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Discovery of a Novel Potent Na^+/Ca^{2+} Exchanger Inhibitor: Design, Synthesis and Structure–Activity Relationships of 3,4-Dihydro-2(1*H*)-quinazolinone Derivatives

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Abstract—Design, synthesis and structure–activity relationships for 3,4-dihydro-2(1*H*)-quinazolinone derivatives with the inhibitory activities of the Na^+/Ca^{2+} exchanger are discussed. These studies based on lead compound **1a** lead to the discovery of a structurally novel and highly potent inhibitor against the Na^+/Ca^{2+} exchanger **4f** (**SM–15811**), which directly inhibited the Na^+-dependent Ca^{2+} influx via the Na^+/Ca^{2+} exchanger in cardiomyocytes with a high potency.

Introduction

Coronary reperfusion by thrombolytic therapy or percutaneous transluminal angioplasty has emerged as a fundamental strategy in the management of ischemic heart disease. Nonetheless, it has been suggested that sometimes early restitution of blood flow after a period of hypoxia results in the deteriorous effects called reperfusion injury.[1] Intracellular Ca^{2+} overload via activation of the Na^+/Ca^{2+} exchanger after ischemic reperfusion has been indicated as a potential cause of this, which induces post-ischemic cardiac injury.[2–4] Thus, we anticipated that Na^+/Ca^{2+} exchanger inhibitors could become a new approach for the treatment of ischemic reperfusion injury. Our research therefore focused upon the discovery of an inhibitor of the Na^+/Ca^{2+} exchanger with high potency and selectivity.

To date, although a number of inhibitors against the Na^+/Ca^{2+} exchanger have been reported, most are weak and non-selective inhibitors.[3] Recently, Val-Met-Arg-Met-NH_2 with IC_{50} value of 1.5 μM has been reported, which is a peptidic inhibitor.[5] As a non-peptidic inhibitor, an aroylguanidine derivative with IC_{50} value of 3.4 μM has been reported which is a modified amiloride derivative like dimethylamiloride.[6] Furthermore, KB-R7943[7] and SEA0400[8] have also been reported as non-peptidic inhibitors. SEA0400 shows more than 80% inhibition at 1 μM against the Na^+/Ca^{2+} exchanger, which is a 10-fold higher potency than that of KB-R7943 (Fig. 1).

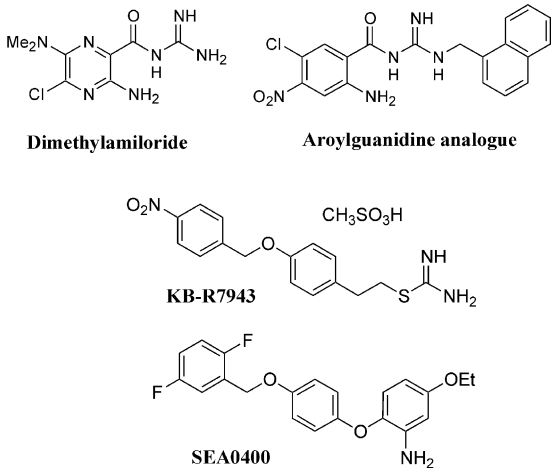

Figure 1. Chemical structures of non-peptidic inhibitor of Na^+/Ca^{2+} exchanger.

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In our research, we identified that 3,4-dihydro-2(1*H*)-quinazolinone derivative **1a** in our chemical library, through a random screening, had a moderately potent inhibitory activity against the Na^+/Ca^{2+} exchanger. Since **1a** has a non-peptidic, structurally novel, and quite different skeleton from other inhibitors, we therefore used **1a** as a lead compound and synthesized derivatives to find a more potent compound as well as to evaluate the structure–activity relationships (SARs) in three parts systemically (boxed regions in Fig. 2). These studies lead to the discovery of a structurally novel and highly potent inhibitor against the Na^+/Ca^{2+} exchanger **4f** (**SM-15811**). Herein, we wish to report these studies.

Chemistry

Compounds **1a–l**, **1n–q** were prepared using Yamamoto's procedure.[9,10] Trichloroacetylation of 2-aminobenzophenone derivatives **5a** and **5b** with trichloroacetyl chloride, followed by treatment with primary amines in DMSO gave directly cyclized products **7**, with accompanying rearrangement of the trichloromethyl group. Removal of trichloromethyl group leading to the 3,4-dihydro-2(1*H*)-quinazolinone **1a–l**, **1n–q** was effected by $NaBH_4$ in DMF (Scheme 1).

Primary amine **1m** was prepared by debenzylation of **1l** with ammonium formate in the presence of Pd/C in MeOH (Scheme 2). **2** was prepared from **1a** by reaction with NaH followed by iodomethane (Scheme 3). Thiocarbonyl analogue **3** was prepared by treating **1b** with P_2S_5 in xylene (Scheme 4).

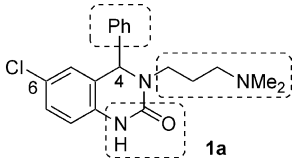
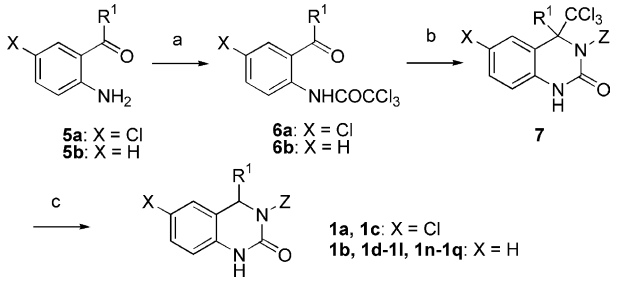
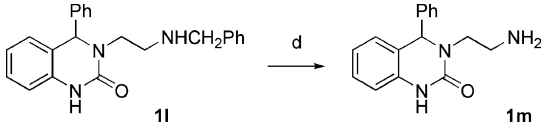


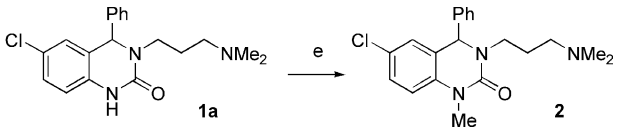
Figure 2. Chemical structure of 3,4-dihydro-2(1*H*)-quinazolinone derivative **1a**.



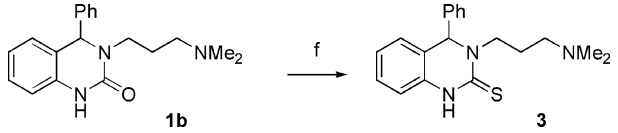
Scheme 1. Reagents and conditions: (a) CCl_3COCl, Et_3N, CH_2Cl_2, 71–99%; (b) *Z*-NH_2, DMSO, 12–94%; (c) $NaBH_4$, DMF, 11–96%.



Scheme 2. Reagents and conditions: (d) HCO_2NH_4, Pd/C, MeOH, reflux, 84%.



Scheme 3. Reagents and conditions: (e) NaH, MeI, DMF, 94%.

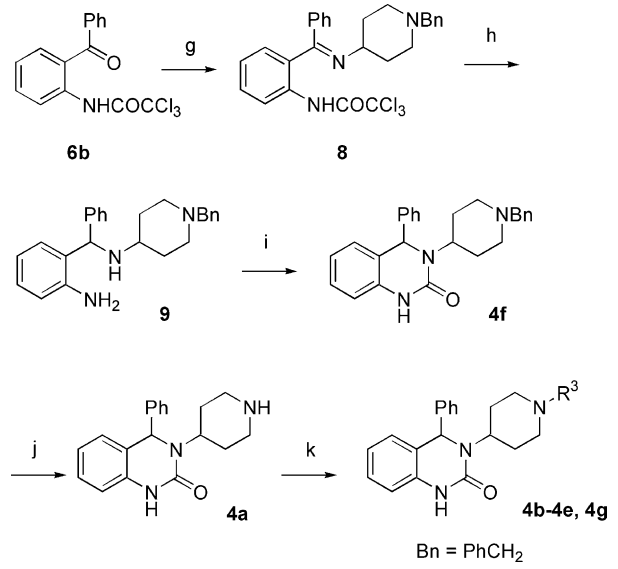


Scheme 4. Reagents and conditions: (f) P_2S_5, xylene, reflux, 36%.

In order to synthesize 4-phenyl-3-(4-piperidinyl)-3,4-dihydro-2(1*H*)- quinazolinone derivatives having cyclic amines **4a–g**, we developed a more versatile procedure. Treatment of trichloroacetylamide **6b** with 4-amino-1-benzylpiperidine in DMSO gave imine **8**. Reduction of the imine and removal of the trichloroacetyl moiety with $NaBH_4$ gave diamine **9**. Treatment of diamine **9** with 1,1′-carbonyldiimidazole lead to cyclization to afford 3-[4-(1-benzyl)piperidinyl]-4-phenyl-3,4-dihydro-2(1*H*)-quinazolinone **4f**. Debenzylation of **4f** gave **4a**, which was subsequently alkylated or reductively alkylated to afford **4b–e, 4g** (Scheme 5).[11]

Pharmacological Results and Discussion

In cardiomyocytes, fura 2 fluorescene ratio (an index of $[Ca^{2+}]_i$) increases by Na^+-dependent Ca^{2+} influx via the Na^+/Ca^{2+} exchanger after Na^+-free treatment. The lead compound **1a** concentration-dependently attenuated the



Scheme 5. Reagents and conditions: (g) 4-amino-1-benzyl piperidine, DMSO, 66%; (h) $NaBH_4$, MeOH, 86%; (i) 1,1′-carbonyldiimidazole, THF, reflux, 81%; (j) HCO_2NH_4, Pd/C, MeOH, reflux, 84%; (k) aldehyde, $NaBH_3CN$, HCl, MeOH or R^3-X, K_2CO_3, DMF (X = Br, I).

increase in the Na^+-free-induced fura 2 fluorescene ratio (Fig. 3).[12]

It is well known that an increase in the $[Ca^{2+}]_i$ is correlated with the development of cardiac contractile force. Therefore, we evaluated the inhibitory activity against Na^+- and K^+-free contracture after 30 min of K^+ free incubation in isolated left atria from guinea pigs.[13] The inhibitory activities were calculated as IC_{30} values.

The lead compound **1a** showed inhibitory activity with IC_{30} value of 0.46 μM. We first examined the 3,4-dihydro-2(1*H*)-quinazolinone skeleton of **1a** (Table 1). Removal of the chlorine atom at the 6-position did not affect the activity (**1b** vs **1a**). But introduction of a methyl group at the 1-position (**2** vs **1a**) reduced the activity markedly as did conversion of the cyclic urea to cyclic thiourea (**3** vs **1b**). Therefore these results suggested that the 3,4-dihydro-2(1*H*)-quinazolinone skeleton was essential for activity.

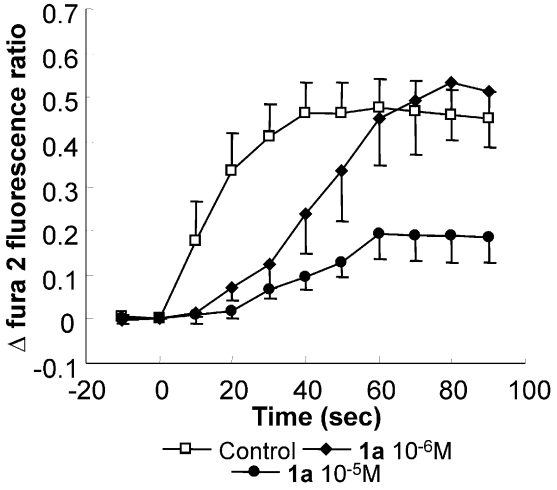
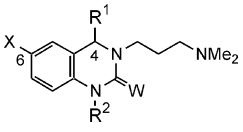


Figure 3. Effect of **1a** on the Na^+/Ca^{2+} exchange activity in rat cardiomyocytes. The Na^+/Ca^{2+} exchange activity was estimated as the increase in fura 2 fluorescene ratio induced by exposing to the Na^+-free HEPES-based buffer using a Ca^{2+} sensitive fluorescent indicator fura 2. Each point represents the mean ± SE of five experiments.

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



Compd[a]	X	R^1	W	R^2	IC_{30} (μM)
Dimethylamiloride					30
Val-Met-Arg-Phe-NH_2					
1a[b]	Cl	Ph	O	H	10
1b	H	Ph	O	H	0.46
2	Cl	Ph	O	Me	0.35
3[c]	H	Ph	S	H	>10
1c	Cl	2-Furyl	O	H	3.8
1d	H	2-Thienyl	O	H	0.69
1e	H	2-Pyridyl	O	H	0.72
1f	H	Me	O	H	0.51
1g	H	Cyclohexy	O	H	5.3
1h	H	Benzyl	O	H	0.57

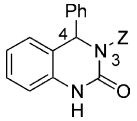
[a]All the compounds tested were racemic.
[b]Compound tested as the citrate.
[c]Compound tested as the hydrochloride.

Next we replaced the phenyl group at the 4-position (Table 1). Replacement of the phenyl group with heteroaryl groups such as 2-furyl **1c**, 2-thienyl **1d** and 2-pyridyl **1e**, which are comparable to the phenyl ring in bulkiness, reduced the activity slightly. Introduction of a methyl group **1f** resulted in a drop in inhibitory activity by almost 10-fold, although the cyclohexyl **1g** had only a slightly reduced activity. A larger group such as benzyl **1h** abolished the activity. These results suggested that the hydrophobic pocket at the 4-position was not so large. The phenyl group was the most suitable for high activity.

For further exploration of the SARs, modifications of side chain at the 3-position were carried out (Table 2). Replacement of a nitrogen atom with a carbon atom (**1i** vs **1b**) or oxygen atom (**1j** vs **1o**) resulted in complete loss in activity, showing that the nitrogen atom is essential. Furthermore, the amide derivative also had diminished activity (**1k** vs **1o**). Hence a basic nitrogen atom was essential for activity. Actually, the calculated pKa values of the amino group of the side chain in **1k** and **1o** were −0.58 and 10.23, respectively, which means that 3,4-dihydro-2(1*H*)-quinazolinones show activities in protonated form.[14]

The secondary amino group showed stronger activity than the primary amino group, but the tertiary amino group showed the strongest activity (**1o** vs **1n** and **1m**), suggesting that the tertiary amino group was preferred. In terms of the length between nitrogen atom in the side chain and nitrogen atom at the 3-position of the 3,4-dihydro-2(1*H*)-quinazolinone, two carbon atoms **1o** and three carbon atoms **1p** showed strong activity, but four carbon atoms **1q** reduced the activity, suggesting either two or three carbon atoms were preferred. These results suggested that amine at the 3-position was very important and tertiary amine with either a length of two or three carbons were preferred.

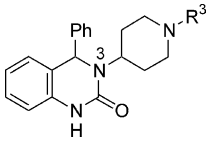
Table 2. Inhibitory activity of 3,4-dihydro-2(1*H*)-quinazolinones against the Na^+/Ca^{2+} exchanger[a]



Compd	Z	IC_{30} (μM)
1i	$(CH_2)_3CH(Me)_2$	>10
1j	$(CH_2)_2OEt$	>10
1k	CH_2CONEt_2	>10
1m	$(CH_2)_2NH_2$	>10
1n	$(CH_2)_2NHEt$	0.58
1o	$(CH_2)_2NEt_2$	0.069
1p	$(CH_2)_3NEt_2$	0.04
1q	$(CH_2)_4NEt_2$	1.9

[a]All the compounds tested were racemic.

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



Compd[a]	R^3	IC_{30} (μM)
4a	H	1.1
4b[d]	Me	0.087
4c[c,d]	Et	0.053
4d[c,e]	*n*Pr	0.093
4e[c,f]	Cyclohexylmethyl	4.0
SM-15811 (**4f**)[b]	$PhCH_2$–	0.017
4g[c,f]	$Ph(CH_2)_2$–	3.6

[a]All the compounds tested were racemic.
[b]Compound tested as the citrate.
[c]Compound tested as the hydrochloride.
[d]Corresponding alkyl iodes were used for *N*-alkylation.
[e]*n*PrBr was used for *N*-alkylation.
[f]Corresponsing aldehydes were used for *N*-alkylation.

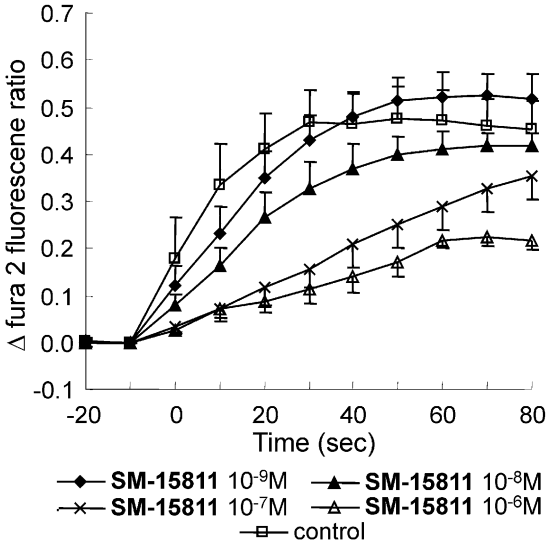


Figure 4. Effect of **SM–15811** (**4f**) on the Na^+/Ca^{2+} exchange activity in rat cardiomyocytes. The Na^+/Ca^{2+} exchange activity was estimated as the increase in fura 2 fluorescene ratio induced by exposing to the Na^+-free HEPES-based buffer using a Ca^{2+} sensitive fluorescent indicator fura 2. Each point represents the mean±SE of five experiments.

Based on these results, we therefore focused our attention on the introduction of a cyclic amine into the side chain at the 3-position of this derivative so as to increase the inhibitory activity. Table 3 summarizes our results in introducing the 4-piperidinyl as a cyclic amine. We synthesized the unsubstituted **4a** and various substituted piperidine derivatives **4b–4g**. Among them, nonsubstituted **4a** was weaker than **4b**. But introduction of chain alkyl groups enhanced the activities (**4b–4d**). Further introduction of a bulky alkyl group such as cyclohexylmethyl **4e** reduced the inhibitory activity. In contrast to cyclohexylmethyl **4e**, the benzyl group **4f** (**SM-15811**) showed strong activity, with IC_{30} value of 0.017 μM, suggesting in this case there may be a π-π interaction between the inhibitor and the binding site. However, phenylethyl group **4g** decreased the activity drastically, suggesting location of the phenyl ring was important for activity (**SM-15811** vs **2g**).

We found that **SM-15811** had strong inhibitory activity against Na^+- and K^+-free contracture after 30 min of K^+ free incubation in isolated left atria from guinea pigs. Moreover, we evaluated **SM-15811** by fura 2 fluorescene ratio [an index of $(Ca^{2+})_i$] increased by Na^+-dependent Ca^{2+} influx via Na^+/Ca^{2+} exchanger after Na^+-free treatment in cardiomyocytes. Figure 4 shows the results. **SM-15811** concentration-dependently attenuated the increase in Na^+-free induced fura 2 fluorescene ratio, indicating **SM-15811** discovered by our systemic modification of 3,4-dihydro-2(1*H*)-quinazolinone derivative directly inhibited the Na^+-dependent Ca^{2+} influx via Na^+/Ca^{2+} exchanger after Na^+-free treatment in cardiomyocytes. The inhibitory activity of **SM-15811** was almost 2 orders stronger than the lead compound **1a** (Figs. 3 vs 4). These results suggested that **SM-15811** found by our systemic SARs studies showed strong inhibitory activity against the Na^+/Ca^{2+} exchanger.

Conclusion

In summary, we found that 3,4-dihydro-2(1*H*)-quinazolinone derivative **1a** in our library concentration-dependently attenuated the increase in Na^+-free-induced fura 2 fluorescene ratio and exhibited inhibitory activity against the Na^+/Ca^{2+} exchanger with IC_{30} value of 0.46 μM. We disclosed the SARs of lead compound **1a** by systemic modification in three parts. The results of the SARs studies are as follows: (1) Removal of chlorine atom at the 6-position of 3,4-dihydro-2(1*H*)-quinazolinone did not affect the activity. (2) 3,4-Dihydro-2(1*H*)-quinazolinone skeleton was essential for activity. (3) Phenyl group at the 4-position of 3,4-dihydro-2(1*H*)-quinazolinone was preferred. (4) Amine at the 3-position of 3,4-dihydro-2(1*H*)-quinazolinone was very important and tertiary amine with either 2 or 3 carbon chain length were preferred. (5) Introduction of *N*-alkyl-4-piperidine at the 3-position of 3,4-dihydro-2(1*H*)-quinazolinone further enhanced the activity. (6) Introduction of a benzyl group that interacted in a π–π manner at the 1-position on the 4-piperidine improved the activity and gave the highly potent inhibitor of Na^+/Ca^{2+} exchanger **SM-15811** with IC_{30} value of 0.017 μM. (7) The location of phenyl ring is important for activity.

SM-15811 discovered by this SARs study concentration-dependently attenuated the increase in Na^+-free induced fura 2 fluorescene ratio, indicating **SM-15811** directly inhibited the Na^+-dependent Ca^{2+} influx via Na^+/Ca^{2+} exchanger in cardiomyocytes, whose activity was almost 2 orders stronger than the lead compound **1a**.

Thus we discovered the structually novel 3,4-dihydro-2(1*H*)-quinazolinone derivative as the highly potent inhibitor of Na^+/Ca^{2+} exchanger. In particular, **SM-15811** could have therapeutic potential in the treatment

of ischemic reperfusion injury as well as a powerful tool for further studies on the role of the Na^+/Ca^{2+} exchanger in the heart. Further pharmacological evaluation is currently in progress in our laboratory.

Acknowledgements

We wish to thank Ms. Hitomi Sakanaka for evaluating the inhibitory activity of compounds against the Na^+/Ca^{2+} exchanger. We also wish to thank Ms. Mikiko Ohmori for evaluating the effect of **1a** and **SM-15811** on the Na^+/Ca^{2+} exchange activity in cultured cardiomyocytes.

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10. The starting material of **1c** was synthesized according to ref 15. The starting material of **1d**, **1g**, and **1h** were synthesized according to ref 16. The starting material of **1e** was synthesized according to ref 17. The starting materials (**5a** and **5b**) of other 3,4-dihydro-2(1*H*)-quinazolinone derivatives **1** and **4** were commercially available.
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