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RESEARCH LETTER

Discovery of novel PC-PLC activity inhibitors

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Abstract

Phosphatidylcholine-specific phospholipase C (PC-PLC) is one of the important members of phospholipase family which is capable of specifically hydrolyzing the third phosphate linker of glycerophospholipid molecules, releasing phosphocholine and diacylglycerols (DAG). It is a crucial virulence factor of bacteria contributed to cell-to-cell spread and leading multiple diseases in mammals. Moreover, PC-PLC has a wide range of biological functions and involves in various cell signaling pathway, including apoptosis, proliferation, differentiation, and metastasis. In this study, we have synthesized 2 chiral compounds (*(R)-7-amino-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-3-ol*, called R-7ABO, and *(S)-7-amino-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-3-ol*, called S-7ABO) and discovered their inhibitory effect on PC-PLC activity which derived from *Bacillus cereus* (*B. cereus*) and human umbilical vein endothelial cells (HUVEC). Therefore, as two novel efficient PC-PLC inhibitors, R-7ABO and S-7ABO might become favorable tools of antibacterial therapy in *B. cereus* infection diseases and researching the function of PC-PLC in HUVECs.

KEYWORDS

Bacillus cereus, PC-PLC inhibitor, R-7ABO, S-7ABO, vascular endothelial cells

1 | INTRODUCTION

As we know, phosphatidylcholine-specific phospholipase C (PC-PLC) which hydrolyzes phosphatidylcholine (PC) to generate 1, 2-diacylglycerol (DAG) and phosphocholine is one of the important members of phospholipase family. PC-PLC also can catalyze the hydrolysis of sphingomyelin (SM) and phosphatidylethanolamine (PE) (Szumilo & Rahden-Staron, 2008). Researches show that bacterial PC-PLC has been purified, whereas gene sequence of mammalian PC-PLC has not been determined (Adibhatla, Hatcher, & Gusain, 2012). Clark *et al.* have demonstrated that *Bacillus* PC-PLC could interact with proteins of mammalian cells via cross-reaction of antibodies although there is not conspicuous sequence homology between *Bacillus* PC-PLC encoding genes and eukaryotic genes (Celandroni, Salvetti, Senesi, & Ghelardi, 2014). It has been reported that *Bacillus cereus* (*B. cereus*) is a kind of aerobic-to-facultative and Gram-positive bacteria which is widely distributed in the environment, including soil, sediments, dust, plants, water, and food (Ribeiro et al., 2010). As common pathogenic bacteria, *B. cereus* contributes to plentiful diseases. For instance, *B. cereus* plays a key role in gastrointestinal infections caused by food poisoning (Drobniewski, 1993). Additionally, *B. cereus* may cause various severe regional and systemic infections, such as endophthalmitis (Beecher, Olsen, Somers, & Wong, 2000), necrosis of the burn wounds (Ribeiro et al., 2010), endocarditis (Block, Levy, & Fritz, 1978), pneumonia, septicemia (Pitt,

McClure, Parker, Amezquita, & McClure, 2015), and so on. It has previously reported that *B. cereus* separated from hospital environment can readily attach to the surface of catheters via producing biofilms which resulting in the formation of microcolonies (Kuroki et al., 2009). Furthermore, it has been strongly proved that PC-PLC is one of the major contributors to retinal toxicity in vitro and in vivo among the multiple toxins and enzymes secreted by *B. cereus* (Beecher et al., 2000). Therefore, it is significant to resist bacterial infections through inhibiting PC-PLC.

Until now, it is suggested that PC-PLC is crucially involved in multiple aspects of cancer biology such as cell metabolism, proliferation, survival, and differentiation. PC-PLC activation could account for 20%–50% of the intracellular phosphocholine (PCho) production in ovarian and breast cancer cells of different subtypes (Podo et al., 2016). PC-PLC showed two- to sixfold activation in breast cancer compared with non-tumoral cells. Inhibition PC-PLC activity was associated with progressive decreases of mesenchymal traits in breast cancer cells (Abalsamo et al., 2012). PC-PLC inhibition could also reduce proliferation of epithelial ovarian cancer cells and in vivo tumor growth in a highly tumorigenic ovarian cancer model (Paris et al., 2017). In addition, inhibition PC-PLC interferes with proliferation, invasion, and glycolysis in U87MG glioma cells (Mercurio et al., 2017). And in squamous carcinoma A431 cells, the PC-PLC activity was higher compared with immortalized non-tumoral keratinocytes (HaCaT) (Cecchetti et al., 2015). These data all suggested that the inhibition of PC-PLC activity might represent a new therapeutic approach to tumor.

Currently, tricyclodecane-9-yl-xanthogenate (D609) has been widely employed for inhibiting PC-PLC activity. As an efficient PC-PLC activity inhibitor, D609 has been used to investigate different functions of PC-PLC during the regulation of a variety of physiological and pathological processes. On the one hand, a study shows that D609 inhibits *B. cereus* PC-PLC via competing with the phosphorylcholine residue of PC for binding to PC-PLC rather than with virus coded enzymes and then interferes with cellular regulation mechanisms. This is a novel construct of antiviral therapy (Amtmann, 1996). On the other hand, D609 also involves in regulating the pathological process of cells by inhibiting PC-PLC activity. For example, the increase in PC-PLC activity causes neuron apoptosis and inflammatory responses, while D609 protects nerve cells from brain pathological process. However, D609 can effectively not only inhibit the activity of PC-PLC but also inhibit the activity of sphingomyelin synthase (SMS), phospholipase D (PLD) (Kiss & Tomono, 1995), and group IV cytosolic phospholipase A2 (cPLA2) (Kang et al., 2008). Chiara Luberto *et al*. have proved that SMS activity was remarkably inhibited by D609 at the concentrations of 10–50 mg/ml when these concentrations were used to study PC-PLC previously (Luberto & Hannun, 1998). Therefore, D609 does not exclusively inhibit PC-PLC activity. It is urgent to explore much more novel, effective, and specific PC-PLC inhibitors to prevent and treat the diseases which are induced by or correlated with bacteria-derived PC-PLC or the abnormal PC-PLC activity in cells.

Here, we have synthesized 2 chiral compounds (*(R)-7-amino-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-3-ol,* called R-7ABO, and *(S)-7-amino-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-3-ol*, called S-7ABO) and identified that they can effectively inhibit the activity of PC-PLC that derived from *B. cereus* and HUVECs. Therefore, R-7ABO and S-7ABO can not only become new potential substances of antibacterial therapy due to their effect on *B. cereus* PC-PLC activity, but also can be utilized to study the function of PC-PLC in vascular endothelial cells.

2 | METHODS AND MATERIALS

2.1 | Cell culture

Human umbilical vein endothelial cells (HUVEC) were obtained as previously described (Jaffe, Nachman, Becker, & Minick, 1973) and then cultured on gelatin-coated plastic dishes in M199 medium (Gibco, USA) with 10% (V/V) fetal bovine serum (Hyclone, USA) and 10 IU/ml fibroblast growth factor 2 (FGF-2) in a humidified incubator at 37°C with 5% CO_2.

2.2 | PC-PLC activity assay

PC-PLC activity assay was performed by using Amplex Red PC-PLC-specific assay kit (Molecular Probes Inc., A12218) according to the manufacturer's instructions. *B. cereus* PC-PLC activity was determined after treated the protein of *B. cereus* PC-PLC (Molecular Probes Inc., A12218) with 0.1% DMSO (Ctrl), R-7ABO, S-7ABO, or D609. For PC-PLC activity in HUVECs, cells were separated into two groups, cultured in normal condition (Norm) and cultured deprived of serum and FGF-2 for 6h. Cell lysates were collected in RIPA lysis buffer (Beyotime, P0013B) containing 1 mM PMSF. The protein concentration was examined by Enhanced BCA Protein Assay Kit (Beyotime, P0009). Then, the protein isolated from cells cultured deprived of serum and FGF-2 was separated into control (Ctrl), R-7ABO-treated, S-7ABO-treated, and D609-treated groups. Incubate 20 μg protein with 0.1% DMSO (Ctrl), different concentrations of R-7ABO, S-7ABO (5, 10, 25, and 50 μM), or D609 (5, 10, 25, 40 μM), respectively, at 37°C for 3 hr and dilute all samples to 100 μl by adding 1X PBS. PC-PLC activity was measured by using Amplex Red PC-PLC-specific assay kit.

2.3 | Cell viability assay

HUVECs cultured deprived of serum and FGF-2 were seeded into 96-well plates and were treated with 0.1% DMSO (v/v) (Ctrl), R-7ABO (5, 10, 25, 50 μM) and S-7ABO (5, 10, 25, and 50 μM), respectively, for 6h. SRB assay was performed to determine cell viabilities. The results were the mean values of triplicate assays.

2.4 | SMS activity assay

An ELISA kit (Jiangsu Jingmei Biological Technology Co. Ltd, JM-04570H1) was used to quantify the concentration of SMS in culture media of HUVECs according to the manufacturer's instructions. HUVECs cultured deprived of serum and FGF-2 were treated with 0.1% DMSO (v/v) (Ctrl), R-7ABO (5, 10, 25, and 50 μM), and S-7ABO (5, 10, 25, and 50 μM), respectively. Light absorption was measured at 450 nm with use of the SpectraMAX 190 microplate spectrophotometer (GMI Co.). Data are expressed relative to a standard curve for SMS.

2.5 | Statistical analysis

All data were presented as means ± *SEM* from at least three independent experiments and analyzed by SPSS (Statistical Package for the Social Sciences) software. When p value was $< .05$, differences were recognized as statistically remarkable.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis of compounds

To a solution of 2, 4-dinitrophenol (**1**, 87 mmol) in DMF (150 ml), NaH (87 mmol) was added. After stirring for 20 min, (*R*)-glycidyl tosylate or (*S*)-glycidyl tosylate (**2**, 87 mmol) was added. The mixture was heated to 80–90°C for 8 hr. After cooling, CH_2Cl_2 (200 ml) and water (150 ml) were added to the mixture. The organic phase was separated and washed with saturated brine (100 ml × 1) and water (100 ml × 4), respectively. The water phase was extracted with CH_2Cl_2 (100 ml × 1). The combined organic phase was dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using CH_2Cl_2 as an eluent to afford yellow oil. The oil was dissolved in mixed solvent of ethyl acetate and petroleum ether. The solution was frozen to give (*R*)-2-((2,4-dinitrophenoxy)methyl)oxirane, or (S)-2-((2,4- dinitrophenoxy)methyl)oxirane, compound **3** as yellow solid. To a solution of HOAc (3 ml), EtOH (360 ml), and H_2O (40 ml), compound **3** (3 mmol) was added. The mixture was stirred and warmed until compound **3** was dissolved. Then, iron powder (18 mmol) was added. The mixture was heated to reflux for 1 hr with vigorous stirring. After cooling, Na_2CO_3 (15 g) was added to the solution. The mixture was stirred for 30 min. After filtration, the solvent was removed under reduced pressure. The residue was dispersed in CH_2Cl_2 (100 ml) and stirred for 1 hr. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (15/1, v/v) as an eluent to afford compound **4 (R-7ABO or S-7ABO)** as white solid (Figure 1). The X-ray crystal structures of compounds R-7ABO and S-7ABO were shown in Figure 2.

3.1.1 | (R)-7-amino-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-3-ol (R-7ABO)

White solid, yield 59.2%; m.p. 187–188°C;$[\alpha]_D^{20} = +53.1°$ (C = 0.08 g/100 ml, CH_3OH); IR (ν_{max} cm^{-1}): 3,395, 2,919, 1614, 1519, 1,214, 1,108; ^1H NMR (d_6-DMSO, 400 MHz): δ (ppm) 2.89 (dd, 1 H, $J_1 = 12.7$, $J_2 = 7.6$, CH), 3.20 (dd, 1 H, $J_1 = 12.7$, $J_2 = 4.6$, CH), 3.59 (dd, 1 H, $J_1 = 11.8$, $J_2 = 5.6$, CH), 3.77 (m, 1 H, CH), 4.02 (dd, 1 H, $J_1 = 11.8$, $J_2 = 3.9$, CH), 4.49 (s, 2 H, NH_2), 4.83 (d, 1 H, $J = 5.2$, OH), 4.98

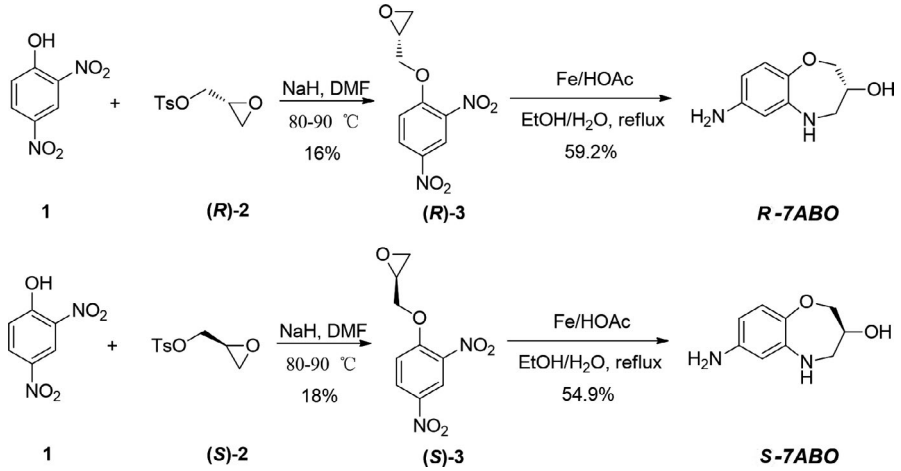

FIGURE 1 Synthesis process of compounds R-7ABO and S-7ABO

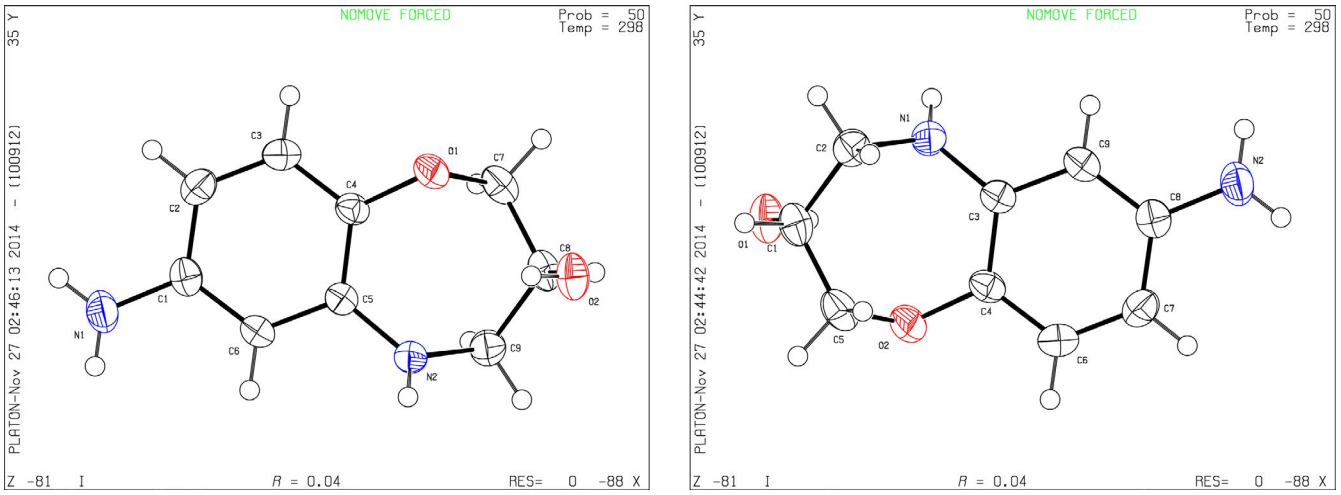

FIGURE 2 The X-ray crystal structures of compounds R-7ABO and S-7ABO [Colour figure can be viewed at wileyonlinelibrary.com]

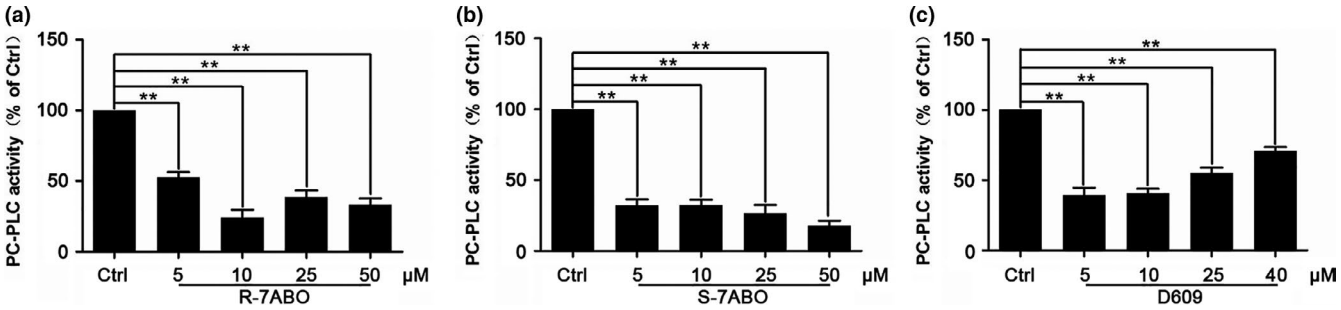

FIGURE 3 R-7ABO and S-7ABO inhibit *Bacillus cereus* PC-PLC in a dose-depended manner. Incubate 0.015 U (0.15 U/ml) *B. cereus* PC-PLC with R-7ABO (5, 10, 25, and 50 μM) (a), S-7ABO (5, 10, 25, and 50 μM) (b), or D609 (5, 10, 25, and 40 μM) (c), dilute samples by adding 1X PBS to 100 μl. The control group (Ctrl) was treated with 0.1% DMSO (V/V). Samples were incubated at 37°C for 3 hr, and PC-PLC activity was determined by utilizing Amplex Red PC-PLC-specific assay kit (Molecular Probes Inc., A12218). (*$p < .05$, **$p < .01$ versus Ctrl, $n = 3$)

(s, 1 H, NH), 5.83 (dd, 1 H, $J_1 = 8.3$, $J_2 = 2.6$, ArH), 5.94 (d, 1H, $J = 2.6$, ArH), 6.45 (d, 1H, $J = 8.3$, ArH). ^{13}C NMR (d_6-DMSO, 100 MHz): δ (ppm) 51.5, 69.6, 76.2, 104.4, 105.5, 121.2, 140.9, 142.7, 144.5. DEPT-135 (d_6-DMSO): δ (ppm) 51.5 (CH_2), 69.6 (CH), 76.2 (CH_2), 104.4 (CH), 105.5 (CH), 121.2 (CH). HRMS calcd for $[M + H]^+$ $C_9H_{13}N_2O_2$: 181.0977, found 181.0969.

3.1.2 | (S)-7-amino-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-3-ol (S-4)

White solid, yield 54.9%; m.p. 187–189°C; $[\alpha]_D^{20} = -53.1°$ (C = 0.08 g/100 ml, CH_3OH); IR (ν_{max} cm^{-1}): 3,397, 2,920, 1,612, 1,515, 1,217, 1,106; ^{1}H NMR (d_6-DMSO, 400 MHz): δ (ppm) 2.89 (dd, 1 H, $J_1 = 11.2$, $J_2 = 7.9$, CH), 3.20 (dd, 1 H, $J_1 = 11.2$, $J_2 = 4.5$, CH), 3.59 (dd, 1 H, $J_1 = 11.8$, $J_2 = 5.6$, CH), 3.78 (m, 1 H, CH), 4.02 (dd, 1 H, $J_1 = 11.8$, $J_2 = 3.9$, CH), 4.47 (s, 2 H, NH_2), 4.82 (d, 1 H, $J = 5.5$, OH), 4.98 (s, 1 H, NH), 5.83 (dd, 1 H, $J_1 = 8.3$, $J_2 = 2.4$, ArH), 5.95 (d, 1H, $J = 2.4$, ArH), 6.45 (d, 1H, $J = 8.3$, ArH). ^{13}C NMR (d_6-DMSO, 100 MHz): δ (ppm) 51.5, 69.6, 76.2, 104.4, 105.5, 121.2, 140.9, 142.7, 144.5. DEPT-135 (d_6-DMSO): δ (ppm) 51.5 (CH_2), 69.6 (CH), 76.2 (CH_2), 104.4 (CH), 105.5 (CH), 121.2 (CH). HRMS calcd for $[M + H]^+$ $C_9H_{13}N_2O_2$: 181.0977, found 181.0990.

3.2 | R-7ABO and S-7ABO inhibit *B. cereus* PC-PLC in a time-depended manner

First, we investigated whether R-7ABO and S-7ABO inhibited the activity of PC-PLC from *B. cereus*. Previous researches indicated that D609 inhibits bacterial PC-PLC specifically (Amtmann, 1996). Therefore, we utilized D609 as a positive control (≤10 mg/ml, Figure 3c) when *B. cereus* PC-PLC activity was determined after treatment with R-7ABO or S-7ABO. The results showed that both R-7ABO (Figure 3a) and S-7ABO (Figure 3b) inhibited the activity of *B. cereus* PC-PLC significantly.

Next, we detected whether R-7ABO and S-7ABO inhibited the activity of *B. cereus* PC-PLC in a time-dependent manner. After incubating *B. cereus* PC-PLC (0.015 U) with 5 μM R-7ABO or S-7ABO for 1.5 hr, 3, and 6 hr at 37°C,

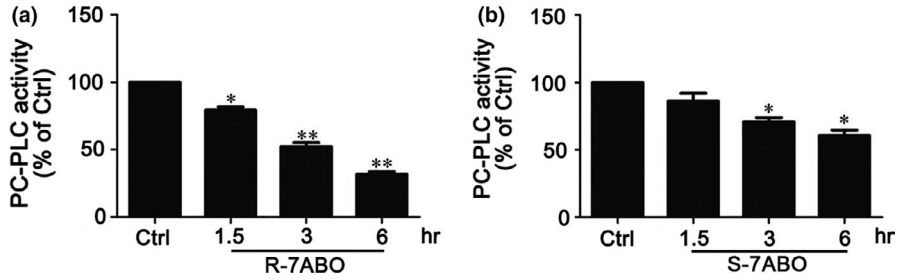

FIGURE 4 R-7ABO and S-7ABO inhibit *Bacillus cereus* PC-PLC in a time-depended manner. Incubate 0.015 U (0.15 U/ml) *B. cereus* PC-PLC with R-7ABO (a) or S-7ABO (b) at the concentration of 5 μM, dilute samples to 100 μl by adding 1X PBS. The control group (Ctrl) was treated with 0.1% DMSO (V/V). Samples were incubated at 37°C for 1.5, 3, or 6 hr, respectively. PC-PLC activity was determined by utilizing Amplex Red PC-PLC-specific assay kit. (*$p < .05$, **$p < .01$ versus Ctrl, $n = 3$)

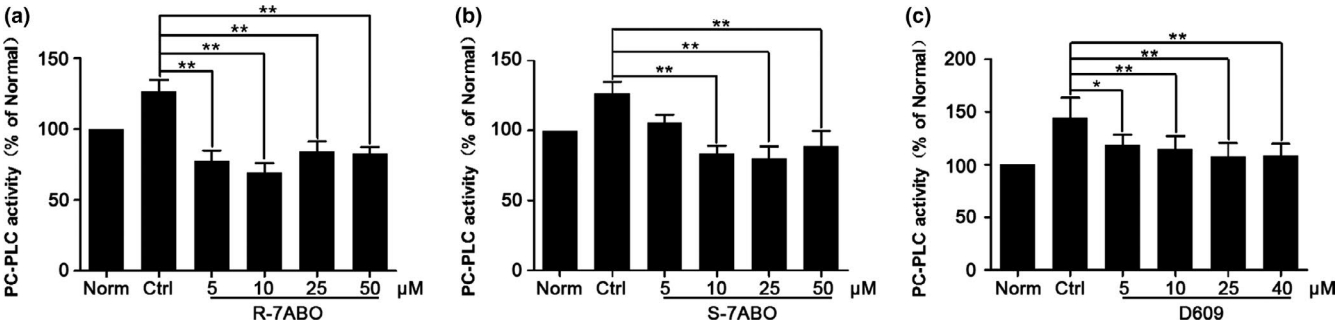

FIGURE 5 R-7ABO and S-7ABO inhibit HUVEC-derived PC-PLC activity. After serum and FGF-2 starvation for 6 hr, cells were lysed and the protein supernatant was collected after centrifugation. In the normal group (Norm), the cells were cultured in the medium with 10% FBS and 10 IU/ml FGF-2. The cells of control group (Ctrl) were treated with 0.1% DMSO (V/V). Incubate 20 μg protein with different concentrations of R-7ABO, S-7ABO (5, 10, 25, and 50 μM), or D609 (5, 10, 25, and 40 μM), respectively, at 37°C for 3 hr, dilute samples to 100 μl by adding 1× PBS. PC-PLC activity was measured by using Amplex Red PC-PLC-specific assay kit. (*$p < .05$, **$p < .01$ versus Ctrl, $n = 3$)

we measured PC-PLC activity by using Amplex Red PC-PLC-specific assay kit. The results indicated that R-7ABO and S-7ABO inhibited the activity of *B. cereus* PC-PLC in a time-dependent manner (Figure 4a,b).

More than 40 years ago, *B. cereus* has already been recognized as a pathogenic agent of food poisoning-related diseases and different serious infections (Wright, 2016). *Bacillus cereus* is ubiquitous in the environment, and it has been isolated from various polluted crude and processed food products (Ghelardi et al., 2002). Santos. C.A. *et al.* suggested that *B. cereus* which obtained from food are commonly connected with food contamination matters and diarrhea (Santos et al., 2011). PC-PLC has been considered as one of the primary virulence factors produced by *B. cereus,* and it is encoded by *plc*A gene. It has been suggested that *Bacillus* PC-PLC is a kind of monomeric enzyme, and there are three Zn^{2+} atoms in its active site which participates in the binding to the substrate and strongly associated with enzymatic activity as well as stability (Gonzalez-Bulnes et al., 2010). At the moment, D609 is still the only one recognized model compound which is broadly applied to inhibit PC-PLC activity. D609 has been demonstrated to be a potent inhibitor of *B. cereus* PC-PLC (Amtmann, 1996) and *C. perfringens* α-toxin (Preuss, Kaiser, & Gehring, 2001). Gonzalez-Roura, Casas, and Llebaria (2002 reported that D609-inhibited *B. cereus* PC-PLC activity was related to change the harmony of Zn^{2+} atoms in the active sites of PC-PLC enzyme. Our data suggested that R-7ABO and S-7ABO performed better inhibitory effect on *B. cereus* PC-PLC. They could inhibit *B. cereus* PC-PLC activity more effectively than D609 in a time- and dose-depended manner. However, the exact mechanisms by which R-7ABO and S-7ABO inhibit *B. cereus* PC-PLC activity need to be investigated in our next study.

3.3 | R-7ABO and S-7ABO inhibit the activity of HUVEC-derived PC-PLC

Because mammalian PC-PLC has not been purified and the gene sequence of it has not yet been resolved, researchers take advantage of PC-PLC inhibitor D609 as a pharmacological tool to study this enzyme. In mammals, PC-PLC not only participates in many important cell signaling pathways but also implicates in different physiological and pathological processes. During the progress of cerebral ischemic injury, PC-PLC activity increased occurs with neuron apoptosis and the expression of pro-inflammatory cytokines (Li et al., 2015). Furthermore, PC-PLC contributes to endothelial dysfunction

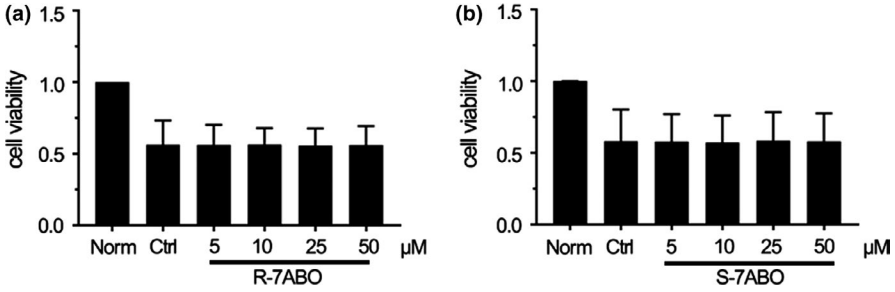

FIGURE 6 R-7ABO and S-7ABO did not decrease cell viability. Viabilities of HUVECs were analyzed by SRB after treatment with R-7ABO (a) and S-7ABO (b). Data are mean ± *SEM*

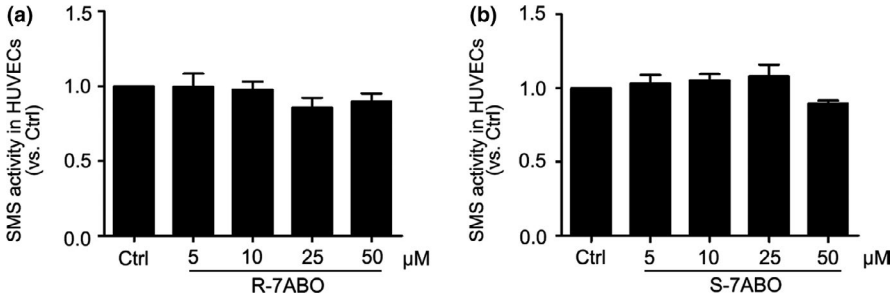

FIGURE 7 R-7ABO and S-7ABO did not inhibit SMS activity in HUVECs. HUVECs cultured deprived of serum and FGF-2 were treated with 0.1% DMSO (v/v) (Ctrl), R-7ABO (5, 10, 25, and 50 μM) (a), and S-7ABO (5, 10, 25, and 50 μM) (b), respectively, for 6h. Data are mean ± *SEM*

and the development of atherosclerosis. We have found that PC-PLC activity induced by LPS and oxLDL resulted in IL-8 secretion, leukocyte adhesion, and stabilizing plaque. Thus, D609 has the effect of anti-atherogenic (Li, Zhang, Yin, Zhang, & Miao, 2010). And our previous study found that PC-PLC regulated human umbilical vein endothelial cell (HUVEC) autophagy negatively and played an important role in the development of atherosclerosis (Wang et al., 2013). PC-PLC could be activated after deprivation of FGF-2 and serum in HUVECs. And the inhibitor D609 could inhibit the activity of PC-PLC in HUVECs deprived of FGF-2 and serum (Miao, Kaji, Hayashi, & Araki, 1997). Thus, in this study, we used this model. Firstly, cells were separated into two groups, cultured in normal condition (Norm) and cultured deprived of serum and FGF-2 for 6h to increase the activity of PC-PLC. Cell lysates were collected. Then, the protein isolated from cells cultured deprived of serum and FGF-2 was separated into control (Ctrl), R-7ABO-treated, S-7ABO-treated, and D609-treated groups. Incubate 20 μg protein with 0.1% DMSO (Ctrl), different concentrations of R-7ABO, S-7ABO (5, 10, 25, and 50 μM), or D609 (5, 10, 25, and 40 μM), respectively, at 37°C for 3 hr. PC-PLC activity was measured by using Amplex Red PC-PLC-specific assay kit. The data suggested that R-7ABO (Figure 5a) and S-7ABO (Figure 5b) inhibited the activity of PC-PLC that derived from HUVEC effectively. D609 was used as the positive control (Figure 5c).

3.4 | R-7ABO and S-7ABO did not decrease the cell viability

Although R-7ABO and S-7ABO inhibited the activity of HUVEC-derived PC-PLC (Figure 5), it might be due to the decreased cell viability induced by cytotoxic effect. To exclude the possibility, we performed SRB assay to detect cell viability. The data showed that R-7ABO and S-7ABO did not decrease the cell viability (Figure 6). Thus, the inhibition of PC-PLC activity is not due to a more general cytotoxic effect.

3.5 | R-7ABO and S-7ABO did not inhibit the activity of SMS in HUVECs

It is suggested that D609 can effectively not only inhibit the activity of PC-PLC but also inhibit the activity of sphingomyelin synthase (SMS), phospholipase D (PLD) (Kiss & Tomono, 1995) and group IV cytosolic phospholipase A2 (cPLA2) (Kang et al., 2008). Chiara Luberto *et al.* have proved that SMS activity was remarkably inhibited by D609. Thus, furthermore, we examined the SMS activity in HUVECs treated with R-7ABO and S-7ABO. The results showed that R-7ABO and S-7ABO did not inhibit the activity of SMS in HUVECs (Figure 7).

4 | CONCLUSIONS AND FUTURE DIRECTIONS

In summary, we synthesized 2 chiral compounds and discovered their negative regulation on the activity of PC-PLC which stemmed from *B. cereus* and HUVEC, respectively. Moreover, R-7ABO and S-7ABO performed better inhibitory effect on *B. cereus* and HUVEC-derived PC-PLC activity than D609. And compared with D609, R-7ABO and S-7ABO did not inhibit the activity of SMS. In our next step, we will clarify the mechanisms of action of these inhibitors (competitive, uncompetitive, or mixed). Not long in

the future, R-7ABO and S-7ABO might become new potential substances of antibacterial therapy due to their effect on *B. cereus* PC-PLC activity. Moreover, R-7ABO and S-7ABO could be two favorable tools for researching the function of PC-PLC in endothelial cells.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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