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A novel class of sodium/calcium exchanger inhibitors: Design, synthesis, and structure–activity relationships of 4-phenyl-3-(piperidin-4-yl)-3,4-dihydro-2(1*H*)-quinazolinone derivatives

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Abstract—Design, synthesis, and structure–activity relationships of 3,4-dihydro-2(1*H*)-quinazolinone derivatives as sodium/calcium (Na^+/Ca^{2+}) exchanger inhibitors are described. In these studies, optimization of the substituents at the 3-position of this series of compounds was carried out and dramatic effects of the substituent on the activities were observed. Based on these SAR studies, a highly potent inhibitor of the Na^+/Ca^{2+} exchanger, which showed single-digit-nanomolar activity, was discovered.

The sodium/calcium (Na^+/Ca^{2+}) exchanger is a transmembrane carrier that plays a critical role in maintaining calcium balance in cardiac myocytes. It is well known that reperfusion injury is associated with a large increase in intracellular Ca^{2+} content.[1,2] Therefore, inhibition of this Ca^{2+} overload is considered to be one of the pharmacological interventions to prevent reperfusion injury. It is proposed that the Na^+/Ca^{2+} exchanger plays an important role when Ca^{2+} overload occurs.[3,4] We have been interested in developing novel inhibitors of the Na^+/Ca^{2+} exchanger with high potency and selectivity, because we considered that inhibitors of the Na^+/Ca^{2+} exchanger would inhibit Ca^{2+} overload during reperfusion and thus prevent reperfusion injury.

A number of compounds, including peptidic and non-peptidic, have been reported as Na^+/Ca^{2+} inhibitors (Fig. 1). As a peptidic inhibitor, Val-Met-Arg-Phe-NH_2 (**1**) with an IC_{50} value of 1.5 μM has been reported, which is a non-selective inhibitor.[5] As a non-peptidic inhibitor, aroylguanidine derivative (**2**) with an IC_{50} value of 3.4 μM has been reported which is a modified amiloride derivative like dimethylamiloride (**3**).[6] Furthermore, KB-R7943 (**4**),[7] SEA0400 (**5**),[8] benzyloxyphenyl derivative (**6**)[9], and SN-6 (**7**)[10] have been reported.

We have already reported design, synthesis, and structure–activity relationships of 3,4-dihydro-2(1*H*)-quinazolinone derivatives with the inhibitory activities of the Na^+/Ca^{2+} exchanger.[11] In the previous article, we disclosed that these studies based on lead compound **8** with a moderate potent inhibitory activity led to the identification of a structurally novel and highly potent inhibitor against the Na^+/Ca^{2+} exchanger **9** (SM-15811), which directly inhibited the Na^+-dependent Ca^{2+} influx via the Na^+/Ca^{2+} exchanger in cardiomyocytes with high potency and exerted the protective effect against myocardial ischemic reperfusion injury (Fig. 2). We further performed the optimization of this compound to find more highly potent inhibitors of the Na^+/Ca^{2+} exchanger. In this study, we found the dramatic effect of substituents of 4-phenyl-3-(piperidin-4-yl)-3,4-dihydro-2(1*H*)-quinazolinone derivatives and that based on this study highly potent 4-phenyl-3-(piperidin-4-yl)-3,4-dihydro-2(1*H*)-quinazolinone derivative was identified. Herein, we wish to report interesting results.

Synthesis of a series of 4-phenyl-3-(piperidin-4-yl)-3,4-dihydro-2(1*H*)-quinazolinone derivatives is illustrated in Scheme 1. Treatment of trichloroacetylamide **10** with 4-amino-1-benzylpiperidine in DMSO, followed by

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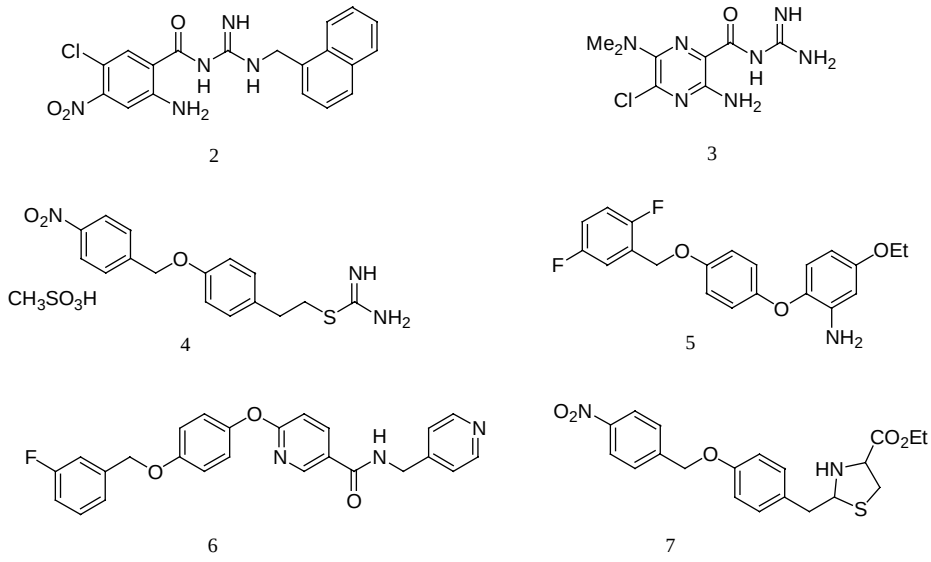


Figure 1. Chemical structures of non-peptidic inhibitor of the Na^+/Ca^{2+} exchanger: (**2**) aroylguanidine derivative; (**3**) dimethylamiloride; (**4**) KB-R7943; (**5**) SEA0400; (**6**) benzyloxyphenyl derivative; (**7**) SN-6.

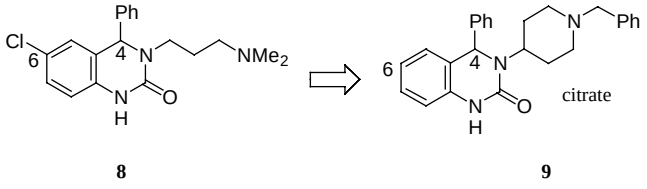


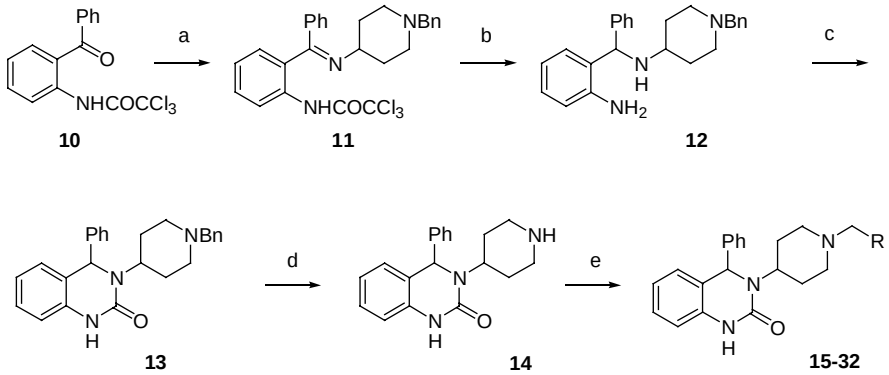
Figure 2. Chemical structures of 3,4-dihydro-2(1*H*)-quinazolinone derivatives **8** and **9**.

reduction of imine and removal of the trichloroacetyl **11** with $NaBH_4$, gave diamine **12**. Treatment of diamine **12** with 1,1′-carbonyldiimidazole led to cyclization to afford 3-[4-(1-benzyl)piperidinyl]-2(1*H*)-quinazolinone **13**. Debenzylation of **13** gave **14**,[11] which was subsequently reductively alkylated to afford **15**–**32**.

The inhibitory activity of test compounds on the Na^+/Ca^{2+} exchange was measured by the inhibition of Na^+- and K^+-free contracture in isolated guinea pig left atria, performed as described previously.[12] The inhibitory activities were calculated as IC_{30} values. In this system, Val-Met-Arg-Phe-NH_2 (**1**) and dimethylamiloride (**3**), which are known inhibitors of the Na^+/Ca^{2+} exchanger, showed inhibitory activities with IC_{30} values of 10 μM and 30 μM, respectively.

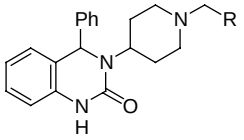
Table 1 summarizes our results in exploring heteroaryl groups like pyridyl, thienyl, or furyl as replacements for the phenyl group. 2-, 3-, or 4-pyridyl methyl (**15**–**17**) were almost 10–30 times weaker than **9**. 2- or 3-Thienyl methyl (**18**, **19**) were almost 2 orders weaker than **9**. Interestingly, although 2-furyl methyl **20** reduced activity markedly, 3-furyl methyl **21** was almost equipotent as **9**.

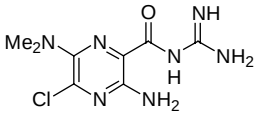
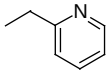
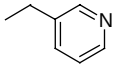
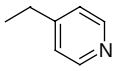
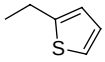
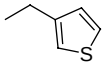
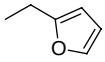
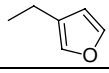
The effect of substituents on the phenyl ring of compound **9** was then investigated. Table 2 summarizes the results of this investigation. At first, both methoxy groups, which are electron-donating groups, and chlorine atoms, which are electron-withdrawing groups, were introduced at each position of the phenyl ring. Introduction of chlorine atoms at the 2-, 3-, or 4-positions (**22**–**24**) reduced the activity dramatically. On the other hand, concerning the methoxy groups, introduction at the 4-position **27** resulted in decrease in activity and introduction at the 2-position **25** resulted in a complete loss of activity. Introduction at the 3-position **26**,



Scheme 1. Reagents: (a) 4-amino-1-benzyl piperidine, DMSO; (b) $NaBH_4$, EtOH; (c) 1,1′-carbonyldiimidazole, THF, reflux; (d) HCO_2NH_4, Pd/C, MeOH, reflux; (e) R-CHO, $NaBH_3CN$, HCl, MeOH.

Table 1. Inhibitory activity of 3,4-dihydro-2(1*H*)-quinazolinones against the Na^+/Ca^{2+} exchanger



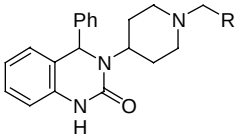
Compound [a]	RCH_2-	IC_{30} (μM)[b]
1	Val-Met-Arg-Phe-NH_2	10
3		30
9[c]	$PhCH_2$-	0.017
15[d]		0.39
16[d]		0.16
17		0.48
18[d]		1.7
19[d]		3.9
20[d]		8.3
21		0.017

[a] All the compounds tested were racemic.
[b] IC_{30} values are expressed as means from three to five preparations.
[c] Compound tested as the citrate.
[d] Compound tested as the hydrochloride.

however, enhanced the activity with an IC_{30} value of 0.0084 μM. Introduction of a methyl group **32** to see if steric effects would alter the activity reduced the activity, indicating that alkoxyl groups were important. These results including the fact that only 3-furyl methyl **21** showed strong activity among the heteroaryl groups discussed above suggest that there might be interaction of oxygen atom with amino acid(s). To investigate further the steric effects of alkoxyl groups, various kinds of alkoxyl groups were introduced. 3,5-Dimethoxy group **28** and 3,4,5-trimethoxy group **29** diminished the activity. One carbon extended substituent 3-ethoxy group **30** reduced the activity. 3-Methoxymethyl **31** lowered the activity. These results suggested that although introduction of an alkoxyl group at the 3-position on the phenyl ring is preferred, the size of the substituent is limited and only 3-methoxy group enhanced the activity.

In summary, we synthesized various types of compounds to replace a benzyl group with heteroarylalkyl and to introduce chlorine atom and alkoxyl groups as substituents on the phenyl group, to optimize the 3,4-dihydro-2(1*H*)-quinazolinone derivative **9**. (1) Concerning the replacement of the phenyl group with heteroaryl group like pyridyl, thienyl, and furyl groups, most of the heteroaryl groups reduced the activity. Only 3-furyl was as potent as phenyl group. (2) Concerning the introduction of substituents, both chlorine atoms which are electron-withdrawing groups and alkoxyl groups which are electron-donating groups were introduced to investigate the preferred substituent systematically. These studies led to the finding of the highly potent Na^+/Ca^{2+} exchanger inhibitor in which methoxy group was substituted at the 3-position of the phenyl group. (3) According to results of (1) and (2), there is a possibility that the oxygen atom might interact with amino acid(s). (4) Based on these results, various types of alkoxyl groups were introduced; however, all introduced substituents reduced the activities, suggesting that the size of the substituent is limited. (5) In the course of this study, dramatic effects of the substituents on the inhibitory activity were observed. These compounds showed great variations in activities, ranging from compounds with no activities even at a concentration of up to 10 μM to a compound with an activity in the nanomolar range. Thus, we discovered the structurally novel 3,4-dihydro-2(1*H*)-quinazolinone derivative as the highly potent inhibitor of the Na^+/Ca^{2+} exchanger (**26**) with an IC_{30} value of 0.0084 μM, which is a single-digit-nanomolar activity. Compound (**26**) would be a useful tool for studying the Na^+/Ca^{2+} exchanger inhibitor as well as indicating the potential for generating a novel class of highly potent Na^+/Ca^{2+} exchanger inhibitors.

Table 2. Inhibitory activity of 3,4-dihydro-2(1*H*)-quinazolinones against the Na^+/Ca^{2+} exchanger



Compound[a]	RCH_2-	IC_{30} (μM)[b]
9[c]	$PhCH_2$-	0.017
22	2-Cl-$PhCH_2$-	9.0
23	3-Cl-$PhCH_2$-	0.54
24	4-Cl-$PhCH_2$-	0.62
25	2-MeO-$PhCH_2$-	>10
26	3-MeO-$PhCH_2$-	0.0084
27	4-MeO-$PhCH_2$-	0.25
28[d]	3,5-Di-MeO-$PhCH_2$-	>10
29[d]	3,4,5-Tri-MeO-$PhCH_2$-	>10
30[d]	3-EtO-$PhCH_2$-	0.58
31[d]	3-$MeOCH_2$-$PhCH_2$-	0.49
32	3-Me-$PhCH_2$-	0.56

[a] All the compounds tested were racemic.
[b] IC_{30} values are expressed as means from three to five preparations.
[c] Compound tested as the citrate.
[d] Compound tested as the hydrochloride.

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