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2-Aminoethyl diphenylborinate (2-APB) analogues: Regulation of Ca^{2+} signaling

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ABSTRACT

In order to obtain compounds with modified 2-APB activities, we synthesized number of 2-APB analogues and analyzed their inhibitory activities for SOCE. The IC_{50} of 2-APB for SOCE inhibition is 3 μM while IC_{50} of some of our 2-APB analogues range 0.1–10 μM. The adducts of amino acids with diphenyl borinic acid have strong inhibitory activities. By using these compounds, we will be able to regulate intracellular Ca^{2+} concentration and consequent cellular processes more efficiently than with 2-APB.

1. Introduction

Extracellular signal molecules attach to the plasmatic membrane where they are recognized by cell surface receptors. Upon binding of the ligand to the appropriate receptor, activation of G protein activates in turn phospholipase C. Active phospholipase C hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) giving rise to two products: 1,2-diacylglycerol and inositol 1,4,5-trisphosphate (IP_3). IP_3 stimulates the release of Ca^{2+} from the intracellular stores in the endoplasmic reticulum through IP_3 receptor while regulating a wide range of cellular processes [1–20].

In 1997, we identified 2-aminoethyl diphenylborinate (2-APB) as being an IP_3 receptor inhibitor and regulate IP_3-induced calcium release [21,22]. This discovery rose a substantial interest and had a great impact as it gained more than 600 citations and more than 1000 studies on 2-APB (examples are references [23–37]) have been published so far. This was supported by supply of 2-APB by Sigma–Aldrich as membrane-permeable modulator of intracellular IP_3-induced cellular calcium release. In this study, we aimed to generate better modulator of calcium signaling than 2-APB.

We synthesized several 2-APB analogues and measured their inhibitory activities on Store-Operated Calcium Entry (SOCE). We found that inhibitory effect of bis boron compound DBP 162-AE and DBP 163-AE were much more effective than 2-APB [38–40]. Previously, we studied bis boron compounds in more detail [39,40]. We extended these studies and synthesized 493 2-APB analogues [38–43] increasing the number of borons, changing diphenyl to diaryl, mono-aryl mono-aliphatic, dialiphatic compounds, substitutions of aminoethyl to amino acid derivative as well as aminoethanole to aminoethylthiol and studied the structure/activity correlation.

Here we analyzed SOCE inhibitory activities of our mono-boron compounds collection.

We believe that if we would regulate intracellular Ca^{2+} concentration and associated cellular processes by boron compounds with various Ca^{2+} related activities, we could therapeutically intervene in many diseases, such as heart diseases and Alzheimer's disease.

2. Materials and methods

2.1. 2-APB analogues

2-APB was first synthesized by Ronderstvent et al. [44] in 1954 from triphenylboranes and ethanol amine. Later, hydroxy diphenyl boran and ethanol amine methods for 2-APB synthesis were reported by Weidman and Zimmermann [45], Letsinger and Skoog [46], Povlock and Lippincott [47].

We have synthesized 493 2-APB analogues [38–43] using methods described by us [38–43] and others [44–55]. The structures, names and synthetic methods of the 493 compounds are in example 1–493 of Ref. [43]. The adducts of diphenyl borinic acid and

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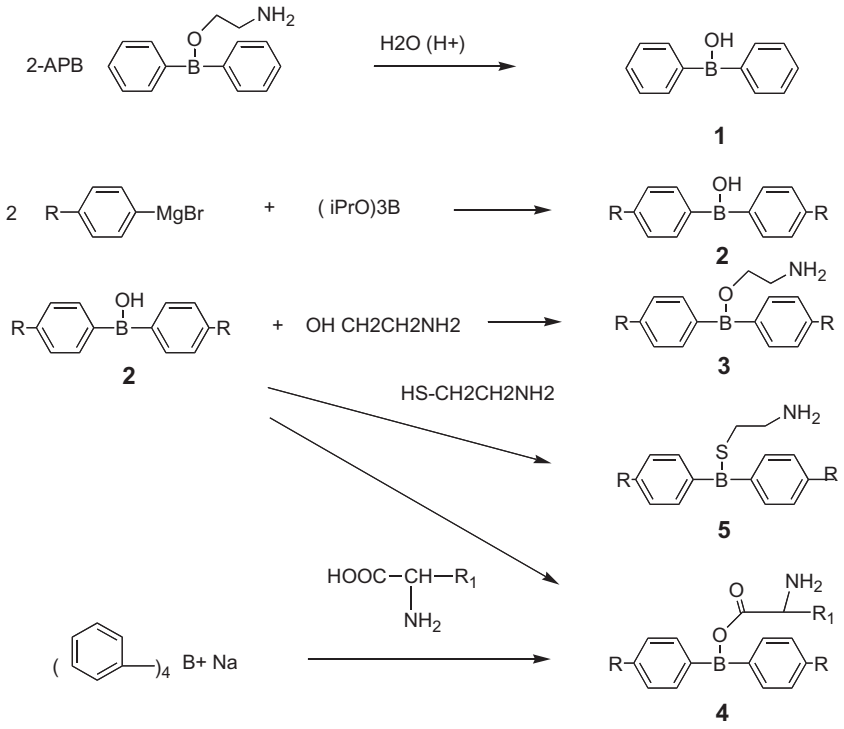
amino acid are well known [50,54,55] and we could obtain these compounds simply by heating in water at 80 °C for 2 h [43].

Hydrolysis of commercially available 2-APB afforded white crystalline diphenylborinic acid **1**. The reaction of aryl magnesium bromide and triisoproxy boron afforded diaryl borinic acid **2**. The reaction of boronic acid **1** or **2** with amino ethanol at room temperature for 6 h afforded 2-aminoethyl diaryl borinate **3**. The reaction of boronic acid **1** with amino acids at 80 °C for 2 h afforded diaryl (aminoacidenate N,O)borone **4**. We also employed another method to get **4** by incubation of sodium tetraphenyl borate and amino acids at 80 °C for 1 h in water. The reaction of boronic acid **1** or **2** with 2-aminoethyl thiol at 40 °C for 3 h afforded diaryl aminoethyl thioborane **5**.



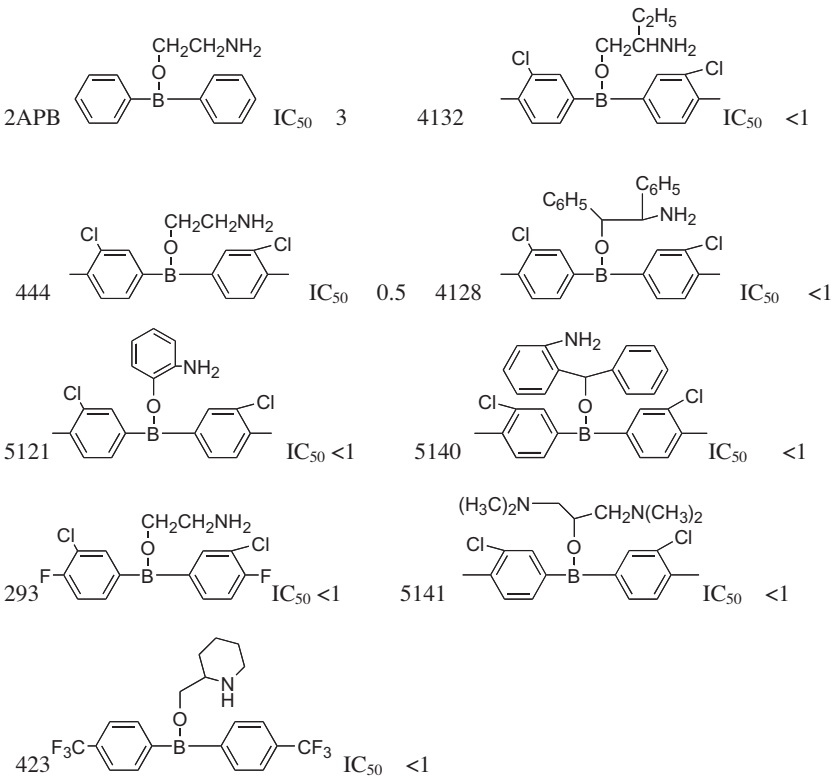
(a) Synthesis of diphenyl borinic acid **1**. 2-Aminoethyl diphenyl borinate (Sigma–Aldrich) 2.25 g was dissolved in 1 N hydrochloric acid 60 ml and stirred for 50 min. The solution was extracted with 30 ml and 20 ml of diethyl ether. The combined ether solution was washed twice with 10 ml water and once with 10 ml of brine. The ether layer was dried with sodium sulphate. Ether was evaporated to give 1.660 g of **1** as white crystalline solid.

(b) Synthesis of 911 Diphenyl(2,6-diaminohexanate-O,N)borane from. Diphenyl borinic acid (**1**) 49 mg (0.269 mmol) and L-lysine hydrochloride 49 mg (0.269 mmol) were stirred with heating in a mixture of ethanol (1.5 ml) and water (0.5 ml) at 80 °C. 911 (44 mg) was obtained as white powder. Spectroscopic data for 911 Diphenyl(2,6-diaminohexanate-O,N)borane. ^{1}H NMR (DMSO-d_6, 500 MHz) 7.95(s,4H), 7.43(m,4H), 7.27(m,4H), 7.29(m,2H), 2.76(m,1H), 2.76(m,1H), 1.76(m,2H), 1.64(m,2H), 1.54(m,4H). ^{13}C NMR (DMSO-d_6 500 MHz) 174.612, 131.486, 131.401, 127.500, 127.452, 126.468, 126.341, 55.256, 38.727, 29.274, 26.709, 22.838.HREMS(ESI-Q-TOF) $(M+H)^+$ found 311.1927, theoretical for $C_{18}H_{23}BN_2O_2$ 311.1925.

(c) Synthesis of 919. Diphenyl(2,3-diaminopropionate O,N)borane A mixture of D-2,3-diaminopropionic acid monohydrochloride 59.4 mg, (0.423 mmol), diphenylborinic acid 79 mg (0.423 mmol), 1 N NaOH aqueous solution 0.42 ml, ethanol 1.5 ml was heated at 80–90 °C for 2 h with stirring. After cooling, hexane 10 ml was added. 46 mg of 919 came out as white precipitate.

(d) Synthesis of 2040. Diphenyl(2,5-diaminopentanoate O,N) borane. A mixture of ornithine dihydrochloride 98 mg (0.478 mmol), diphenyl borinic acid 87 mg (0.488 mmol), 1 N NaOH solution, ethanol 1.5 ml was stirred at 90 °C overnight to obtain white solid substance. This substance was washed with hexane and 46 mg of 2040 was obtained.

(e) Synthesis of 8073. A mixture of diisopropylaminoethanethiol (from diisopropylaminoethanethiol monohydrochloride and NaOH) 29.2 mg (0.18 mmol), diphenyl borinic acid 32.2 mg (0.176 mmol), ethanol 0.5 ml was stirred at 40 °C for 7 h. After cooling, addition of ether and hexane gave 17.8 mg of 8073.



2.2. SOCE inhibitory activities measurement

The measurement method of SOCE inhibitory activities in CHO-K1 cells is identical to the reference which we previously reported. Shortly, CHO-K1 cells were plated in 96-well plate 2days before experiment and grown in DMEM containing 10% FBS. The cells were washed with BSS(+)[115 NaCl, 5.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 20 Hepes (pH 7.4) (in mM)], loaded Fura-2-AM for a hour, and washed again with BSS(-) which was substituted $CaCl_2$ with 0.5 mM. The Ca^{2+} imaging was performed by FDSS-3000 system (Hamamatsu Photonics, Japan). For measurement of SOCE inhibitory activities, the cells were added the drug of interest, 1 μM of thapsigargin for depleting ER calcium stores, and external Ca^{2+} (2 mM in final concentration) to induce SOCE. The peak height of the fluorescence ratio (F_{340nm}/F_{380nm}) was measured and estimated the SOCE inhibitory activity of each compound [38–40].

3. Results and discussion

We measured inhibitory activities of the 2-APB analogues in CHO cells for SOCE. The results are shown in Supplementary Table 1.

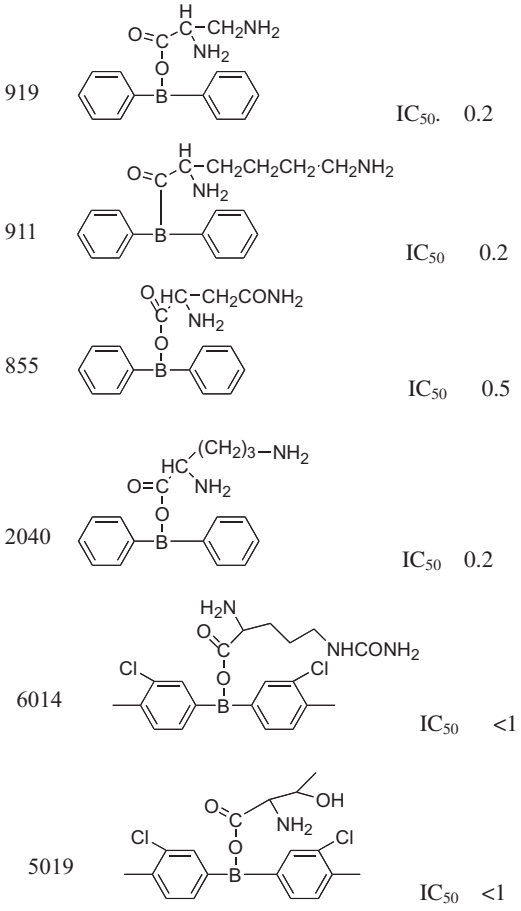
*3.1. Aminoalcohol adduct compounds **3***

IC_{50} of 2-APB for SOCE inhibition is 3 μM, but depending on the phenyl group substitution, the IC_{50} was reduced. For example, when 3-chloro-4-methyl phenyl (compound 444), the IC_{50} became 0.5 μM. IC_{50} of di(3-chloro-4-methylphenyl) 2-aminoethyl borinate is 0.5 μM, IC_{50} of compound 424, di(4-trifluoromethylphenyl) 2-aminoethyl borinate, is 0.7 μM and IC_{50} of 4132 di(3-chloro-4-methylphenyl) 2-aminobutylthyl borinate is 0.5 μM.

*3.2. Amino acid adduct compounds **4***

The amino acid borane type compounds have high inhibitory activities for SOCE.

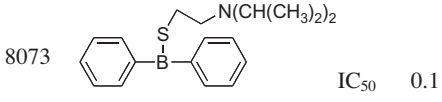
IC_{50} of the compound 919 – Diphenyl(2,3-diaminopropionateO,N)borane for SOCE inhibition is 0.2 μM, IC_{50} of the compound 911 – Diphenyl(2,6-diaminohexanoate O,N)borane is 0.2 μM, and that of the compounds 2040 – Diphenyl(2,5-diaminopetanoateO,N)borane is 0.2 μM. IC_{50} of the compound 855 – diphenyl-(asparaginate O,N)borinate is 0.5 μM.



When diphenyl borinate is changed to phenyl benzyl borinate, for example in compound number 8075 – phenyl benzyl 2-aminoethyl borinate, the inhibitory activity for SOCE is markedly reduced. IC_{50} of compounds 8075, 8110 – phenyl butyl borinate, 8105 – phenyl phenethyl thioborinate, and 3044 – phenyl naphtyl 2–aminoethyl borinate is over 10 μM. IC_{50} of 8154 – dinaphtyl 2-aminoethyl borinate for SOCE inhibition is 2 μM.

3.3. Aminothiol adduct compounds **5**

The thioborinate type of compounds show broad scale of IC_{50}. For example, compound 8061-2-aminoethylthio diphenyl borane has IC_{50} of 10 μM, while IC_{50} of the compound 8073-2-di isopropylaminoethylthio diphenyl borane is 0.1 μM. This compound displayed the highest inhibitory activity for SOCE amongst all tested analogues.



We synthesized three types of 2-APB analogues. Some of them had as much as 10 times higher inhibiting effect on SOCE as compared to 2-APB. The adducts of aminoacid with diphenyl borinic acid had the highest activity.

As mentioned above, the IC_{50} of 2-APB for SOCE inhibition is 3 μM. The IC_{50} of some of our synthesized 2-APB analogues is around 0.1 μM. These compounds can thus regulates the intracellular Ca^{2+} concentration and consequent cellular response more efficiently than 2-APB at druggable concentrations.

It is essential to study in detail the IC_{50} of IICR of each compound comparing with SOC inhibition.

The 2-APB analogues presented in this study could be proven to be excellent lead compounds for many human diseases including heart disorders [56], Alzheimer's [57,58] and Huntington's disease [59,60].

We have shown different kinds of active compounds with IC_{50} ranging 0.1–5 μM. By choosing the compound we can control the intracellular Ca^{2+} concentration and regulate many cellular processes.

We believe that many investigators will find these reagents regulating not only for SOCE but for IICR and related cellular processes very useful.

4. Summary

We synthesized many kinds of 2-APB analogues, differing inhibitory activities. Some of which displayed as much as 10 times higher activities for SOCE inhibition than 2-APB. Among them, adducts of amino acids and aminothiols with borinic acid showed high activity. 911 Diphenyl(2,6–diaminohexanoate O,N)borane, 919 Diphenyl (2,3-diaminopropionate O,N)borane, 2040 Diphenyl(2,5-diaminopentanoate O,N)borane, and 8075 2-di isopropylaminoethylthio diphenyl borane are good candidates for regulation of intracellular Ca^{2+} concentration and consequent cellular processes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.08.102.

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