2-Aminoethyl diphenylborinate (2-APB) analogues: part 2. regulators of Ca2+ release and consequent cellular processes

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Abstract
In order to obtain compounds with modified 2-APB activities, we synthesized number of bis-boron 2-APB analogues and analyzed their inhibitory activities for SOCE and IICR. Adducts of amino acids with bis-borinic acid showed the highest activity. The IC50 of 2-APB for SOCE inhibition was 3 µM, while the IC50 of 2051 bis(4,4'(phenyllysineboryl)benzyl) ether was 0.2 µM. By using these compounds, we may be able to regulate Ca2+ release and consequent cellular processes more efficiently than with 2-APB.

Keywords: 2-APB, 2-APB analogue, regulator of Ca2+ release, regulator of cellular processes

Introduction
Extracellular signal molecules attach to the plasma membrane where they are recognized by cell surface receptors. Upon binding of the ligand to the appropriate receptor, activation of a G protein in turn activate an enzyme such as phospholipase C involved in second messenger system. Active phospholipase C hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to give rise to two products: 1,2-diacylglycerol and inositol 1,4,5-triphosphate (IP3). IP3 stimulates the release of Ca2+ from the intracellular stores in the endoplasmic reticulum through IP3 receptors thus regulating a wide range of cellular processes [1-20].

In 1997, we identified 2-aminoethyl diphenylborinate (2-APB) as being an IP3 receptor inhibitor which regulates IP3-induced calcium release [21-22]. This discovery led to substantial interest as it led to more than 600 citations and more than 1000 studies on 2-APB have been published so far (examples are references 23-37). This was supported by increasing sales of 2-APB by Ronderstvent et al., [46] from triphenylboranes and ethanol amine. Later, hydroxy diphenyl boran and ethanol amine methods for 2-APB synthesis were reported by Weidman and Zimmermann [47], Letsinger and Skoog [48], Povlock and Lippincott [49].

We have synthesized 493 2-APB analogues [38-44] using methods described by us [38-44] and others [46-56]. The structures, names and synthetic methods of the 493 compounds are in example 1-493 of Ref. 44. Detail of synthetic methods to get bis boron compounds of this paper are described in Ref. 39. We will show one example to prepare best sample 1024. Other compounds can be obtained by similar methods.

Preparation of bis-(4,4’-(hydroxyphenylboryl)phenyl) ether 1012
To a solution of bromobenzene (1055 µL, 10.00 mmol) in diethyl ether (40 mL), we added 0.99 M sec-BuLi (10.6 mL, 10.5 mmol) at -98°C, gradually warmed to -78°C to complete lithiation, and then added triisopropoxyborane (2.4 mL, 10.00 mmol). The reaction mixture was stirred for 80 min at -78°C. At the same time, bis[p-bromophenyl]ether (1640 mg, 5. mmol) was dissolved in diethyl ether (50 mL), then reacted with 0.99M sec-BuLi (10.5 mL, 10.6 mmol) and stirred for 1 h at -78°C. The reaction mixture was

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added to the mixture of diisopropoxyphenylborane, gradually warmed to room temperature, and then stirred overnight. The reaction was quenched with 1N HCl, the diethyl ether layer was collected, and the water layer then extracted twice with diethyl ether. The combined diethyl ether layers were dried over MgSO₄ and concentrated. The crude residue was purified by flash column chromatography on a silica gel (n-hexane/EtOAc = 3:1) to give bis-(4,4’-(hydroxyphenylboryl) phenyl) ether 1012 (1122 mg, 3.15 mmol, 58.8%) as an oil.

**Preparation of bis(4,4’-(phenylglutamineboryl)phenyl) ether 1024**

4,4’-(hydroxyphenylboryl)diphenyl ether 1012 (22 mg, 0.0583 mmol) was dissolved in 0.2 mL ethanol and 2 mL water. Glutamine 19 mg, 0.017 mmol was then added. The reaction mixture was heated for 17 hrs at 80°C. 10 mL ether was added to get compound 1024 (17 mg, 46%) as a white precipitate.

**Methods**

We have assayed the inhibitory activity of the 2-APB analogues for SOCE and IICR using our improved assays described previously [45].

**Results and discussion**

We measured inhibitory activities of the 2-APB analogues for SOCE and IICR. The results are shown in (Supplement Table S1).

**Amino acid adduct on bis(4,4’-(hydroxyphenyl boryl)phenyl) ether**

This combination gave high activity compounds. Bis((4,4’-phenylglutamineboryl)phenyl) ether obtained by the reaction of bis((4,4’-hydroxyphenylboryl)phenyl) ether and glutamine was best sample showing IC₅₀ 0.2 µM. 1023 bis((4,4’-(phenylasparagineboryl)phenyl) ether obtained by the reaction of bis((4,4’-(hydroxyphenylboryl)phenyl) ether and asparagine was also good sample showing IC₅₀ 0.3 µM.

We can verify the efficiency of these sample by comparing with IC₅₀ of 2-APB for SOCE inhibition 3 µM, and IC₅₀ of 919 :best sample of our previous paper (45) 0.2 µM.

**2-Aminoethanol adduct on bis borinic acid**

Many compounds were prepared. best three compounds are picked up

IC₅₀ of 1022 bis-(4,4’(phenyl aminoethoxy boryl)phenyl) ether is 0.2. IC₅₀ of 4020 bis-(4,4’(phenyl aminoethoxy boryl)benzyl) ether is 0.2. IC₅₀ of 162AE bis-(3,3’(phenyl aminoethoxy boryl)benzyl) ether is 0.5.
Comparison of 2APB, mono-boron compounds and bis-boron compounds

The IC_{50} of 2-APB for SOCE inhibition is 3 µM. The IC_{50} of best mono-boron compound (example 919) at previous paper (45) is 0.2 µM. The IC_{50} of best bis-boron compound reporting at this paper (example 1024) is 0.2µM. That is, the bis-boron compound reporting at this paper and mono-boron compound reported at previous paper (45) showed almost same activity and about 10 times stronger activity than 2APB. Mono-boron compounds are easy to prepare. But bis-boron compounds are somewhat difficult to prepare.

Comparison of bis–phenyl ether and bis-benzyl ether

When we compare compounds mentioned at 3.1 and at 3.2 and when we compare 1022, 4020, 162AE, we can tell that there is not so much difference between bis-phenyl ether type compound (1022) and bis-benzyl ether type compounds (4020,162AE).

Comparison of amino acids and ethanol amine

As a reagent to add on to the dihydroxy boron compound, we used amino acid 3.1 and ethanol amine (3.3). Activities of both compounds were quite similar, but the stabilities of the compound obtained are different. Amino acid adducts are much more stable and easy to purify. We recommend amino acids derivatives over ethanol amine derivatives as regulators of Ca^{2+} and cellular process. Also among amino acid, basic amino acid having extra amino group or amide group like lysine, ornithine, asparagine, glutamine gave compounds with strong activity.

We have synthesized many bis-boron compounds. These compounds showed as active as mono-boron compounds on molar basis. But when we consider on a weight basis, bis-boron compounds are half as active as mono-boron compounds, because the molecular weight of bis boron compound is about twice that of mono-boron compound.

We listed the chemical structures of the best 9 compounds

Top three of these were 2051 bis(4,4'(phenyllysineboryl)benzyl) ether, 1024 bis-((4,4'-phenylglutamineboryl)phenyl) ether, 1023 bis-((4,4'-phenylasparagineboryl)phenyl) ether.

These compounds can thus regulates the Ca^{2+} release and consequent cellular response more efficiently than 2-APB at pharmacological concentrations.

Some of these compounds were shown to inhibit the calcium dependent enzyme transglutaminase [44]. Transglutaminase inhibitors block the abnormal cross-link of protein [43,44] and therefore they might slow down or even stop the progression of diseases caused by misfolded proteins, such as Huntington’s disease.

The 2-APB analogues presented in this study could be proven to be excellent lead compounds for many human diseases including heart disorders [59], Alzheimer’s [60,61] and Huntington’s disease [62,63].

We have shown different kinds of active compounds with IC_{50} ranging 0.1 to 5 µM. By choosing the compound we would be able to control the release of Ca^{2+} and regulate many cellular processes such as secretion, cardiac contraction, fertilization, proliferation, synaptic plasticity, atrial arrhythmias [31], inhibition of calcium entry channel [25], excitation-contraction coupling in the heart [32], arrhythmogenic action of endothelin-1 on ventricular cardiac myocytes [34], dysregulation of neural calcium signaling in Alzheimer disease [61], Huntington aggregation [62,63] and protein cross-link by transglutaminase [43].

We believe that many investigators will find these reagents regulating Ca^{2+} release and related cellular processes very useful.

Conclusion

We synthesized bis-boron 2-APB analogues of differing inhibitory activities. Some of which displayed as much as 10 times higher activity for SOCE inhibition than 2-APB. Among them, adducts of amino acids with bis-borinic acid showed the highest activity. 2051 bis(4,4'(phenyllysineboryl)benzyl) ether, 1024 bis-((4,4'-phenylglutamineboryl)phenyl) ether, 1023 bis-((4,4'-phenylasparagineboryl)phenyl) ether are best 3 candidates for regulation of Ca^{2+} release and consequent cellular processes.

Additional files

Supplement Table S1

Competing interests

The author declares that he has no competing interests.

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