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Benzimidazol-2-yl or benzimidazol-2-ylthiomethyl benzoylguanidines as novel Na^+/H^+ exchanger inhibitors, synthesis and protection against ischemic-reperfusion injury

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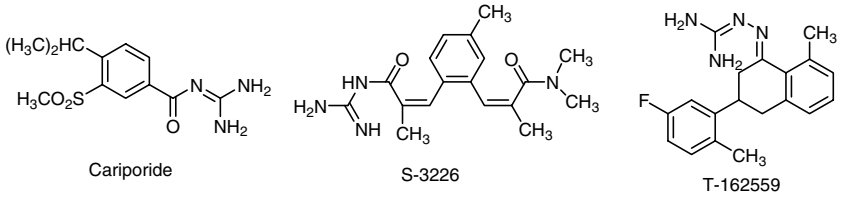
Abstract—A novel series of benzimidazol-2-yl or benzimidazol-2-ylthiomethyl benzoylguanidines were designed and synthesized as Na^+/H^+exchanger inhibitors. Most of them were found to inhibit NHE1-mediated platelet swelling in a concentration-dependent manner, and to have significant cardioprotective effect against myocardial ischemic-reperfusion injury, among which compounds **10a** and **34** were more potent than cariporide in both in vivo and in vitro tests.

The Na^+/H^+ exchanger (NHE), an integral membrane protein ubiquitously expressed in mammalian cells, maintains intracellular pH homeostasis by exchanging one intracellular H^+ for an extracellular Na^+. The biological function of NHE also incorporates its participation in cell proliferation and differentiation, roles in cytoskeletal organization and cell migration, regulation of cell volume, and cooperation on ion transportation. Among the nine isoforms[1] that have been identified so far, NHE-1 is the most intensively studied one, for its over-activation is implicated in a series of pathological processes such as essential hypertension, myocardial ischemic-reperfusion injury, post-ischemic dysfunction, and cellular death. This is because excessive activation of NHE1 during ischemia and reperfusion leads to increased intracellular Na^+ that results in increased intracellular calcium through the Na^+/Ca^{2+} exchanger (NCE) and ultimately cell damage and cell death.



NHE inhibitors developed thus far can be structurally categorized into three major classes, i.e. aroyl guanidines (including monocyclic and bicyclic), exemplified by cariporide;[2] non-aryl acylguanidines, exemplified by S-3226,[3] an NHE3 selective inhibitor; and non-acylguanidines, exemplified by T-162559.[4] In most cases, the acylguanidine functional group is considered as the active site of the inhibitors, and thus is applied by many drug designers. The bioisosteres of acylguanidines such as aminoimidazoles have also been investigated.[5]

Recognizing its importance in NHE inhibition, we chose benzoylguanidine as main structure, and introduced a

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Table 1. In vivo and in vitro test results of target compounds

Compound[a]	CK[b] (U/ml)	Infarct size [c] (%)	PSA IC_{50}[d] (nM)
Control	67.27 ± 4.82	66.70 ± 4.26	–
Cariporide	37.73 ± 3.70**	44.55 ± 3.45**	65.0
9a	45.48 ± 4.64**	49.16 ± 4.14**	$>10^4$
9b	58.46 ± 3.83*	51.28 ± 3.74**	$>10^4$
9c	57.61 ± 7.56	58.50 ± 2.28*	295
10a	32.05 ± 4.40**	39.34 ± 2.72**	11.0
10b	50.66 ± 7.41*	59.72 ± 3.23*	1.42
10c	46.02 ± 5.97**	47.56 ± 2.53**	4.05
11a	49.37 ± 3.42**	43.65 ± 3.38**	264
11b	53.69 ± 4.75*	61.05 ± 5.40	267
11c	45.66 ± 5.60**	50.61 ± 7.15*	$>10^4$
31a	60.94 ± 8.21	60.17 ± 4.05	$>10^4$
31b	53.95 ± 7.49*	54.73 ± 4.28*	332
32a	43.59 ± 4.79**	48.75 ± 1.34**	79.0
32b	37.84 ± 4.68**	46.52 ± 0.57**	40.2
33	55.25 ± 4.87*	63.08 ± 3.57	147
34	34.20 ± 3.27**	35.49 ± 4.53**	27.8
35	38.39 ± 5.83**	45.20 ± 5.40**	27.1

*$p < 0.05$, **$p < 0.01$ compared with baseline value.

[a] Cariporide and the tested compounds were injected intravenously 5 min before LAD occlusion at the dose of 0.01 mmol/kg.

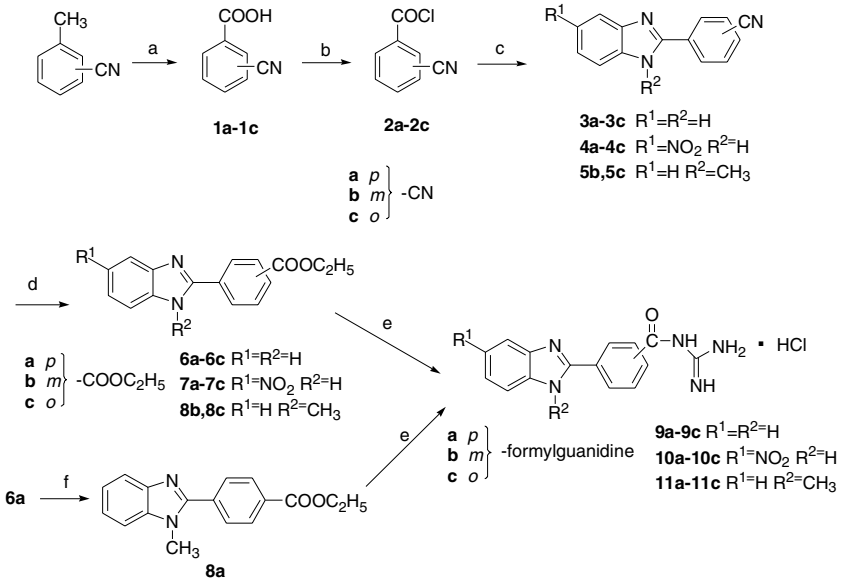
[b] The amount of creatine kinase (CK) was determined using a CK-NAC kit (Nanjing JianchengBioengineering Institute, Nanjing, China) and a 722 grating photospectrometer (Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, China). Serum CK activity was expressed as U/ml. Values are means ± SD, $n = 6$ or higher.

[c] Infarct size was expressed as the ratio of myocardial infarct area to area at risk. Values are means ± SD, $n = 6$ or higher.

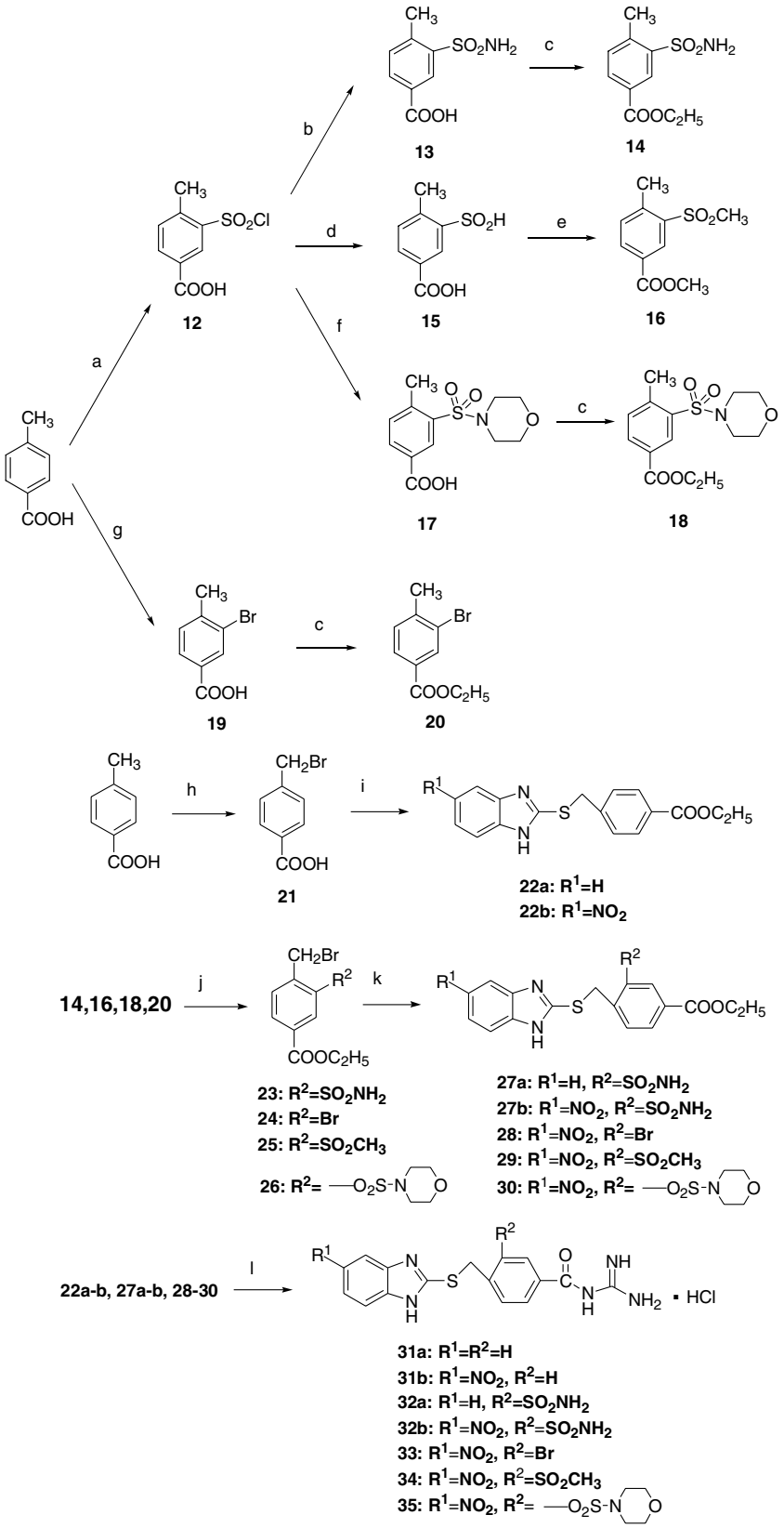
[d] Drug concentration to achieve half-maximal inhibition of acid-induced swelling in rat platelets.

benzimidazole ring (1*H*-benzo[*d*]imidazol-2-yl), respectively, at its ortho, meta, and para position to form compounds **9a**–**c**, **10a**–**c**, and **11a**–**c**. Primary pharmacological test gave more favorable results for para substituted compounds, especially compounds with 5-nitro substituente at the benzimidazole moiety. In light of this finding, we tried to increase the flexibility of the molecule through extending the hinge between the benzimidazole ring and benzoylguanidine moiety by inserting a thiomethyl group there and at the para position of benzoylguanidine, thus formed compound, **31a**–**b**, **32a**–**b**, and **33**–**35**. Various sulfonyl groups which were present in many successful NHE inhibitors were also employed in compound, **32a**–**b**, **34**, and **35** (Table 1).

Synthetic routes of target compounds are depicted in Schemes 1 and 2. Cyanobenzoic acids **1a**–**c**, obtained by oxidation of corresponding cyanotoluene, were treated with $SOCl_2$ to offer benzoylchlorides **2a**–**c**, which were then cyclocondensed with 4- or 1N-substituted



Scheme 1. Reagents and conditions: (a) i—$KMnO_4$, KOH, H_2O; ii—20% HCl; (b) toluene, DMF, reflux; (c) 1,2-diaminobenzene or 4-nitro-1,2-diaminobenzene or 2-methylaminoaniline, anhyd acetone, 0–5 °C; (d) i—20% NaOH, reflux; ii—20% HCl; iii—anhyd C_2H_5OH, H_2SO_4, reflux; (e) i—guanidine, isopropanol, 80 °C; ii—satd HCl in absolute ethanol; (f) CH_3I, KOH, anhyd acetone, reflux.



Scheme 2. Reagents and conditions: (a) $ClSO_3H$, 95 °C, 4 h; (b) NH_4OH, 50 °C,1.5 h; (c) anhyd C_2H_5OH, H_2SO_4, reflux; (d) i—Na_2SO_3, NaOH, H_2O; ii—H_2SO_4; (e) CH_3I, DMA, NaOH; (f) morpholine, 50 °C, 2 h; (g) i—Br_2, $AgNO_3$, HNO_3, acetic acid, ii—Na_2CO_3; iii—HCl; (h) Br_2, chlorobenzene, hv; (i) i—2-mercapto-1H-benzimidazole or 2-mercapto-5-nitro-1*H*-benzimidazole, NaOH, PEG-600, sealed system, 120 °C, ii—anhyd C_2H_5OH, H_2SO_4, reflux; (j) NBS, benzoyl peroxide, anhyd CCl_4, hv; (k) 2-mercapto-1*H*-benzimidazole or 2-mercapto-5-nitro-1H-benzimidazole, NaOH, PEG-600, sealed system, 120 °C; (l) i—guanidine, isopropanol, 80 °C; ii—HCl.

1,2-diaminobenzene to give benzimidazolyl phenylnitriles **3a**–**c**, **4a**–**c**, and **5a**–**c**. Subsequent hydrolysis and esterification converted the phenylnitriles to corresponding ethyl benzoates. One exception is intermediate **8a**, which was prepared by N-methylation of **6a** with CH_3I in the presence of KOH in anhydrous acetone. Target compounds **9a**–**c**, **10a**–**c**, and **11a**–**c** were obtained by treatment of the corresponding ethyl benzoates with excessive guanidine in absolute isopropanol, followed by hydrochlorination with gaseous HCl. The salts thus formed were further purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, 7:1).

Synthesis of compounds **31a**–**b**, **32a**–**b**, and **33**–**35** is illustrated in Scheme 2. Introduction of a chlorosulfonyl or bromo group into the 3-position of 4-methyl benzoic acid resulted in **12** and **19**. Further alteration of the chlorosulfonyl group of **12** obtained **13**, **15**, and **17**. Then the acids **13**, **17**, and **19** were esterified to give their ethyl esters **14**, **18**, and **20**, while **16** was obtained by CH_3I methylation of both the carboxyl and the sulfinic acid group of **15**. These esters were bromized with NBS to afford corresponding benzyl bromines **23**–**26**, which were subsequently treated with 2-mercapto-(5-nitro)-1*H*-benzimidazole to give intermediates **27a**–**b** and **28**–**30**. Intermediates **22a**–**b** came from the nucleophilic substitution of *p*-carboxylbenzylbromide with 2-mercapto- (5-nitro)-1H-benzimidazole followed by esterification. The synthesis of 2-mercapto-(5-nitro)-1H-benzimidazoles referred to literature method.[6] The final benzoylguanidine products were obtained similarly with the above compounds **9a**–**c**, **10a**–**c**, and **11a**–**c**.

NHE1 inhibitory activity of 16 target compounds and cariporide was evaluated in rat platelet swelling assay (PSA), in which the swelling of rat platelets was induced by a propionate buffer (pH 6.7). The experiment was performed as described by Rosskopf et al.,[7] with minor modifications. The half-maximal inhibitory concentration (IC_{50}) value of the tested compounds was obtained from the linear part of the relationship between the log concentration and NHE activity using linear regression analysis.

All the target compounds were also tested for the protection against myocardial ischemic-reperfusion injury in SD rat hearts. The pharmacological model was created by ligating the left anterior descending coronary artery (LAD) for 1 h and then releasing the ligature for 2 h, followed by immediate euthanization and heart-excision. The hearts were stained by Evans blue injection and TTC immersion respectively to measure the area at risk and infarct area. Infarct size was expressed as the ratio of infarct area to area at risk. Besides, blood sample was taken and blood serum was prepared to undergo creatine kinase (CK) activity determination.

The PSA results showed that most of the tested compounds inhibited rat platelet NHE-1 in a concentration-dependent manner. Compounds **10a**–**c**, **32b**, **34**, **35** were superior to cariporide in NHE inhibition. The IC_{50} values of **10b** and **10c** were 1.42 and 4.05 nM, respectively, making them 45 and 15 times more potent than cariporide, the IC_{50} of which was 65 nM in the same test.

In the in vivo study, 15 of the 16 tested compounds (except for **31a**) had cardioprotective activity against IR injury at various degree ($p < 0.05$), among which the infarct size and the CK level of **9a**, **10a**, **10c**, **11a**, **32a**–**b**, **34**, **35** were significantly lower than those of the control group ($p < 0.01$). The infarct size of **11a**, **32b**, and **35**, the CK level of **32b** and **35** were comparable with those of cariporide. Both the parameters of **10a** and **34** consistently implied a more favorable activity than cariporide.

In concern with the structure–activity relationship, it is obvious that the *para*-benzimidazolyl substituted benzoylguanidines were more potent than the ortho- and meta-substituted ones, the order being para > ortho > -meta. As mentioned earlier, that is the reason we chose the para-substituted compounds for further chemical investigation. Also mentioned earlier is that 5-nitro benzimidazolyl benzoylguanidines were more active than their non-substituted counterparts. This is reconfirmed by compound **31a** versus **31b** and **32a** versus **32b**. Among compounds **31a**–**b**, **32a**–**b**, and **33**–**35**, the 3-methylsulfonyl (**34**), 3-morpholinylsulfonyl (**35**), and 3-aminosulfonyl (**32a**–**b**) benzoylguanidines were better than non-substituted ones (**31a**–**b**), whereas the 3-bromo substituted analog (**33**) represented even less activity. By comparing compound **9a** versus **31a**, **10a** versus **31b**, the prolongation of the hinge seemed to diminish the activity. Thus, we may infer that the remarkably good results of compound **34** and other compounds possessing a sulfonyl group may come mainly from the various sulfonyl substituents.

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