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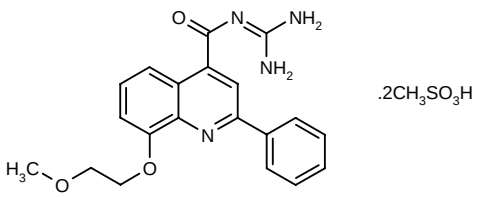
MS-31-038

Na^+/H^+ Exchange Inhibitor

N[1]-[8-(2-Methoxyethoxy)-2-phenylquinolin-4-ylcarbonyl]guanidine bis(methanesulfonate)



$C_{20}H_{20}N_4O_3.2CH_4O_3S$ Mol wt: 556.6142

CAS: 181048-36-2 (as free base)
CAS: 181049-21-8 (as monomethanesulfonate)

EN: 275395

Synthesis

The general synthetic route to 2-phenyl-4-quinolylguanidine derivatives (VII) is outlined in Scheme 1 (1). Key intermediates, 2-phenylquinoline-4-carboxylic acid derivatives (VI), can be prepared by using classic quinoline synthetic methods (2, 3).

As described in Scheme 2, the synthesis of MS-31-038 (XI) can be accomplished as follows: reaction of 2-(2-methoxyethyloxy)aniline (VIII) in refluxing ethanol with benzaldehyde and pyruvic acid gives 2-phenyl-8-(2-methoxyethoxy)-4-quinolinecarboxylic acid (IX). Condensation of (IX) with guanidine using 1,1'-carbonyldiimidazole (CDI) in dimethylformamide affords the corresponding 4-quinolylguanidine (X), which is finally converted to its bismethanesulfonate (XI) by treatment with methanesulfonic acid in ethanol.

Introduction

The Na^+/H^+ exchanger is an ubiquitous membrane ion transporter which primarily regulates intracellular Na^+ concentration, as well as intracellular pH (pHi). It is driven by Na^+ gradient and extrudes H^+ from the cytosol in exchange extracellular Na^+ with a stoichiometry of 1:1 (4). The exchanger is stimulated by a decrease in pHi (5) and is activated by a variety of endogenous mediators (*e.g.*, catecholamines, thrombin, angiotensin II, endothelin) (6-9).

It has been reported that the Na^+/H^+ exchanger may be a key mediator of cardiac injury process during myocardial ischemia and reperfusion (10). The exchanger has been shown to be relatively inactive at normal pHi but is excessively stimulated by intracellular acidification during myocardial ischemia and the early period of reperfusion (11). Under ischemic conditions, activation of this exchanger causes accumulation of cytosolic Na^+ concentration, which, in turn, leads to an increase in intracellular Ca^{2+} concentration via the Na^+/Ca^{2+} exchanger (12). The cytosolic Ca^{2+} overload is associated with myocardial cell injury and several cardiac dysfunctions (13). It has been suggested that inhibition of Na^+/H^+ exchange could be a new approach in the therapy of cardiac injury during ischemia and reperfusion.

Several studies have shown that Na^+/H^+ exchange inhibitors have significant efficacy against the development of myocardial necrosis during ischemia and reperfusion. Rohmann *et al.*, in a porcine model of ischemia and reperfusion-induced injury, reported that pretreatment with the selective Na^+/H^+ exchange inhibitor Hoe-694 before ischemia significantly reduced myocardial infarct size. Furthermore, administration of the drug shortly before the onset of reperfusion decreased infarct size (14). Klein *et al.* also reported that preischemic treatment with Hoe-694 produced protective effects against cardiac injury in pigs subjected to ischemia and reperfusion. However, the compound had no significant effect on reduction of infarct size when administered shortly before reperfusion (15). Garcia-Dorado *et al.* examined the effects of another selective Na^+/H^+ exchange inhibitor, cariporide, on cardiac injury in pigs and reported that pretreatment with the drug before coronary occlusion reduced ischemia and infarct size and attenuated arrhythmias (16). Several studies in rabbits have reported the efficacy of cariporide in reducing myocardial infarct size during ischemia and reperfusion. Linz *et al.* showed that infarct size was decreased by both preischemic and postischemic (prereperfusion) treatment with cariporide (17). In contrast, Bugge *et al.* reported that EIPA, an analog of amiloride, only had protective effects against

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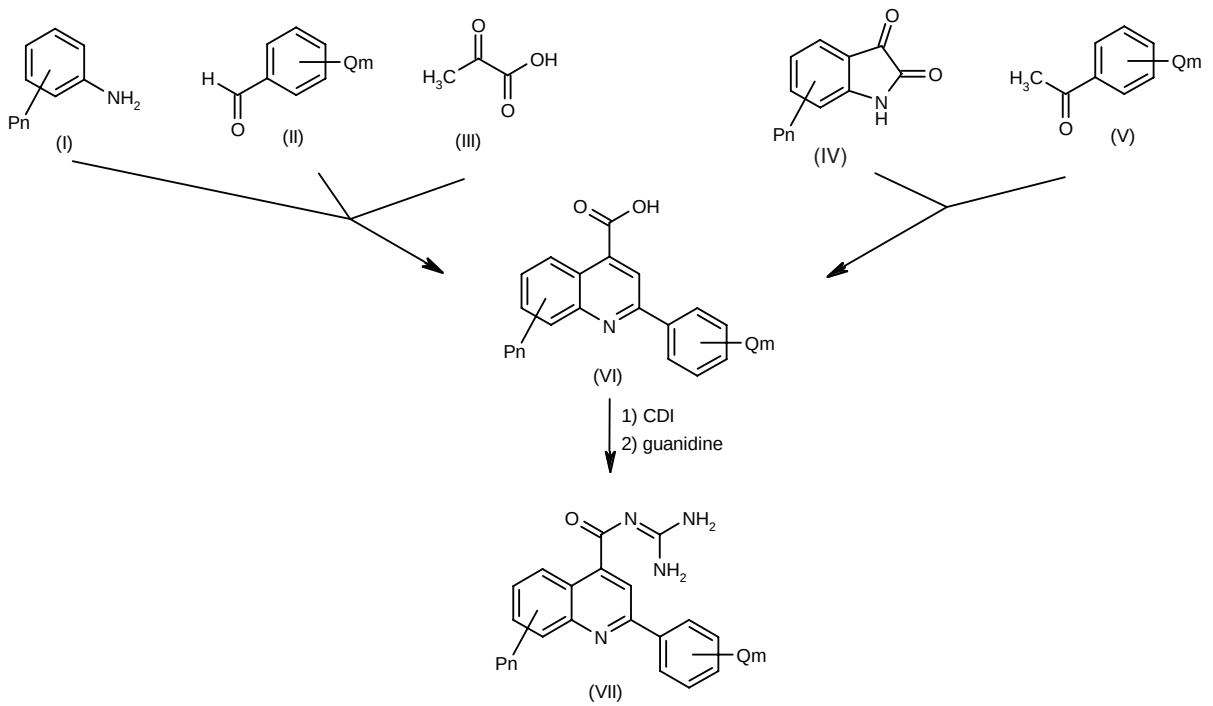
Scheme 1: The General Synthetic Route to 2-Phenyl-4-Quinolylguanidine Derivatives



Scheme 2: Synthesis of MS-31-038

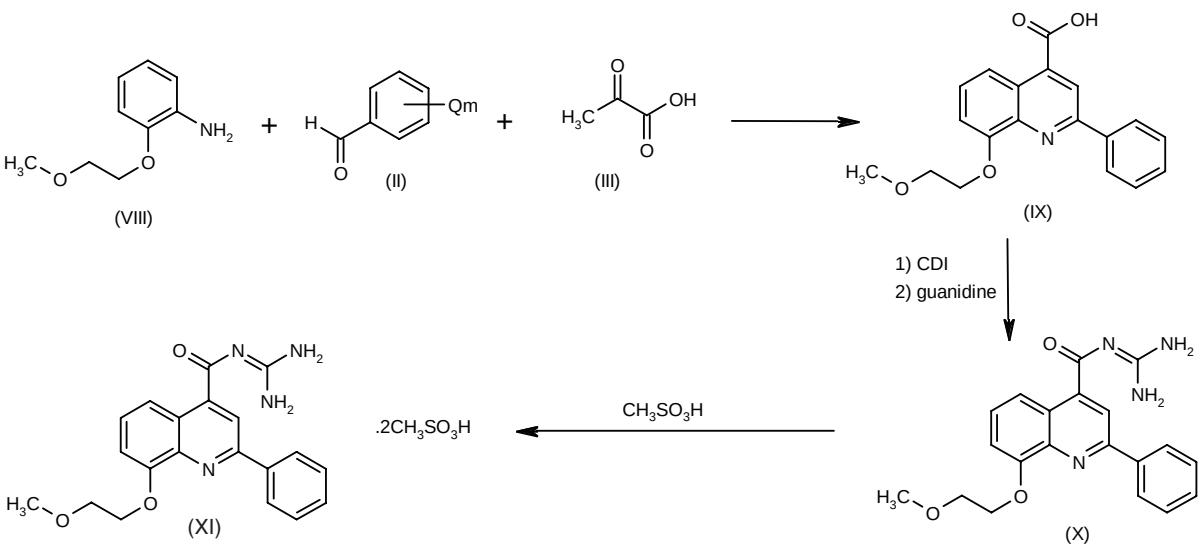
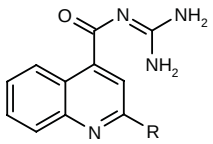
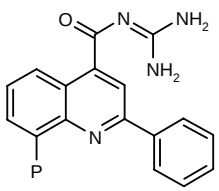


Table I: Effects of substituent R in the 2-position of 4-quinolylguanidine on Na^+/H^+ exchange activity of rat mesenteric artery.



Compound	R	Inhibition (%) at 10 μM
XII	H	14.8
XIII	Me	0
XIV	*n*-Hexyl	9.9
XV	Ph	69.8

Table II: Effects of substituent P in the 8-position of 2-phenyl-4-quinolylguanidine on Na^+/H^+ exchange activity of the rat mesenteric artery.



Compound	P	NHE inhibition (%) 0.1 μM	1 μM
XV	H	5.2	69.8
XVI	OMe	47.4	63.6
XVII	Cl	12.0	57.0
XVIII	Me	8.1	50.0
XI (MS-31-038)	OCH_2CH_2OMe	25.1	60.6

cardiac injury when administered prior to ischemia (18). In rats, preischemic administration of cariporide and FR-16888, a novel selective Na^+/H^+ exchange inhibitor, was shown to reduce myocardial infarct size (19, 20). In an isolated rat heart model of regional ischemia and reperfusion, preischemic treatment with EIPA was shown to significantly reduce infarct size; however, when EIPA was administered only during reperfusion, no cardiac protection was observed (21).

Discovery of MS-31-038

In order to obtain a novel Na^+/H^+ exchange inhibitor, our research program focused on heterocycles bearing an acylguanidyl moiety. The abilities of the synthesized compounds to inhibit Na^+/H^+ exchange were studied by measuring the recovery of pHi after intracellular acidification in rat mesenteric artery segments. The pHi was decreased by using a NH_4Cl prepulse technique, as described previously by Foster *et al.* (22).

During the synthesis and the screening of the acylguanidine derivatives, we discovered the interesting nature of the 4-quinolylguanidine derivatives (XII-XV). As shown in Table I, the inhibitory activity of the compounds on Na^+/H^+ exchange could be modulated by manipulating the substituent R in the quinoline nucleus. Among compounds (XII-XV), the 2-phenyl analog (XV) was the most potent and was selected for further chemical modifications.

Extensive chemical modifications were performed in order to improve the potency and the aqueous solubility of (XV). By introducing a hydrophilic substituent to the quinoline nucleus, we found that the 8-position was suitable for substitution and 8-methoxy derivative (XVI) resulted in an increase in potency at 0.1 μM (Table II).

Although replacement of a methoxy group in (XVI) with a chloro atom or a methyl group decreased potency, the 8-methoxyethyloxy analog (XI), or MS-31-038, was moderately active and increased water solubility.

Pharmacological Actions

The inhibitory effects of MS-31-038, cariporide and MS-31-050 [2-(2-methylphenyl)-5,7-dimethoxy-4-quinolylcarbonylguanidine dihydrochloride] were compared in segments of rat mesenteric artery. As shown in Table III, MS-31-038 and cariporide were equally potent in inhibiting Na^+/H^+ exchange, whereas MS-31-050 exhibited the most potent effects (23).

The cardioprotective effects of MS-31-038, cariporide and MS-31-050 on myocardial infarct size were examined in rats subjected to ischemia and reperfusion. In this model, the main coronary artery was occluded in anesthetized rats for 20 min and then reperfused for 2 h. The infarct size was determined by staining with triphenyltetrazolium chloride and was expressed as a percentage of the area at risk. The infarct size was dramatically decreased by postischemic treatment with MS-31-038. Even when given 1 min before the onset of reperfusion, MS-31-038 at doses of 3 and 10 mg/kg i.v. reduced infarct

Table III: Inhibitory effects of MS-31-038, cariporide and MS-31-050 on Na+/H+ exchange activity of rat mesenteric artery (23).

	Conc. (μM)	n	Inhibition (%)
MS-31-038	0.1	3	25.1 ± 4.4
	1	3	60.6 ± 1.5
	10	3	72.1 ± 5.7
Cariporide	0.1	3	23.9 ± 3.0
	1	3	45.4 ± 1.8
	10	3	70.6 ± 5.8
MS-31-050	0.03	3	38.4 ± 2.5
	0.1	3	67.4 ± 0.8

Results are expressed as mean ± SE.

Table IV: Effects of postischemic treatment of MS-31-038 and cariporide on myocardial infarction (23).

	Dose (mg/kg i.v.)	n	Infarct size (%)
Control		5	65.4 ± 7.4
MS-31-038	3	5	29.9 ± 11.6*
	10	6	9.8 ± 3.4***
Cariporide	10	5	48.1 ± 7.6

Results are expressed as mean ± SE. *$p < 0.05$, ***$p < 0.001$ *vs.* control (Tukey-Kramer test).

Table V: Effects of preischemic and postischemic treatment of MS-31-050 on myocardial infarction (23).

	Dose (mg/kg i.v.)	n	Infarct size (%)
Control		6	73.6 ± 3.6
MS-31-050			
Preischemic	10	5	22.3 ± 13.4**
Postischemic	10	5	70.8 ± 5.5
	30	5	50.6 ± 9.4

Results are expressed as mean ± SE. **$p < 0.01$ *vs.* control (Tukey-Kramer test).

size. In contrast, cariporide, whose inhibitory effect on Na^+/H^+ exchange was comparable to that of MS-31-038, showed no effect on infarct size at 10 mg/kg i.v. in the same procedure (Table IV) (23). MS-31-050, which was more potent than MS-31-038 and cariporide in inhibiting Na^+/H^+ exchange, significantly reduced the infarct size at 10 mg/kg i.v. when given prior to the onset of ischemia. However, postischemic treatment with MS-31-050 failed to protect the heart even at a higher dose of 30 mg/kg i.v. (Table V) (23).

MS-31-038 did not affect the measured hemodynamic parameters, including mean arterial pressure, heart rate, left ventricular pressure (LVP), and both the positive and negative values of the first derivative of LVP, in anesthetized rats at 10 mg/kg i.v., which was an effective dose for cardioprotection (23).

Amiloride is a prototype Na^+/H^+ exchange inhibitor that has a blocking action on the Na^+ channel and Na^+/Ca^{2+} exchange (24). Furthermore, 5-(*N,N*-hexamethylene) amiloride (HMA), an amiloride analog, has been reported to exert a depressant action on cardiac Na^+ channel and prolong cardiac action potential duration (APD) at concentrations that presumably affect Na^+/H^+ exchange (25). Thus, we examined the effects of MS-31-038 on cardiac action potentials in isolated dog Purkinje fiber *in vitro* using conventional microelectrodes. Results of a preliminary electrophysiologic study showed that MS-31-038 had no effect on V_{max}, APD_{50} and APD_{90} at 10 μM, a concentration which significantly inhibited Na^+/H^+ exchange, and at an even higher concentration of 30 μM (unpublished data).

Pharmacokinetics

The single-dose pharmacokinetics of MS-31-038 (5 mg/kg i.v.) were examined in fasting dogs. Plasma concentrations were determined using an HPLC assay and pharmacokinetic parameters were assessed using a one- or two-compartment analysis. In a preliminary study, plasma concentrations of MS-31-038 declined biphasically. Half-life of MS-31-038 obtained for β phase was 1.85 h. Oral bioavailability was about 70% based on AUC value obtained from intravenous study (unpublished data).

Conclusions

The inhibitory effect of MS-31-038 on Na^+/H^+ exchange in rat mesenteric artery was as potent as that of cariporide, a selective Na^+/H^+ exchange inhibitor. Compared to MS-31-038 and cariporide, MS-31-050 exhibited the most potent effect.

Like the selective Na^+/H^+ exchange inhibitors cariporide and FR-168888, MS-31-050 significantly reduced myocardial infarct size in rats subjected to ischemia-reperfusion when administered before ischemia. However, it showed no cardioprotective effects when administered postischemia. In contrast, postischemic administration of MS-31-038 had protective effects on myocardial infarct size. In addition, it did not change hemodynamic parameters. These results indicate that MS-31-038 may be an effective treatment for patients with ischemic heart disease.

Manufacturer

Mitsui Chemicals, Inc. (JP).

References

1. Fujiwara, J., Mori, H., Yamashita, H., Kitamori, T., Hosoya, J., Banno, H. (Mitsui Chemicals, Inc.). *Quinoline-4-carbonylguanidine derivs., process for producing the same and pharmaceutical preparations containing the cpds.* JP 96277269, EP 726254, US 5627193.

2. Doebner, O. *Ueber α-alkylcinchoninsauren.* Ber 1887, 20: 277-80.

3. Pfitzinger, W. *Chinolinederivate aus isatinsaure.* J Prakt Chem 1886, 33: 100.

4. Mahnensmith, R.L., Aronson, P.S. *The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological process.* Circ Res 1985, 56: 773-88.

5. Wakabayashi, S., Fafournoux, P., Sardet, C., Pouyssegur, J. *The Na/H antiporter cytoplasmic domain mediates growth factor signals and controls "H-sensing".* Proc Natl Acad Sci USA 1992, 89: 2424-8.

6. Hori, M., Nakatsubo, N., Kagiya, T., Iwai, K. Sato, H., Iwakura, K., Kitabatake, A., Kamada, T. *The role of Na^+/H^+ exchange in*

norepinephrine-induced protein synthesis in neonatal cultured rat cardiomyocytes. Jpn Circ J 1990, 54: 535-9.

7. Siffert, W., Akkerman, J.W.N. *Protein kinase C enhances Ca^{2+} mobilization in human platelets by activating Na^{+}/H^{+} exchange.* J Biol Chem 1988, 263: 4223-7.

8. Kaibara, M., Mitarai, S., Yano, K., Kameyama, M. *Involvement of Na^{+}-H^{+} antiporter in regulation of L-type Ca^{2+} channel current by angiotensin II in rabbit ventricular myocytes.* Circ Res 1994, 75: 1121-5.

9. Simonson, M.S., Wann, S., Mene, P., Dubyak, G.R., Kester, M., Nakazato, Y., Sedor, J.R., Dunn, M.J. *Endothelin stimulates phospholipase C, Na^{+}/H^{+} exchange, c-fos expression, and mitogenesis in rat mesangial cells.* J Clin Invest 1989, 83: 708-12.

10. Karmazyn, M., Moffat, M.P. *Role of Na^{+}/H^{+} exchange in cardiac physiology and pathopysiology: Mediation of myocardial reperfusion injury by pH paradox.* Cardiovasc Res 1993, 27: 915-24.

11. Noel, J., Pouyssegur, A. *Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na^{+}/H^{+} exchanger isoforms.* Am J Physiol 1995, 268: C283-C96.

12. Tani, M., Neely, J.R. *Role of intracellular Na^{+} in Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat hearts. Possible involvement of Na^{+}-H^{+} and Na^{+}-Ca^{2+} exchange.* Circ Res 1989, 65: 1045-56.

13. Opie, L.H. *Reperfusion injury and its pharmacologic modification.* Circulation 1989, 80: 1049-62.

14. Rohmann, S., Weygant, H., Minck, K.O. *Preischemic as well as postischemic application of a Na^{+}/H^{+} exchange inhibitor reduces infarct size in pigs.* Cardiovasc Res 1995, 30: 945-51.

15. Klein, H.H., Pich, S., Bohle, R.M., Wollenweber, J., Nebendahl, K. *Myocardial protection by Na^{+}-H+exchange inhibition in ischemic, reperfused porcine hearts.* Circulation 1995, 92: 912-7.

16. Garcia-Dorado, D., Gonzalez, M.A., Barrabes, J.A., Ruiz-Meana, M., Solares, J., Lidon, R.M., Blanco, J., Puigfel, Y., Piper, H.M., Soler-Soler, J. *Prevention of ischemic rigor contracture during coronary occlusion by inhibition of Na^{+}-H^{+} exchange.* Cardiovasc Res 1997, 35: 80-9.

17. Linz, W., Albus, U., Crause, P., Jung, W., Weichert, A., Scholkens, B.A., Scholz, W. *Dose-dependent reduction of myocardial infarct mass in rabbits by the NHE-1 inhibitor cariporide (HOE 642).* Clin Exp Hypertens 1998, 20: 733-49.

18. Bugge, E., Munch-Ellingsen, J., Ytrehus, K. *Reduced infarct size in the rabbit heart in vivo by ethylisopropyl-amiloride. A role for Na^{+}/H^{+} exchange.* Basic Res Cardiol 1996, 91: 203-9.

19. Aye, N.N., Komori, S., Hashimoto, K. *Effects and interaction of cariporide and preconditioning on cardiac arrhythmias and infarction in rat in vivo.* Br J Pharmacol 1999, 127: 1048-55.

20. Ohara, F., Sugimoto, T., Yamamoto, N., Ohkubo, K., Ozaki, T., Maeda, K., Seki, J., Goto, T. *Protective effect of FR168888, a new Na^{+}/H^{+} exchange inhibitor, on ischemia and reperfusion-induced arrhythmia and myocardial infarction in rats in comparison with other cardioprotective compounds.* Jpn J Pharmaol 1999, 80: 295-302.

21. Bugge, E., Ytrehus, K. *Inhibition of sodium-hydrogen exchange reduces infarct size in the isolated rat heart - A protective additive to ischaemic preconditioning.* Cardiovasc Res 1995, 29: 269-74.

22. Foster, C.D., Hill, W.A.G., Honeyman, T.W., Scheid, C.R. *Characterization of Na^{+}-H^{+} exchange in segments of rat mesenteric artery.* Am J Physiol 1992, 262: H1651-6.

23. Banno, H., Fujiwara, J., Hosoya, J., Kitamori, T., Mori, H., Yamashita, H., Ikeda, F. *Effects of MS-31-038, a novel Na^{+}-H^{+} exchange inhibitor, on the myocardial infact size in rats after postischemic administration.* Arzneim-Forsch Drug Res 1999, 49: 304-10.

24. Kleyman, T.R., Cragoe, E.J. Jr. *Amiloride and its analogs as tools in the study of ion transport.* J Membr Biol 1988, 105: 1-21.

25. Lai, Z.-F., Hotokebuchi, N., Cragoe, E.J. Jr., Nishi, K. *Effects of 5-(N,N-hexamethylene) amiloride on action potentials, intracellular Na, and pH of guinea pig ventricular muscle in vitro.* J Cardiovasc Pharmacol 1994, 23: 259-67.