in a 79% yield: mp 244−247 °C (EtOAc/MeOH); NMR (DMSO-d_6) δ 2.55 (s, 3H), 3.47 (s, 3H), 8.57 (s, 1H), 14.10 (s br, 1H); IR (KBr) 1703, 1550, 1324, 1298, 1142 cm^{-1}. Anal. ($C_9H_8ClNO_6S$) C, H, Cl, N, S.

4-Chloro-2-methyl-5-(methylsulfonyl)-3-nitrobenzoic Acid Methyl Ester (155). Starting from **154** method F gave pale yellow crystals in a 95% yield: mp 156−157 °C (EtOAc); NMR (DMSO-d_6) δ 2.54 (s, 3H), 3.46 (s, 3H), 3.93 (s, 3H), 8.56 (s, 1H); IR (KBr) 1737, 1549, 1319, 1293, 1265, 1148, 1132 cm^{-1}. Anal. ($C_{10}H_{10}ClNO_6S$) C, H, Cl, N, S.

3-Amino-4-chloro-2-methyl-5-(methylsulfonyl)benzoic Acid Methyl Ester (156). Hydrogenation of **155** in analogy to the method described for **65** gave pale yellow crystals of the title compound in a 34% yield: mp 188 °C (EtOAc); NMR (DMSO-d_6) δ 2.38 (s, 3H), 3.33 (s, 3H), 3.85 (s, 3H), 5.89 (s br, 2H), 7.59 (s, 1H); IR (KBr) 3447, 3367, 1725, 1289, 1210, 1135 cm^{-1}. Anal. ($C_{10}H_{12}ClNO_4S$) C, H, Cl, N, S.

4-Chloro-2-methyl-5-(methylsulfonyl)-3-(1-pyrrolyl)-benzoic Acid Methyl Ester (157). This compound was prepared from **156** in a manner analogous to that described for **153** above to give a 76% yield of **157** as pale yellow crystals: mp 136−137 °C (*t*-BuOMe/CH_2Cl_2); NMR (DMSO-d_6) δ 2.19 (s, 3H), 3.43 (s, 3H), 3.91 (s, 3H), 6.32 (t, 2.1, 2H), 6.85 (t, 2.1, 2H), 8.49 (s, 1H); IR (KBr) 1711, 1315, 1237, 1147, 735 cm^{-1}. Anal. ($C_{14}H_{14}ClNO_4S$) C, H, N.

3-Amino-2-methyl-5-(methylsulfonyl)benzoic Acid Methyl Ester (158). Hydrogenation of **155** in a similar manner as described for **65** using a Pd/C (10%) catalyst in a MeOH/THF/Et_3N mixture (30:20:1) gave a 67% yield of the title compound as yellow crystals: mp 121 °C (EtOAc/*t*-BuOMe); NMR (DMSO-d_6) δ 2.25 (s, 3H), 3.12 (s, 3H), 3.84 (s, 3H), 5.70 (s, 2H), 7.30 (d, 2.0, 1H), 7.34 (d, 2.0, 1H); IR (KBr) 3485, 3378, 1711, 1263, 1130 cm^{-1}. Anal. ($C_{10}H_{13}NO_4S$) C, H, N, S.

2-Methyl-5-(methylsulfonyl)-3-(1-pyrrolyl)benzoic Acid Methyl Ester (159). This compound was prepared from **158** in a manner analogous to that described for **153** to give a 79% yield of **159**: mp 116−117 °C (*t*-BuOMe); NMR (DMSO-d_6) δ 2.34 (s, 3H), 3.32 (s, 3H), 3.91 (s, 3H), 6.30 (t, 2.1, 2H), 7.04 (t, 2.2, 2H), 7.95 (d, 2.0, 1H), 8.27 (d, 2.0, 1H); IR (KBr) 1722, 1329, 1150, 1121 cm^{-1}. Anal. ($C_{14}H_{15}NO_4S$) C, H, N, S.

4-Bromo-2-(bromomethyl)-5-(methylsulfonyl)benzoic Acid Methyl Ester (160). To a solution of 4-bromo-2-methyl-5-(methylsulfonyl)benzoic acid methyl ester (**53**, 40 g, 130 mmol) in dry CH_2Cl_2 (280 mL) was added NBS (27.2 g, 152 mmol) in portions over a period of 5 h. During this time the mixture was refluxed and irradiated by means of an ordinary 400-W UV lamp. Afterward the reaction mixture was washed with H_2O (3 × 100 mL) and the organic layer dried and evaporated, yielding a yellow crystalline crop. This was chromatographed on silica gel with hexane/EtOAc (4:1). The polar fractions were combined to give **160** (30.4 g, 61%). An analytical sample of white crystals was prepared by recrys-

tallization from EtOAc: mp 136−137 °C; NMR (DMSO-d_6) δ 3.43 (s, 3H), 3.92 (s, 3H), 5.04 (s, 2H), 8.24 (s, 1H), 8.47 (s, 1H); IR (KBr) 1730, 1545, 1432, 1310, 1292, 1259, 1150, 1095 cm^{-1}. Anal. ($C_{10}H_{10}Br_2O_4S$) C, H, S.

4-Bromo-2-((diethylamino)methyl)-5-(methylsulfonyl)-benzoic Acid Methyl Ester (161). A mixture of 4-bromo-2-(bromomethyl)-5-(methylsulfonyl)benzoic acid methyl ester (**160**, 5 g, 13 mmol) and Na_2CO_3 (8.7 g, 82 mmol) was stirred in Et_2NH (35 mL, 335 mmol) under an N_2 atmosphere at room temperature for 24 h. The reaction mixture was then taken up in EtOAc (150 mL) and washed with H_2O (3 × 75 mL). The organic layer was dried and evaporated, and the residue was recrystallized from (*i*-Pr)$_2$O to give **161** (3 g, 61%): mp 94 °C; NMR (DMSO-d_6) δ 0.94 (t, 7.1, 6H), 2.46 (q, 7.2, 4H), 3.40 (s, 3H), 3.85 (s, 5H), 8.14 (s, 1H), 8.27 (s, 1H); IR (KBr) 1725, 1323, 1313, 1249, 1149, 1094 cm^{-1}. Anal. ($C_{14}H_{20}BrNO_4S$) C, H, Br, N, S.

Method N. (4-Chloro-2-methyl-5-(methylsulfonyl)benzoyl)guanidine (166). Free guanidine base was prepared by consecutive addition of Na (6.56 g, 285.5 mmol) and guanidine hydrochloride (30 g, 314 mmol) to dry MeOH (80 mL). After being stirred for 30 min at room temperature the mixture was filtered under N_2 protection. 4-Chloro-2-methyl-5-(methylsulfonyl)benzoic acid methyl ester (**48**, 15 g, 57.1 mmol) was added to the filtrate, and the mixture was stirred for 2.5 h at 50 °C. After the mixture was cooled to room temperature, H_2O (250 mL) was added, and the solution was stirred for 30 min and an additional 30 min with ice cooling while crystallization took place. The product was collected and recrystallized from MeOH, yielding **166** as white crystals (9.5 g, 57%): mp 207−208 °C; NMR (DMSO-d_6) δ 2.56 (s, 3H), 3.32 (s, 3H), 7.57 (s, 1H), 8.38 (s, 1H); IR (KBr) 1660, 1615, 1589, 1522, 1377, 1317, 1293, 1135 cm^{-1}. Anal. ($C_{10}H_{12}ClN_3O_3S$) C, H, Cl, N, S.

Method O. (4-Isopropyl-2-methyl-5-(methylsulfonyl)-benzoyl)guanidine (182). 4-Isopropyl-2-methyl-5-(methylsulfonyl)benzoic acid (**34**, 10.0 g, 39 mmol) was chlorinated with $SOCl_2$ (50 mL, 689 mmol) at 120 °C for 2 h. Excessive $SOCl_2$ was removed by the aid of a water-jet pump, and the remaining acid chloride was used without further purification. The preparation of guanidine base (180 mmol) in MeOH was carried out as described in the preceding instruction. The MeOH was removed in vacuo and the residue taken up in 1,2-dimethoxyethane (200 mL). The acid chloride was also taken up in 1,2-dimethoxyethane (200 mL) and then added to the guanidine solution. After the mixture was stirred for 1 h at room temperature, the inorganic precipitate was removed and the filtrate was evaporated. The residue was purified by silica gel chromatography (EtOAc → MeOH) to give compound **182** (9.0 g, 79%) after recrystallization from EtOAc: mp 220−223 °C; NMR (DMSO-d_6) δ 1.26 (d, 6.7, 6H), 2.56 (s, 3H), 3.17 (s, 3H), 3.75 (sept, 6.6, 1H), 7.42 (s, 1H), 8.26 (s, 1H). Anal. ($C_{13}H_{19}N_3O_3S \cdot H_2O$) C, H, N, S.

Methods P and Q. (4-(*tert*-Butylthio)-2-methyl-5-(methylsulfonyl)benzoyl)guanidine Methanesulfonate (202). 4-(*tert*-Butylthio)-2-methyl-5-(methylsulfonyl)benzoic acid (**87**, 800 mg, 2.65 mmol) and 2-chloro-1-methylpyridinium iodide (750 mg, 2.94 mmol) in NMP (7.3 mL) were stirred for 20 min. After addition of guanidine hydrochloride (1.15 g, 12 mmol), *N*-ethyldiisopropylamine (3.3 mL, 19.4 mmol) was dropped in with slight cooling, and stirring was continued for an additional 1 h. The mixture was poured onto ice water (50 mL), acidified, washed with EtOAc (2 × 20 mL), alkalified, and extracted with EtOAc (2 × 40 mL). The combined organic extracts were dried, evaporated, and triturated with Et_2O to give **202** (400 mg, 44%) as the free base: mp 112−115 °C; IR (KBr) 3427, 3370, 2966, 1662, 1598, 1527, 1302 cm^{-1}.

To the solution of the above free base (350 mg, 1.02 mmol) in Me_2CO (5 mL) $MeSO_3H$ (0.075 mL, 1.05 mmol) was added with a suitable pipet. Then Et_2O was added until the solution became cloudy and crystals of the methanesulfonate of **202** (350 mg, 78%), separated on cooling, were collected: mp 200−202 °C; NMR (DMSO-d_6) δ 1.46 (s, 9H), 2.42 (s, 3H), 2.54 (s, 3H), 3.40 (s, 3H), 7.75 (s, 1H), 8.20 (s, 1H), 8.40 (s br, 2H), 8.56 (s br, 2H), 11.69 (s, 1H). Anal. ($C_{14}H_{21}N_3O_3S_2 \cdot CH_4O_3S \cdot 0.5H_2O$) C, H, N, S.

Method R. [4-(4-Amino-1-piperidyl)-2-methyl-5-(methylsulfonyl)benzoyl]guanidine Dihydrochloride (232). To a solution of guanidine in MeOH (15 mL) prepared from guanidine hydrochloride (4 g, 41.9 mmol) in an analogous manner to that described in method N 4-[4-[(*tert*-butoxycarbonyl)amino]-1-piperidyl]-2-methyl-5-(methylsulfonyl)benzoic acid methyl ester (**119**, 3.5 g, 8.2 mmol) was added and the mixture was stirred for 3 h at 50 °C. After addition of H_2O (30 mL) the precipitate was collected and recrystallized from MeCN, yielding [4-[4-[(*tert*-butoxycarbonyl)amino]-1-piperidyl]-2-methyl-5-(methylsulfonyl)benzoyl]guanidine (1.4 g, 38%) as white crystals: mp 176 °C dec; NMR (DMSO-d_6) δ 1.40 (s, 9H), 1.58 (q br, 10, 2H), 1.82 (d br, 9.5, 2H), 2.53 (s, 3H), 2.79 (t br, 10.3, 2H), 3.14 (d br, 11.2, 2H), 3.32 (s, 3H), 3.40 (s br, 1H), 6.85 (d br, 7.1, 1H), 7.33 (s, 1H), 8.26 (s, 1H); IR (KBr) 1713, 1600, 1525, 1449, 1347, 1291, 1160, 1124 cm^{-1}.

A suspension of the foregoing Boc compound (1.3 g, 2.9 mmol) was stirred in 2 N HCl/dioxane (30 mL) at room temperature for 3 h. After dilution with Et_2O (30 mL) the precipitate was collected by filtration. Recrystallization from MeOH yielded **232** (800 mg, 65%) in the form of the white dihydrochloride: mp 305−310 °C dec; NMR (DMSO-d_6) δ 1.81 (q br, 10.5, 2H), 2.04 (d br, 8.8, 2H), 2.52 (s, 3H), 2.87 (t br, 10.4, 2H), 3.36 (s, 3H), 3.1−3.4 (m, 3H), 7.49 (s, 1H), 8.13 (s, 1H), 8.31 (s br, 2H), 8.49 (s br, 2H), 8.68 (s br, 2H); IR (KBr) 1714, 1691, 1596, 1307, 1293, 1255, 1142 cm^{-1}. Anal. ($C_{15}H_{23}N_5O_3S \cdot 2HCl$) C, H, Cl, N, S.

Method S. (2-Methyl-5-(methylsulfonyl)-4-(phenylamino)benzoyl)guanidine Hydrochloride (248). HCl (1 N, 1.15 mL) was dropped into a stirred suspension of (2-methyl-5-(methylsulfonyl)-4-(phenylamino)benzoyl)guanidine (400 mg, 1.15 mmol) in H_2O (100 mL), which was prepared according to method O from compound **139**. The filtered solution was frozen and then lyophilized to give the title compound (420 mg, 95%): mp 260 °C; NMR of the base (DMSO-d_6) δ 2.47 (s, 3H), 3.30 (s, 3H), 7.01 (s, 1H), 7.11 (t, 7.1, 1H), 7.25 (d, 8.1, 2H), 7.38 (t, 7.7, 2H), 7.90 (s, 1H), 8.36 (s, 1H). Anal. ($C_{16}H_{18}N_4O_3S \cdot HCl$) C, H, Cl, N, S.

Method T. [4-(1,4-Dihydro-4-oxo-1-pyridyl)-2-methyl-5-(methylsulfonyl)benzoyl]guanidine (279). In a small, sealed, round-bottomed flask were heated (4-fluoro-2-methyl-5-(methylsulfonyl)benzoyl)guanidine (**165**, 1 g, 3.66 mmol), 4-[(trimethylsilyl)oxy]pyridine[30] (10 g, 59.8 mmol) and K_2CO_3 (2 g, 14.5 mmol) at 135 °C for 4.5 h. The liquid silyl compound was decanted after cooling. The residue was washed with Et_2O and chromatographed (silica gel, gradient elution, EtOAc → MeOH). The homogeneous fractions were combined and recrystallized from MeOH to give **279** (800 mg, 61%): mp 267−268 °C; NMR (DMSO-d_6) δ 2.59 (s, 3H), 3.11 (s, 3H), 6.17 (d, 7.7, 2H), 7.54 (s, 1H), 7.73 (d, 7.7, 2H), 8.36 (s, 1H); IR (KBr) 3559, 3382, 1643, 1600, 1551, 1350, 1303 cm^{-1}. Anal. ($C_{15}H_{16}N_4O_4S \cdot 0.5H_2O$) C, H, N, S.

Crystal Data of 246, Methanesulfonate: $C_{15}H_{20}N_4O_6S_2$; M = 416.51; triclinic; $P\bar{1}$; a = 8.559(1) Å; b = 9.597(3) Å; c = 11.580(7) Å; α = 105.84(4)°; β = 98.29(6)°; γ = 91.97(4)°; V = 902.8(6) Å^3; Z = 2; ρ_x = 1.532 g cm^{-3}; μ(Cu Kα) = 0.322 mm^{-1}; $F(000)$ = 436; no. of reflections with $I \geq 3\sigma(I)$ = 3912; no. of refinement parameters = 264; final R values, R = 0.0398; R_w = 0.0482.

Na^+/H^+ Exchange Inhibition Assay: Preparation and Washing of Red Blood Cells. The red blood cells preparation as well as the internal acidification of the red blood cells follows closely the procedures as outlined by Morgan and Canessa.[31b] Blood was obtained from rabbits (e.g. New Zealand White) which were sacrificed and exsanguined. The blood was collected into 50-mL Falcon centrifuge tubes which contained heparin-Na solution (5 mL, 250 units/mL). The blood was mixed well with the heparin solution. The red blood cells were collected by centrifugation at 2000*g* at 4 °C; the plasma and buffy coat was removed. The remaining solution was filtered through 200 μm gaze. The filtrate was resuspended to the original volume with wash buffer [140 nM KCl, 0.15 mM $MgCl_2$, 10 mM tris(hydroxymethyl)aminomethane (Tris)/3-morpholinopropanesulfonic acid (MOPS), pH 7.4]. The red blood cells were again collected by centrifugation and the washing was repeated (2×).

Intraerythrocyte Acidification. For the intracellular acidification the packed, collected red blood cells (5 mL) were resuspended in the acidification buffer [45 mL; 170 mM KCl, 0.15 mM $MgCl_2$, 0.1 mM ouabain, 10 mM glucose, 10 mM sucrose, 20 mM Tris/2-morpholinoethanesulfonic acid (MES), pH 6.2]. The red blood cell suspension was incubated for 10 min at 37 °C with occasional mixing. To clamp the internal pH, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and DIAMOX (acetazolamide) were added up to 200 μM and 1 mM, respectively. The incubation was continued for a further 30 min at 37 °C. Thereafter the red blood cells were collected by centrifugation for 4 min; they were resuspended in ice-cold unbuffered wash solution (170 mM KCl, 40 mM sucrose, 0.15 mM $MgCl_2$) and washed therein (4×).

Na^+/H^+ Exchange Assay. The incubation was carried out in Macrowell-Tube strips in an 8 × 12 format. The incubation was started by adding prewarmed, acidified red blood cell solution (20 μL) to the incubation buffer (200 μL; 160 mM KCl, ^{22}NaCl, 0.16 μCi/well, 10 mM NaCl, 0.15 mM $MgCl_2$, 0.1 mM ouabain, 10 mM glucose, 40 mM sucrose, 10 mM Tris/MOPS, pH 8.0, 0.5 mM DIAMOX, 1% DMSO). Substances to be tested were dissolved in DMSO and were subsequently diluted to the appropriate concentrations in the incubation buffer. The incubation was carried out at 37 °C for 5 min. The incubation was stopped by adding ice-cold stop solution (800 μL; 112 mM $MgCl_2$, 0.1 mM ouabain). The red blood cells were collected by centrifugation for 7 min. The supernatant was sucked off by using an aspirator which allowed for the simultaneous aspiration of four adjacent tubes. The red blood cells were washed (3×) with ice-cold stop solution (900 μL) by repeating the resuspension/centrifugation step as described before. After the last wash H_2O (200 μL) was added to the red blood cell pellet. The tubes were then sonified for 2 × 30 min. Thereafter the Macrowell-tube strips were taken apart; each tube was added top to bottom to a separate scintillation vial; and a slight shaking caused the hemolyzed red blood cell solution to empty into the scintillation vial. To each vial the scintillant Aquasafe 300 PS (3 mL, Zinsser Analytic, Frankfurt/M, Germany) was added; the vials were capped and well mixed. The radioactivity taken up into the red blood cells was determined in a Packard scintillation counter by following the β-decay.

Evaluation of Results. Each substance concentration was determined in triplicate. From each value the mean of the count determination in the presence of EIPA (10 μM) was subtracted, accounting for the non-Na^+/H^+-dependent $^{22}Na^+$ uptake into the erythrocytes. The mean of the remaining counts in the absence of a substance was taken as 100% control; the mean values in the presence of the compounds to be tested were expressed as percent of that control value. The percent uptake data were plotted in a semilogarithmic plot; IC_{50} values were obtained by subjecting the data to a nonlinear curve fitting procedure using the equation $E = (E_{max} - E_{min})/(1 + IC_{50}/x)$, where x corresponds to the concentrations of the compound to be tested. The standard deviation of the IC_{50} values was 14% on the average.

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Supporting Information Available: X-ray crystallographic data, including positional parameters, bond distances, bond angles, and anisotropic displacement parameter expressions, for **246** (9 pages). Ordering information is given on any current masthead page.

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