2-Aminoethoxydiphenyl borate as a prototype drug for a group of structurally related calcium channel blockers in human platelets

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Running title: 2-APB analogs inhibit calcium influx in human platelets

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ABBREVIATIONS: SOCE, store-operated Ca\textsuperscript{2+} entry; CRAC, Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+} current; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; 2-APB, 2-aminoethoxydiphenyl borate; TRPC, transient receptor potential canonical; TRPV, transient receptor potential vanilloid; TRPM, transient receptor potential melastatin; ERK, extracellular regulated kinase; SNAP, synaptosome-associated protein; EGTA, ethylene glycol-bis(2-aminoethylether)-\(N,N,N',N'\)-tetraacetic acid; N–B; nitrogen boron coordinate bond; NMR, nuclear magnetic resonance; \(d_6\)-DMSO, deuterated dimethyl sulfoxide.
ABSTRACT:

We have synthesized a series of 2-aminoethoxydiphenyl borate (2-APB, 2,2-diphenyl-1,3,2-oxazaborolidine) analogs and tested their ability to inhibit thrombin induced Ca$^{2+}$ influx in human platelets. The analogs were either synthesized by adding various substituents to the oxazaborolidine ring (methyl, dimethyl, tert-butyl, phenyl, methyl phenyl, pyridyl) or increasing the size of the oxazaborolidine ring to 7 and 9 membered rings. NMR analysis of the boron containing analogs suggests that each of them exist as a ring structure through the formation of a N$\rightarrow$B coordinate bond (except for the hexyl analog). The possibility that these boron containing compounds also formed dimers was also considered. All compounds dose-dependently inhibited thrombin induced Ca$^{2+}$ influx in human platelets, with the 2,2-diphenyl-1,3,2-oxazaborolidine-5-one derivative having the weakest activity at 100 µM while the (S)-4-benzyl and (R)-4-benzyl derivatives of 2-APB were approximately 10 times more potent than the parent 2-APB. Two non-boron analogs (3-methyl and 3-tert-butyl 2,2-diphenyl-1,3-oxazolidine) were synthesized; they had approximately the same activity as 2-APB, this implies that the presence of boron was not necessary for inhibitory activity. All of the compounds tested were also able to inhibit thrombin-induced calcium release. It was concluded that extensive modifications of the oxazaborolidine ring in 2-APB can be made and Ca$^{2+}$-blocking activity was maintained.
INTRODUCTION:

2-Aminoethoxydiphenyl borate (2-APB, systematic name: 2,2-diphenyl-1,3,2-oxazaborolidine) was originally described as an inhibitor of inositol 1, 4, 5-trisphosphate receptors (IP$_3$R) in a variety of cells (Maruyama et al., 1997) and has been extensively used as a probe for examining calcium influx processes. 2-APB was inappropriately used in several studies to imply that IP$_3$R's were involved in store operated Ca$^{2+}$ entry (SOCE), (Ma et al., 2000; van Rossum et al., 2000). For a review of our current understanding of the nature of SOCE and how the SOCE channels are regulated see Parekh and Putney (2005). It has been suggested that SOCE is mediated by members of the TRPC (transient receptor potential canonical) family although this has not been universally accepted (Parekh and Putney, 2005).

Upon further investigation it became evident that 2-APB, along with inhibition of IP$_3$R’s, was also inhibiting SOCE directly in several cell types (Dobrydneva and Blackmore, 2001; Dobrydneva et al., 2001; Dobrydneva et al., 2002; Gregory et al., 2001, Bakowski et al., 2001, Braun et al., 2001, Broad et al., 2001, Prakriya and Lewis, 2001; Iwasaki et al., 2001; Diver et al., 2001; Ma et al., 2001; Bootman et al., 2002, Ma et al., 2001). The inhibition of SOCE appeared to be at an extracellular site by a mechanism not involving intracellular IP$_3$ receptors. In our study showing that 2-APB blocked SOCE in human platelets (Dobrydneva and Blackmore, 2001), we tested whether several other structurally related compounds were also inhibitors of SOCE. We found that the non-boron analog of 2-APB, 2, 2-diphenyltetrahydrofuran and diphenylboronic anhydride also inhibited both calcium mobilization and calcium influx (Dobrydneva and Blackmore, 2001). Two other non-boron analogs, phenytoin and
diphenhydramine were very weak inhibitors of calcium influx. From this limited structural analysis it appeared that compounds with calcium blocking activity possessed diphenyl groups, also the presence of a saturated 5 membered ring structure was needed. The presence of boron in the structure was not an absolute requirement, although boron and nitrogen were necessary for ring formation through a N→B coordinate bond (Figure 1). It has also been proposed that 2-APB forms a dimer (Figure 1) (van Rossum et al., 2000). 2-APB, at high concentrations, has also been shown to activate TRPV1, TRPV2 and TRPV3, three heat-gated members of the transient receptor potential vanilloid ion channel subfamily (Hu et al., 2004). 2, 2-Diphenyltetrahydrofuran and diphenylboronic anhydride (Dobrydneva and Blackmore, 2001) were also able to modulate the activity of these channels (Chung et al., 2005).

The calcium channel responsible for store mediated calcium entry in human platelets is believed to be TRPC1 (e.g. Brownlow and Sage, 2003). The regulation of this entry process appears to be very complex and possibly involves the conformational coupling between the IP3 receptor in the endoplasmic reticulum and the store-operated calcium channels located in the plasma membrane (Rosado et al., 2005). There is evidence for the involvement of SNAP-25, pp60src, the actin cytoskeleton and ERK in this process (Rosado et al., 2001, 2004a, 2004b and Redondo et al., 2004). Patch clamp experiments have shown that 2-APB acts extracellularly on various TRP channels (see Xu et al., 2005 for references), so it reasonable to assume that 2-APB also acts extracellularly on TRPC1 in platelets. However 2-APB could also be interacting with any of the other components that regulate TRPC1 activity such as ERK, pp60src and SNAP-25 (Rosado et al., 2001, 2004a, 2004b and Redondo et al., 2004).
The aim of the present study was to synthesize a series of boron containing and non-boron containing analogs of 2-APB to see if we could obtain compounds more potent than the parent 2-APB at inhibiting Ca$^{2+}$ influx in human platelets. We anticipated that this series of compounds would shed light onto the structure of the pharmacophore that was responsible for calcium blocking activity. We succeeded in synthesizing several analogs that were as effective as the parent 2-APB, and two boron containing compounds that were more potent than 2-APB at inhibiting Ca$^{2+}$ influx in platelets.
MATERIALS AND METHODS:

Materials

The following were obtained from Sigma-Aldrich: 2-aminoethoxydiphenyl borate, 2-(Methylamino) ethanol, 2-(t-butylamino) ethanol, 2-amino-1-phenylethanol, 4-amino-1-butanol, 6-amino-1-hexanol, diphenylborinic anhydride, the sodium salt of glycine, ethanolamine, dimethyl sulfoxide, benzophenone, phosphorus pentachloride, ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA). 3,3-diphenyl dihydrofuran-2-one (trivial name: α,α-diphenyl-γ-butyrolactone) was from ACROS. All organic solvents were from Fisher Scientific. Fura-2/AM was from Molecular Probes.

Synthesis of 2-APB analogs

All melting points were determined with a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected. ^1H NMR spectra were determined in a UNITYplus-400 MHz instrument operating at a field strength of 399.89 MHz or a Varian Mercury Vx-400 spectrometer. All C, H, N analyses were performed by Desert Analytics, Tucson, Arizona. A general method was developed for the synthesis of 2-APB and the various 2-APB analogs which is described for each compound. The method involved a reaction between diphenylborinic anhydride and a corresponding amino alcohol. The synthesis of 2,2-diphenyl-1,3,2-oxazaborolidine (1) and (S)-4-benzyl-2,2-diphenyl-1,3,2-oxazaborolidine (11) are shown in Figure 2. All of the reaction solutions were allowed to stir in an ice bath until precipitation occurred (no more than one hour), then they were chilled in an ice bath undisturbed for an additional 15 minutes, which increased the amount of precipitation. All of the products were purified by recrystallization using ethyl acetate as the solvent, except for 2,2,5-triphenyl-1,3,2-oxazaborolidine (8), which was
recrystallized using benzene. All of the products were white solids with sharp melting points. Monomeric structures with an internal N→B coordinate bond are shown for all compounds synthesized (Figure 3). Since we have depicted 2-APB and its derivatives as ring structures in this study, then the commonly used name 2-aminoethoxydiphenyl borate does not describe this ring system. We have therefore used the 1983 IUPAC Hantzsch-Widman system to name the ring forms of 2-APB and its derivatives. The numbering order of the heteroatoms in the oxazaborolidine ring is oxygen number 1, nitrogen number 2 and boron number 3, hence the numbering of this ring is 1,3,2-oxazaborolidine in 2-APB.

2,2-Diphenyl-1,3,2-oxazaborolidine, (1).

Ethanolamine (0.02g) was added to 0.10g of diphenylborinic anhydride in 4 ml of acetonitrile. The solution was stirred for one hour without any signs of precipitation. The solution was refrigerated for 15 hours where the product precipitated and was filtered to give 0.056g of 1 (75% yield). The melting point was 193-194 °C (literature. 190-192 °C). 1H NMR (d6-DMSO): 2.83 (p, CH2), 3.77 (CH2), 6.07 (t, NH2), 7.01 (m, 2H), 7.14 (m, 4H), 7.39 (m, 4H). The broad triplet at 6.07 due to the NH2 rapidly exchanged with the addition of D2O.

3-Methyl-2,2-diphenyl-1,3,2-oxazaborolidine, (2).

2-Methylaminoethanol (0.05g) was added to 0.20g of diphenylborinic anhydride in 8 ml of acetonitrile. The product precipitated to give 0.066g of 2, (46% yield). Melting point: 196-197.5 °C. 1H NMR (d6-DMSO): δ 2.16 (d, CH3), 2.64 (p, CH), 3.03 (p, CH), 3.75 (q, CH), 4.01 (q, CH), 6.67 (m, NH), 7.09 (t, 2H), 7.16 (t, 4H), 7.46 (dd, 4H). Anal.,
Calc. for C_{15}H_{18}BNO: C, 75.33; H, 7.60; N, 5.86. Found: C, 75.32; H, 7.56; N, 5.68.

The multiplet at 6.67 was for the NH rapidly exchanged with the addition of D_{2}O.

**3,3-Dimethyl-2,2-diphenyl-1,3,2-oxazaborolidine, (3).**

Diphenylborinic anhydride (0.01g) was stirred with 0.05 ml of dimethylaminoethanol in 10 ml of acetonitrile. The product slowly precipitated from solution to give 0.195g of 3, (82% yield). Melting point: 165-166 °C. 1H NMR (d_{6}-DMSO): 2.50 (s, 6H, CH_{3}), 2.92 (t, CH_{2}), 4.10 (t, CH_{2}), 7.10 (m, 2H), 7.18 (m, 4H), 7.65 (m, 4H).

**3-tert-Butyl-2,2-diphenyl-1,3,2-oxazaborolidine, (4).**

2-tert-Butylaminoethanol (0.07g) was added to 0.20g of diphenylborinic anhydride in 8 ml of acetonitrile. The product precipitated to give 0.125g of 4, (76% yield). Melting point: 133.1-134.0 °C. 1H NMR (d_{6}-DMSO): δ 1.16 (s, CH_{3}), 2.69 (t, CH_{2}), 3.55 (t, CH_{2}), 6.91 (tt, NH), 6.98 (t, 2H), 7.06 (t, 4H), 7.44 (dd, 4H). Anal., Calc. for C_{18}H_{24}BNO: C, 73.85; H, 8.62; N, 4.31. Found: C, 73.31; H, 8.76; N, 4.68. The peak at 6.91, assigned to the NH, rapidly exchanged with the addition of D_{2}O.

**2,2,5-Triphenyl-1,3,2-oxazaborolidine, (5).**

2-Amino-1-phenylethanol (0.08g) was added to 0.20g of diphenylborinic anhydride in 8.0 ml of acetonitrile. The product precipitated to give 0.072g of 5, (41% yield). Melting point: 194.9-196.0 °C. 1H NMR (d_{6}-DMSO): δ 3.25 (q, CH_{2}), 4.85 (dd, CH), 6.31 (t, NH_{2}), 7.03 (t, 2H), 7.08 (t, 4H), 7.16 (dd, 4H), 7.25 (t, 2H), 7.34 (t, 4H), 7.49 (dd, 4H). Anal., Calc. for C_{20}H_{20}BNO: C, 79.75; H, 6.69; N, 4.65. Found: C, 80.27; H, 6.62; N, 4.65. The triplet peak at 6.31 rapidly exchanged with the addition of D_{2}O.

**2,2-Diphenyl-1,3,2-oxazaborepane, (6).**
4-Amino-1-butanol (0.053g) was added to 0.20g of diphenylborinic anhydride in 8.0 ml of acetonitrile. The product precipitated from solution to give 0.020g of 6, (27% yield). Melting point: 185.60-186.1 °C. $^1$H NMR ($d_6$-DMSO): $\delta$ 1.58 (p, CH$_2$), 1.67 (p, CH$_2$), 2.71 (p, CH$_2$), 3.59 (t, CH$_2$), 5.82 (t, NH$_2$), 7.01 (t, 2H), 7.12 (t, 4H), 7.45 (dd, 4H). Anal., Calc. for C$_{16}$H$_{20}$BNO: C, 75.95; H, 7.98; N, 5.58. Found: C, 76.47; H, 8.04; N, 5.59.

2,2-Diphenyl-1,3,2-oxazaboronane, (7).

6-Amino-1-hexanol (0.073g) was added to 0.20g of diphenylborinic anhydride in 8.0 ml of acetonitrile. The product precipitated to give 0.228g of 7, (32% yield). Melting point: 70.0-71.0 °C. $^1$H NMR ($d_6$-DMSO): $\delta$ 1.28 (p, CH$_2$), 1.35 (p, CH$_2$), 1.42 (p, CH$_2$), 1.63 (p, CH$_2$), 2.63 (q, CH$_2$), 3.33 (broad s, NH$_2$), 3.46 (t, CH$_2$), 7.08 (t, 2H), 7.17 (t, 4H), 7.53 (dd, 4H). Anal., Calc. for C$_{18}$H$_{24}$BNO: C, 72.73; H, 8.26; N, 11.57. Found: C, 72.33; H, 8.08; N, 11.04. The peak at 3.33 rapidly exchanged with the addition of D$_2$O.

2,2,5-Triphenyl-benzo[3,4]-1,3,2-oxazaborolidine, (8).

Sodium borohydride (0.107g) was added to 0.50g of 2-acetylpyridine in 2.0 ml methanol to yield 0.293g of 2-(1-hydroxyethyl)pyridine after stirring for 30 minutes. The precipitate was filtered out of the solution using vacuum filtration and dried. 2-(1-hydroxyethyl) pyridine (0.283g) was added to 0.825g of diphenylborinic anhydride in 31.0 ml acetonitrile. The product precipitated to yield 0.473g of the 8 (yield 69.2%). Melting point: 200-201.1 °C (literature melting point 199-200 °C, Farfan et al., 1992).

5-Methyl-2,2-diphenyl-benzo[3,4]-1,3,2-oxazaborolidine, (9).

0.277g of 2-(1-hydroxyethyl) pyridine was stirred with 0.518g of diphenylborinic anhydride in 30.0 ml acetonitrile. The product precipitated to give 0.381g of 9 (72.8%
yield). Melting point: 164.2-164.9 °C (literature melting point 164-165 °C; Farfan et al., 1992).

**2,2-Diphenyl-1,3,2-oxazaborolidine-5-one, (10).**

Diphenylborinic anhydride (0.2g), dissolved in 20.0 ml of acetonitrile, was stirred at with 0.058g of the sodium salt of glycine. The acetonitrile was removed under nitrogen to give 0.238g of **10** (45% yield). Melting point: 235–238 °C. 1H NMR (d6-DMSO): 3.47 (t, CH2), 7.09 (t, NH2), 7.17 (m, 2H), 7.23 (m, 4H), 7.39 (m, 4H). Anal., Calc. for C14H14BNO2: 70.43; H, 5.80; N, 5.86. Found: C, 70.12; H, 5.83; N, 5.80. The triplet at 7.09 rapidly exchanged with the addition of D2O.

**(S)-4-Benzyl-2,2-diphenyl-1,3,2-oxazaborolidine, (11).**

(S)(-)-2-Amino-3-phenyl-1-propanol (0.50g) was dissolved in 11.0 ml of acetonitrile and treated with a solution of 1.14g of diphenylborinic anhydride in 9.0 ml of acetonitrile. The reaction was stirred for one week and then the solvent was removed with nitrogen gas to give 0.556g of **11** (60% yield). Melting point: 129-135 °C. 1H NMR (d6-DMSO): 2.734 (dd, 1H), 2.94 (dd, 1H), 3.35 (m,1H), 3.55 (t,1H), 3.81 (t,1H), 5.80 (t,1H), 6.42 (t,1H), 7.05-7.45 (mm, 15H). Anal., Calc. for C21H22BNO: C, 80.0; H, 6.98; N, 4.45. Found: C, 79.89; H, 6.95; N, 4.54. The two triplets at 5.80 and 6.42 are assigned to the two non-equivalent NH protons rapidly exchanged with the addition of D2O.

**(R)-4-Benzyl-2,2-diphenyl-1,3,2-oxazaborolidine, (12).**

(R)(+)-2-Amino-3-phenyl-1-propanol (0.50g) was dissolved in 30.0 ml of acetonitrile and treated with a solution of 1.14 g of diphenylborinic anhydride in 9.0 ml of acetonitrile. The solution was stirred at for four days when the solvent was removed...
with nitrogen gas to give 0.510g of 12 (55% yield). Melting point: 120–125 °C. 1H NMR (d6-DMSO): 2.73 (dd,1H), 2.94 (dd,1H), 3.35 (t,1H), 3.81 (t,1H), 5.80 (t,1H), 6.42 (t,1H), 7.05-7.45 (mm, 15H). Anal., Calc. for C21H22BNO: C, 80.0; H, 6.98; N, 4.45. Found: C, 79.66; H, 7.18; N, 5.25. The two triplets at 5.80 and 6.42 are assigned to the two non-equivalent NH protons rapidly exchanged with the addition of D2O.

**Synthesis of Oxazolidine analogs:**

Dichlorodiphenylmethane was prepared from benzophenone and phosphorus pentachloride according to the method of Staudinger and Freudenberger (1943).

**3-Methyl-2,2-diphenyl-1,3-oxazolidine, (13).**

Silver oxide (2.0 g, 8.6 mmol) was added to a mixture of dichlorodiphenylmethane (2.0 g, 9.4 mmol) and 2-(methylamino)ethanol (1.4 ml, 17 mmol). The mixture was warmed on a hot plate until a violent reaction ensued (Reaction 1). The mixture was cooled, and the solid residue was washed repeatedly with hexanes. The combined filtrates were washed with water, dried over sodium sulfate, filtered and concentrated in vacuo. The residue was distilled under high vacuum. The distillate was recrystallized from hexanes giving 0.87 g of 13 (3.6 mmol, 42% yield). NMR spectra were in agreement with published data (Azzena et al., 1993).

**3-tert-Butyl-2,2-diphenyl-1,3-oxazolidine, (14).**

Silver acetate (1.7 g, 10.2 mmol) was added to a mixture of dichlorodiphenylmethane (1.0 g, 4.7 mmol) and 2-(tert-butylamino)ethanol (5.5 g, 47 mmol). The mixture was warmed on a hot plate for 30 min during which a brown solid appeared (Reaction 2). The reaction was allowed to cool, and the solid was washed with hexanes (100 ml). The hexane filtrate was washed three times with water (100 ml...
each), dried over sodium sulfate, and concentrated in vacuo. Crystals formed slowly on standing which were placed on an absorbant tissue to remove the benzophenone side-product that remains in the melt. The product 14 thus obtained was 230 mg (0.82 mmol, 17% yield), mp 66-68 °C; $^1$H NMR (CDCl$_3$) δ 7.59 (m, 4H), 7.27 (m, 6H), 3.76 (t, $J$=6.5 Hz, 2H), 3.31 (t, $J$=6.5 Hz, 2H), 0.92 (s, 9H); $^{13}$C NMR (CDCl$_3$) δ 144.5, 129.6, 127.5, 127.3, 99.7, 63.0, 54.5, 47.6, 29.9. The compound was sublimed under vacuum for analysis. Anal., Calc. for C$_{19}$H$_{23}$NO: C, 81.10; H, 8.24; N, 4.98; Found: C, 80.98; H, 8.33; N, 4.81.

2,2-diphenyl-1,3-oxazolidine, (16).

We were unable to isolate 2, 2-diphenyl-1,3-oxazolidine (16), which is the carbon analog of the ring form of 2-APB, where the boron is replaced by a carbon. Results from the parent oxazolidine are therefore not reported. The compound was however successfully prepared by the condensation of ethanol amine with benzophenone imine. Nevertheless, the oxazolidine 16 exists as an equilibrium mixture with its ring-opened 17 tautomer (Figure 4). Proton NMR analysis suggests a ratio of 14:86 in favor of the ring-opened form. The evidence for the ring-opened structure is the non-equivalence of the phenyl rings. The phenyl rings in the ring-open tautomer are diastereomeric due to the imine double bond. The $^1$H NMR shows four sets of strong aromatic resonances at 7.62, 7.46, 7.34 and 7.17 ppm. The ring-closed structure should show only two strong signals for the sets of four equivalent ortho and meta hydrogens, respectively. In the aliphatic region there are two pairs of triplets. The major pair occur at 3.84 and 3.48 ppm, while the minor pair occur at 3.85 (obscured by the major isomer) and 3.15 ppm. The methylenes adjacent to the nitrogen are the
upfield resonances of each set. The imine structure of the ring-opened isomer gives rise to the shift from 3.15 to 3.48 ppm. Finally, the chemical shifts of the aliphatic protons of the ring-closed methyl derivative 13 are similar to those of the minor isomer. Precedent for ring-opening tautomerization has been reported. Thus the oxazoline from acetophenone and ethanolamine exists as a 50:50 mixture with the ring-opened form (Konkova et al., 1999). It is reasonable to suggest that the conjugation provided by the extra phenyl ring in benzophenone results in a greater proportion of the imine form.

**Blood Donors and Platelet Preparation:**

All donors were healthy non-smoking volunteers (ages 20-60) who had not consumed any medication known to affect platelet function (e.g. calcium channel blockers and aspirin) for at least 10 days prior to the study. Venous blood was collected into 1/10 volume of (74.8 mM sodium citrate, 38.1 mM citric acid and 123 mM dextrose pH 6.4) (Baxter Healthcare Corp.). The blood was centrifuged at 250 x g for 10 min. at room temperature to obtain platelet rich plasma (PRP). The PRP was centrifuged at 550 x g for 12 min. to sediment the platelets. The platelets were then re-suspended in a modified Tyrodes physiological salt solution (NaCl, 145 mM; KCl, 4 mM; MgSO4, 1 mM; Na2HPO4, 0.5 mM; Na/HEPES, 10 mM; glucose, 6 mM; pH 7.4) containing 1.0 mM EGTA to prevent spontaneous aggregation during the various experimental manipulations by binding extracellular Ca2+.

**Platelet loading with fura-2 and measurement of [Ca2+]i.**

Intracellular calcium measurements [Ca2+]i employed the fluorescent dye fura-2, which involved incubating the platelets with the cell permeant acetoxyethyl ester (fura-2/AM). Suspensions of human platelets (isolated as described above) were incubated
with 2 µM fura-2/AM for 1 hour at room temperature (on a rocking platform). Excess fura-2/AM was removed by centrifugation (500 x g for 10 min) and the platelets suspended in modified Tyrodes buffer, without added EGTA. Aliquots of platelet suspension (0.5 ml) were added to 1.0 ml cuvettes containing a Teflon coated stirrer bar (CHRONO-LOG, Havertown, PA). To measure intracellular Ca^{2+} mobilization, Ca^{2+} was not added to the platelet suspension; test compounds were added at approximately 10 seconds, then at 20 seconds 0.01 units ml⁻¹ thrombin was added. To evaluate Ca^{2+} influx, at approximately 30 seconds before [Ca^{2+}], measurements were performed, Ca^{2+} was added back to the buffer to a final concentration of 2.0 mM, then test compounds dissolved in Me₂SO (various concentrations in 2.5 µl) and thrombin 0.01 units ml⁻¹ were added. The measurements of [Ca^{2+}], was performed at room temperature in a SPEX ARCM spectrofluorometer using excitation wavelengths of 340 nm and 380 nm and an emission wavelength of 505 nm. Calibration was performed as previously described (Dobrydneva and Blackmore, 2001). [Ca^{2+}] was calculated by using the SPEX dM3000 software. The data in figure 5 shows a typical experiment. To calculate the % inhibition of thrombin effects by the various analogs the [Ca^{2+}] before thrombin addition was measured; then the [Ca^{2+}] was measured after the thrombin effect had reached a maximum value (which was approximately after 1 minute). The difference was then compared to the effect observed in the absence of any inhibitor (control); which represents 0% inhibition.
RESULTS AND DISCUSSION:

Chemistry:

All of the 2-APB analogs that we synthesized were obtained in 27-76% yields after purification by recrystallization. The reactions were temperature dependent, leading to high yields at cool temperatures and low yields at warm temperatures. It is believed that the low yields at warm temperatures could be caused by the acetonitrile reacting with the diphenylboronic anhydride before the amine could be added. A possible product may be the acetonitrile adduct of diphenylboronic anhydride, formed when the unpaired electrons of acetonitrile forms a N→B coordinate bond (Figure 6). This finding illustrates one possible pitfall when synthesizing boron containing compounds in the presence of solvents (e.g. acetonitrile) or reactants with unpaired electrons that could form coordinate bonds. At warm temperatures the solution of acetonitrile and anhydride developed a golden color, while at cooler temperatures the solution remains colorless which suggests some involvement of the solvent acetonitrile. To avoid the contamination of the product produced when acetonitrile and diphenylboronic anhydride react, the reaction vessel was placed in an ice bath for the duration of the reaction, and the recrystallizing solvents were changed to ethyl acetate and benzene. All of these 2-APB analogs were in agreement with the C, H, N analyses and exhibited the predicted functional group absorptions in the Infrared. The NMR of these compounds was in good agreement with the assigned structures.

One of the intriguing aspects of the structural analysis of 2-APB was observed in the NMR data for this series of compounds. 2-APB can be visualized as either an open chain form or a ring structure (Figure 1) owing to the potential coordinate covalent
bond that might arise between the electron rich amine and the electron deficient boron. The existence of the ring form is indeed confirmed by the crystal structure of 2-APB (Retting and Trotter, 1976) which shows a heterocyclic form in staggered arrays, with each molecule linked to two others through hydrogen bonds. An examination of the NMR of 2-APB in $d_6$-DMSO shows that the NH$_2$ protons are indeed in a highly deshielded position (6-7 ppm) which is also suggestive of a ring rather than as an open chain structure. By contrast the NH$_2$ peak for ethanolamine is found at 3.3 ppm. This series of 2-APB analogs was designed to examine this ring concept for 2-APB and also to see if a 5 membered ring was needed or if larger rings would be active (6 and 7).

The objective was to see if chain extension could resolve the question of the coordination of the NH$_2$ group and the boron. The N-methyl (2,3), and N-butyl (4) analogs as well as the phenyl (5) butyl (6) analogs all showed the NH and NH$_2$ protons to be highly deshielded in the 5.9 to 6.8 ppm region in $d_6$-DMSO. This would suggest that these analogs were also in a similar ring structure due to the proximity of the NH and NH$_2$ groups to the electron deficient boron. However, NMR data for the hexyl analog (7) in $d_6$-DMSO showed the NH$_2$ protons to be at 3.3 ppm which is in keeping with an open chain structure. Thus extending the length of the ethanolamine side chain in 2-APB by adding two extra carbons (ethyl to butyl) did not prevent ring formation based on the NMR data. However extending the length of the side chain by another two carbons (butyl to hexyl) prevented the formation of a ring, based on the NMR data. The reason for this is not clear however the longer side chain decreases the probability that the nitrogens unpaired electrons will be in close proximity to the boron and hence lessen the likelihood that a coordinate bond will be formed. The formation of hexyl
dimers would also be less likely to occur since either one or two coordinate bonds would have to be formed. As seen in Table 1 hexyl-APB (7), butyl-APB (6) and 2APB (1) all have the same ability to inhibit thrombin, despite hexyl-2-APB (7) not forming a ring. However we should caution extrapolating NMR data performed in dimethylsulfoxide and biological data in which cells are incubated in an aqueous buffered salt solution at pH 7.4. Under these aqueous conditions we do not know the proportion of open chain, ring and dimer forms of 2-APB and its analogs.

The presence of a chiral center gives rise to the interesting splitting pattern of the adjacent protons. A distinctive structural feature of the two stereoisomers (11 and 12) produced in this study was observed in their NMR spectra. As might be expected the two \( R \) and \( S \) enantiomers would necessarily have the same basic NMR patterns and indeed this is observed in \( d_6 \)-DMSO. The NMR data further suggests that the two NH\(_2\) protons in the \( R \) and \( S \) compounds are essentially non-equivalent. This non-equivalence produces a triplet arising from the mutual splitting of the two NH protons and subsequent splitting by the adjacent proton on the optically active center. The protons adjacent to the oxygen in these two compounds are also apparently non-equivalent as well and are split into a set of triplets. The protons at the chiral center of this oxazaborolidine ring system are also non-equivalent and are therefore split into a set of doublets of doublets.

The rationale for the synthesis of the 2,2-diphenyl-1,3,2-oxazaborolidine-5-one (10) from the condensation of diphenylborinic anhydride and the sodium salt of glycine was based on the possibility that greater activity might be achieved by creating a better leaving group within the 2-APB structure. This would assume that the activity of this
class of compounds may be related to an interaction of some nucleophilic moiety on the receptor/channel protein with the highly polarized 2-APB ring system. This might lead to the subsequent displacement of the coordinately bound NH$_2$ group from the 2-APB moiety and provide for a reversible attachment to the receptor/channel site. By introducing the ester moiety into the basic 2-APB structure this type of interaction was expected to be enhanced and may produce a more effective interaction with the SOCE receptor protein. However this compound was a very weak inhibitor of thrombin induced Ca$^{2+}$ influx at 100 µM (see below).

**2APB derivatives block thrombin induced Ca$^{2+}$ elevation:**

In the present study we examined the capacity of the various analogs to inhibit the ability of thrombin to increase [Ca$^{2+}$]$_i$ in platelets in the presence of extracellular calcium (Table 1) and absence of extracellular calcium (Table 2). When extracellular calcium was present, approximately 80-90% of the increase in [Ca$^{2+}$]$_i$ induced by low concentrations of thrombin (0.01 units ml$^{-1}$) was due to calcium influx (Dobrydneva and Blackmore, 2001). This was because in the absence of extracellular calcium the increase in [Ca$^{2+}$]$_i$ induced by thrombin was approximately 10-20% of that observed when extracellular calcium was present.

Modification of the oxazaborolidine ring in the 2-APB analogs does not alter activity substantially except for the glycyl ester (10), which had a small 16% inhibitory activity at 100 µM and no activity at lower concentrations (Table 1). Most of the 2-APB (1) analogs that we synthesized possessed approximately the same ability to inhibit thrombin-induced [Ca$^{2+}$]$_i$ increase (Table 1). This included phenyl-APB (5), dimethyl-APB (3), monomethyl-APB (2), t-butyl-APB (4), butyl-APB (6), hexyl-APB (7), and
methyl-pyridyl APB (9). Dose responses are shown for 2-APB and butyl-APB in Figure 7. Each of these compounds produced between 96 and 99% inhibition at 100 µM and between 31 and 74% inhibition at 10 µM and 3 to 16% inhibition at 1 µM. Phenyl-pyridyl APB (8) stimulated Ca\(^{2+}\) influx at 100 µM by itself, however at 10 and 1µM it was inhibitory. The response of 100µM phenyl pyridyl APB to increase [Ca\(^{2+}\)]\(i\) was approximately 50% of the increase observed with 0.01 units ml\(^{-1}\) thrombin (Figure 7). The kinetics of the increase in [Ca\(^{2+}\)]\(i\) was similar to that observed with thrombin and much faster than that seen initially with the SERCA (sarcoplasmic/endoplasmic reticulum calcium ATPase) inhibitor thapsigargin (data not shown). Thapsigargin initially produces a very small and slow increase in [Ca\(^{2+}\)]\(i\), (also see Figure 4 in Dobrydneva and Blackmore, 2001) then after approximately 1 minute there is a very rapid and large increase in [Ca\(^{2+}\)]\(i\), probably secondary to the generation of thromboxane, since the response was largely blocked by treating the platelets with aspirin (data not shown). Thus we do not believe that phenyl pyridyl APB was increasing [Ca\(^{2+}\)]\(i\) by a mechanism involving inhibition of SERCA since the kinetics and magnitude of increase in [Ca\(^{2+}\)]\(i\) are completely different to that seen with the SERCA inhibitor thapsigargin. We have not examined this phenomenon any further. There were two analogs that showed a greater potency than 2-APB to inhibit thrombin, these were R and S benzyl-APB (11 and 12) (Figure 8).

Previous studies in platelets showed that 2-APB inhibited calcium influx channels (Dobrydneva and Blackmore 2001; Diver et al 2001). 2-APB also inhibited calcium mobilization in platelets (Maruyama et al 1997; Dobrydneva and Blackmore 2001; Diver et al 2001). We therefore also examined the effects of the 2-APB analogs
on thrombin-induced calcium mobilization (Table 2) to see if they were more selective at inhibiting either process. When one compares the inhibitory effects of each compound at a 10 µM concentration, 2-APB was the most effective compound at inhibiting internal release with the exception of (S) benzyl APB (12), while at a 1µM concentration 2-APB was clearly the most effective inhibitor with a 36% inhibition (Table 2). When extracellular calcium was present and the inhibitory effects of each compound at a 10 µM concentration were compared (Table 1), 2-APB possessed a similar inhibitory activity to many of the compounds (e.g. 3, 4 and 6), however several analogs were clearly more effective (e.g. 8, 9, 11, 12 and 14). Several of the analogs were also more effective at the 1 µM concentration (e.g. 11, 12 and 14). Thus many of the analogs synthesized had less inhibitory effects on internal release (Table 2) when compared to 2-APB, whereas several analogs were more effective at preventing calcium influx (e.g. 11 and 12). Despite extensive modifications to 2-APB; many of the compounds are able block both calcium influx and internal release, at least in platelets.

Based on this limited number of analogs examined we can propose a common pharmacophore that is responsible for inhibiting thrombin induced calcium influx. This pharmacophore consists of two phenyl groups linked to either tetrahedral carbon or a boron atom. This is confirmed by the fact that replacing the boron with a carbon such as in 13 and 14 resulted in inhibitory activity comparable to that seen with most of the boron containing analogs. Therefore it does not appear that the presence of boron is critical for activity.

All of the most active analogs possessed oxygen at position 1 of the oxazolidine or oxazaborolidine ring. An exception was compound 15, which had oxygen in a
different position in the ring. Compound 15 was still inhibitory, although it was clearly less effective than compounds containing oxygen at position 1; it possessed 59% inhibitory activity at 100 µM and only 17% inhibitory activity at 10 µM and no activity at 1 µM (Table 1). Also, the replacement of the oxygen at position 1 with a carbonyl group such as in phenytoin; resulted in only a 22% inhibition at 100 µM (Dobrydneva and Blackmore, 2001). All of the active analogs possessed nitrogen at position 3 of the oxazolidine or oxazaborolidine ring. Methylation (2), dimethylation (3) and t-butylation (4) or the fusing of a benzo ring to the oxazaborolidine ring at this nitrogen (8, 9) had very little influence on activity. The addition of a phenyl group at position 5 of the oxazaborolidine ring did not influence activity at a concentration of 10 µM (5, 8). At 100 µM, 8 by itself promoted calcium influx, however at 10 µM 8 had no effect on calcium influx by itself however it produced a good inhibition (77%) of thrombin, so that this compound was active. The addition of a carbonyl group at position 5 of the oxazaborolidine ring (10) drastically reduced activity.

Substitution at position 4 of the oxazaborolidine with a benzyl group (11 and 12) actually increased the calcium blocking activity of 2-APB by approximately 1 order of magnitude. This is a curious finding since carbon 4 is a chiral center which would place the benzyl groups in both compounds in different orientations relative to the oxazaborolidine ring. This lack of stereoselectivity between compounds could imply that there must be a large hydrophobic binding pocket that can tolerate binding of the benzyl groups when presented in two different orientations.

Another feature of the boron containing compounds presented in this study that should be considered, is their ability to dimerize. However since comparable calcium
blocking activity was also observed with carbon analogs of 2-APB (13 and 14), which can’t dimerize, therefore dimers do not contribute to their inhibitory activity. We previously showed that diphenyl tetrahydrofuran (non-boron analog of 2-APB without a nitrogen in the tetrahydrofuran ring) possessed the ability to inhibit thrombin induced calcium influx (Table 1 and Dobrydneva and Blackmore, 2001). This compound nevertheless was not as effective as 2-APB at 100 µM; however at lower concentrations it was more effective than 2-APB (Table 1). Thus the absence of nitrogen and boron did not have a deleterious effect on activity. We also tested another non boron analog 3,3-diphenyl dihydrofuran-2-one (15); this compound was not as effective as 2-APB and many of its analogs (Table 1). This compound contains an oxygen at position 4 and a carbonyl at position 3 (relative to oxygen in 1,3-oxazolidine), therefore the location of the oxygen and or the carbonyl group in the ring appears critical for activity. Also the absence of nitrogen in the ring may contribute to weak activity.

Since the 2-APB analogs posses the potential to dimerize and cyclize due to the formation of a N→B coordinate bond it is also possible that they could also form N→B coordinate bonds with various amino groups in the calcium channel. The reaction between acetonitrile and diphenylborinic anhydride (Figure 6) illustrates this phenomenon. A variety of nitrogen adducts of BF₃ have previously been characterized crystallographically, these include amines, imidazole (Barber et al., 2005; Figure 6 D), pyridine and acetonitrile (Swanson et al., 1969; Figure 6 C) adducts (see Barber et al., 2005 for references). It is therefore conceivable that the boron analogs synthesized in the present study can also form coordinate bonds with amino acids (either N→B or O→B). There is precedence for the boron containing organic molecule bortezomib
forming a coordinate bond with the N-terminal threonine residue of the 26S proteasome (Pekol et al 2005). If N→B coordinate bonds were to occur in the cell, then these boron containing compounds would perhaps bind more tightly to the calcium channel than their non boron containing counterparts due to coordinate bond formation. Table 3 summarizes all the modifications to 2-APB that were prepared and what influence they had on activity. It should be pointed out that in the present study we have not attempted to alter the diphenyl groups to see if such modifications would influence activity.

This study shows that 2-APB can undergo significant modifications that have little effect on its ability to inhibit thrombin induced Ca^{2+} influx in human platelets. We did however find that two benzyl derivatives (11, 12) were more potent than the parent 2-APB compound. This gives us encouragement that further modifications to the 2-APB molecule can increase its potency even further. We did find previously that diphenyltetrahydrofuran (Dobrydneva and Blackmore 2001 and Table 1) produced approximately 70% inhibition of the thrombin response at 100 µM, however the IC_{50} was approximately 1 µM. Thus diphenyltetrahydrofuran was the most potent inhibitor that we have found so far, although it was not as effective as 2-APB and many of its analogs which produce approximately 100% inhibition at 100 µM (Table 1).

As indicated by Bootman et al., (2002), 2-APB influences calcium homeostasis in a variety of cells by several different mechanisms. These include the original finding that 2-APB inhibits calcium release via IP_{3} receptors (Maruyama et al., 1997), although this effect is cell-specific (Soulsby and Wojcikiewicz, 2002). In addition 2-APB also inhibits IP_{3} receptor ubiquitination and proteasomal degradation in some cells (Soulsby and Wojcikiewicz, 2002). 2-APB has also been shown to inhibit smooth endoplasmic
reticulum calcium ATPase pumps in some cells, thus causing a slow increase in \([\text{Ca}^{2+}]\) (see Bootman et al., 2002 for references). In addition 2-APB prevents mitochondria from releasing \(\text{Ca}^{2+}\) (Prakriya and Lewis, 2001). The best effect of 2-APB so far characterized is its ability to inhibit store mediated calcium influx in a variety of cells including platelets (Bootman et al., 2002; Dobrydneva and Blackmore, 2001). There are many TRP channels that are inhibited by 2-APB in the 10-100 \(\mu\text{M}\) range, these include TRPC1, TRPC3, TRPC5, TRPC6, TRPV6, TRPM3, TRPM7, TRPM8 and TRPP2 (see Xu et al., 2005 for references). Other members of the TRP channels are either activated or unaffected 2-APB. 2-APB has also been shown to modulate the store-operated calcium release–activated \(\text{Ca}^{2+}\) (CRAC) channels (e.g. Braun et al., 2001) and the \(\text{Mg}^{2+}\)-inhibited cation channel (MIC) (e.g. Prakriya and Lewis, 2002; Kozak et al., 2002).

It will be of interest to compare the effects of the 2-APB analogs used in the current study on other parameters that influence \([\text{Ca}^{2+}]\) in a variety of cell types. Different 2-APB analogs may have selective inhibitory or stimulatory effects on the various TRP channels and they are likely to be very useful in elucidating the nature of the TPR channels present in various cells.
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FOOTNOTES

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MOLPHARM/2005/015701

FIGURE LEGENDS:

Figure 1. Structures of 2-APB: open chain form, five membered (1,3,2-oxazaborolidine) ring form created when a N→B coordinate bond is formed and dimeric form produced when two N→B coordinate bonds are formed between two molecules of 2-APB. Dimeric 2-APB could also exist with the formation of only one N→B coordinate bond (structure not shown).

Figure 2. The general scheme for the synthesis of the diphenyl oxazaborolidine analogs is shown. Two examples are shown. In A the synthesis of the parent 2,2-diphenyl-1,3,2-oxazaborolidine (1) is shown, the starting reactants are diphenylborinic anhydride and ethanolamine. In B the synthesis of (S)-4-benzyl-2,2-diphenyl-1,3,2-oxazaborolidine (11) is shown, the starting reactants in this synthesis are diphenylborinic anhydride and (S) (-) 2-amino-3-phenyl-1-propanol.

Figure 3. Structures of compounds (1 through 14) synthesized in this study. The method for the synthesis of each compound is detailed in the text. Compound 15 (3,3-diphenyl dihydrofuran-2-one) was obtained from a commercial source (ACROS). Compounds 5, 8, 9, 11 and 12 contain a chiral carbon atom. Compounds 5, 8 and 9 are racemic mixtures.

Figure 4. Structures of the proposed tautomeric forms of the non-boron analog of 2-APB, 2,2-diphenyl-1,3-oxazolidine (16) and 2-(diphenylmethyleneamino)ethanol (17).
The formation of 16 and 17 involved condensing ethanol amine with benzophenone imine, following deamination of the 2-(aminodiphenylmethylamino)ethanol intermediate.

**Figure 5.** The effect of (S)-4-benzyl-2,2-diphenyl-1,3,2-oxazaborolidine (11) on the ability of thrombin to increase [Ca^{2+}]_i in the presence of extracellular calcium. Extracellular calcium 2.0 mM was added to the platelet suspension 30 seconds before data collection was started. Approximately 10 seconds after data collection was commenced, various concentrations (1, 10, and 100 µM) of (S)-4-benzyl-2,2-diphenyl-1,3,2-oxazaborolidine (11) were added to the platelet suspension in . Thrombin (0.01 units ml^{-1}) was added approximately 10 seconds later. A representative experiment is shown. The ability of thrombin to increase [Ca^{2+}]_i was inhibited in a dose dependent fashion by (S)-4-benzyl-2,2-diphenyl-1,3,2-oxazaborolidine (11).

**Figure 6.** Proposed structure of the acetonitrile adducts of diphenylborinic acid anhydride. Product A is a diacetonitrile adduct of diphenylborinic anhydride, product B is a monoacetonitrile adduct of diphenylborinic acid which could result from the hydrolysis of adduct A. The structure shown in C is the result of combining acetonitrile with boron trifluoride, this adduct is commercially available (e.g. Sigma, CAS number 420-16-6), while adduct D (which has been crystalized) is the result of reaction between imidazole with boron trifluoride.

**Figure 7.** Effect of phenyl pyridyl APB (8), thrombin and thapsigargin to increase [Ca^{2+}]_i in platelets in the presence of extracellular calcium. The concentration of phenyl pyridyl
APB (8) was 100 µM, thapsigargin was 100 nM and two concentrations of thrombin (0.005 and 0.01 units ml⁻¹) were used. Representative traces are shown.

**Figure 8.** Dose response of 2-APB (1), butyl APB (6), (S) benzyl APB (11) and (R) benzyl APB (12) to inhibit the ability of thrombin (0.01 units ml⁻¹) to increase [Ca²⁺], in platelets in the presence of extracellular calcium. Data are mean ± SEM from four separate experiments.

**Figure 9.** Structure of diphenyl oxazaborolidine and oxazolidine rings; showing the position of each modification used in the present study. Atoms in the oxazaborolidine or oxazolidine are numbered 1 through 5 (Table 3). Substituents to the oxazaborolidine and oxazolidine rings are labeled R₁ through R₅ (Table 3).
TABLE 1.
The effect of 2-APB derivatives and non-boron containing analogs on thrombin-induced increases in \([\text{Ca}^{2+}]_i\) in the presence of extracellular calcium in platelets (predominantly calcium influx). Abbreviated trivial names are shown for simplicity together with a designated number for each compound (Figure 3).

<table>
<thead>
<tr>
<th>Compound name (number)</th>
<th>% inhibition of thrombin response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compound concentration (µM)</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>2-APB (1)</td>
<td>98±6</td>
</tr>
<tr>
<td>monomethyl APB (2)</td>
<td>96±0</td>
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<tr>
<td>dimethyl APB (3)</td>
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</tr>
<tr>
<td>butyl APB (6)</td>
<td>97±3</td>
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<tr>
<td>hexyl APB (7)</td>
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</tr>
<tr>
<td>phenyl pyridyl APB (8)</td>
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<tr>
<td>methyl pyridyl APB (9)</td>
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</tr>
<tr>
<td>glycine ester APB (10)</td>
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<tr>
<td>(R) benzyl APB (11)</td>
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<td>(S) benzyl APB (12)</td>
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<td>t-butoxazolidine (14)</td>
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<td>diphenylbutylactone (15)</td>
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<td>diphenyltetrahydrofuran a</td>
<td>69±9</td>
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</table>

a from Dobrydneva and Blackmore (2001)
b stimulates calcium influx by itself at 100 µM
c not determined
TABLE 2.
The effect of 2-APB derivatives and non-boron containing analogs on thrombin-induced increases in \([\text{Ca}^{2+}]_i\) in the absence of extracellular calcium in platelets (internal calcium mobilization). Abbreviated trivial names are shown for simplicity together with a designated number for each compound (Figure 3).

<table>
<thead>
<tr>
<th>Compound name (number)</th>
<th>% inhibition of thrombin response</th>
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<td>Compound concentration (µM)</td>
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<td>2-APB (1)</td>
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<td>monomethyl APB (2)</td>
<td>90±2</td>
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<td>86±6</td>
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<td>89±2</td>
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<tr>
<td>diphenyltetrahydrofuran</td>
<td>51±7</td>
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\(^a\) from Dobrydneva and Blackmore (2001)
\(^b\) stimulates calcium release by itself at 100 µM
\(^c\) not determined
TABLE 3.
Summary of the modifications in the oxazaborolidine or oxazolidine ring (Figure 9) that influence the ability of thrombin to stimulate Ca\(^{2+}\) influx in human platelets. The introduction of carbonyl groups or oxygen into the 5 membered ring; tend to decrease activity. The introduction of a benzyl on carbon 4 increased activity of the parent 2-APB molecule. There were many modifications and substitutions on the ring that could be tolerated and which had minimal effect on activity.

<table>
<thead>
<tr>
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<th>Active</th>
<th>Less Active</th>
<th>More Active</th>
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<tr>
<td>1</td>
<td>oxygen</td>
<td>carbonyl</td>
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</tr>
<tr>
<td>2</td>
<td>boron, carbon</td>
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<td></td>
</tr>
<tr>
<td>3</td>
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<td>5</td>
<td>carbon</td>
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**Substituent group**

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<td>pyridyl</td>
</tr>
<tr>
<td>R(_3)</td>
<td>benzyl</td>
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<tr>
<td>R(_4)</td>
<td>ethyl, butyl</td>
</tr>
<tr>
<td>R(_5)</td>
<td>methyl, phenyl</td>
</tr>
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</table>
Reaction 1

\[
\text{dichlorodiphenyl methane} + \text{2-(methylamino) ethanol} \xrightarrow{\text{Ag}_2\text{O}} \text{(13)}
\]
Reaction 2

\[
\text{dichlorodiphenyl methane} + \text{2-}(\text{tert-butylamino})\text{ ethanol} \xrightarrow{\text{AgOAc}} \text{(14)}
\]
Figure 2

A

diphenyloborinic anhydride + ethanolamine → 2,2-diphenyl-1,3,2-oxazaborolidine + diphenyloborinic acid

B

diphenyloborinic anhydride + (S)(-) 2-amino-3-phenyl-1-propanol → (S)-4-benzyl-2,2-diphenyl-1,3,2-oxazaborolidine + diphenyloborinic acid
Figure 3

1. 

2. 

3. 

4. 

5. 

6. 

7. 

8. 

9. 

10. 

11. 

12. 

13. 

14. 

15.
Figure 4

\[
\text{benzophenone imine} + \text{2-aminoethanol} \leftrightarrow \text{2-(aminodiphenyl methylamino)ethanol}
\]

\[
\text{NH}_3 \quad \text{16} \quad \text{2,2-diphenyl-1,3-oxazolidine} \leftrightarrow \text{17} \quad \text{2-(diphenylmethylened amino)ethanol}
\]
Figure 6
Figure 9