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The interaction between phenylboronic acid derivatives and active ingredients with diphenol structure of traditional Chinese medicine

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Abstract
Many active ingredients of traditional Chinese medicine with important pharmacological effects always have glycol or diphenol structure, which lays a foundation for the combination with phenylboronic acid (PBA) derivatives to form cyclic boronic esters compounds. Herein, four important pharmacological active ingredients, namely baicalein, baicalin, gallic acid and protocatechuic acid, were chosen to study the interaction with PBA derivatives. Five PBA derivatives of 3-aminophenylboronic acid monohydrate (APBA), 3-acrylaminophenylboronic acid (AAPBA), poly(3-acrylaminophenylboronic acid) (PAAPBA), poly[(poly(ethylene glycol) methacrylate-block-3-acrylaminophenylboronic acid)] (PE₃PB), and poly[(poly(ethylene glycol) methacrylate-random-3-acrylaminophenylboronic acid)] (PE₅PB) were used. The interactions between five PBA derivatives and four active ingredients were explored by fluorescent spectrophotometer using the alizarin red (ARS) method. The fluorescent intensity of PBA derivative-ARS-active ingredient mixture was decreasing with the increasing concentrations of active ingredients. In comparison, the fluorescent intensity of PAAPBA, PE₃PB, and PE₅PB showed an obviously decrease after active ingredients were added, while the fluorescent intensity of APBA and AAPBA showed a gradually decrease after active ingredients were added. These results indicated a stronger interaction between PBA polymers and active ingredients than that of APBA and AAPBA. Simultaneously, PE₃PB and PE₅PB could enhance cellular uptake of baicalin in A549 cells. This research provided new strategies for improving the bioavailability and water solubility, extending the circulation time, and wider application of the active ingredients of traditional Chinese medicine in the prevention and therapy of diseases.

Keywords Phenylboronic acid · Active ingredients · ARS · Fluorescent spectra

Introduction
It is well known that many traditional Chinese medicines with proven curative effects have been widely used in clinical practice and have made indelible contributions to human health (Nahin et al. 2007; Wang et al. 2016; Takayama and Iwasaki 2017; Yu et al. 2017). Under the general trend of international “herbal fever” in the world, active ingredients with special pharmacological effects of traditional Chinese medicine have attracted much attention owing to their pivotal role in the prevention and treatment of diseases (Yang et al. 2013; Liu et al. 2015).

Although many active ingredients of traditional Chinese medicine have important pharmacological effects, their inherent shortcomings limit their wide application. For example, baicalein shows antibacterial (Palierse et al. 2021), antiviral (Lalani et al. 2020), anticancer effects (Bie et al. 2017). However, baicalein has poor water solubility and is easily oxidized. Therefore, taking effective measures
to improve water solubility and protect from oxidation is of great significance for the wide application of baicalein in the treatment of diseases.

Phenylboronic acid (PBA) derivatives are well known to combine with polyol and diphenol compounds to reversibly form cyclic boronic esters (Lorand and Edwards 1959; Van et al. 1984; Sadasivuni et al. 2013; Govindaraj et al. 2017). For example, Melavanki et al. investigated the interaction between boronic acids and sugar, and study the effect of structural change of sugars on binding affinity (Bhavya et al. 2020; Melavanki et al. 2020). As shown in Fig. 1, boronic acid is weak Lewis acid, and it will convert into tetrahedral boronate after boronic acid reacts with water. This process is responsible for the ability of boronic acid to bind reversibly with diols and other Lewis bases (Bhavya et al. 2020; Ni et al. 2012). The reversible boronic ester could be facilitated in the drug delivery systems. For example, phenyboronic acid and catechol can form boronate esters in a drug delivery system that can improve the stability and in response to acidic pH values and cis-diols (Li et al. 2014, 2012).

The unique chemistry of PBA derivatives makes a foundation to design PBA derivatives-based drug delivery systems, which can greatly enhance molecular interactions by multivalent effects with polyol or diphenol compounds (Lan and Guo 2019). Many active ingredients have glycol or diphenol structure, which makes a chance to circumvent their own shortcomings by combining with PBA derivatives in drug delivery systems.

Herein, we chose five PBA derivatives to study their interaction with four important active ingredients of traditional Chinese medicine. Five PBA derivatives were 3-aminophenylboronic acid monohydrate (APBA), 3-acryl aminophenylnoronic acid (AAPBA), poly(3-acryl aminophenylboronic acid) (PAAPBA), poly[(poly(ethylene glycol) methyl ether methacrylate)-block-(3-acryl aminophenylboronic acid)] (PPEGMA-b-PAAPBA), and poly[(poly(ethylene glycol) methyl ether methacrylate)-random-(3-acryl aminophenylboronic acid)] (PPEGMA-r-PAAPBA), respectively. Four active ingredients were baicalin, baicalein, gallic acid, and protocatechuic acid, respectively. The interaction of PBA derivatives with alizarin red (ARS) was studied with UV and fluorescent spectrophotometers, respectively. The interaction between PBA derivatives and active ingredients was explored with a fluorescent spectrophotometer.

**Materials and experiments**

**Materials**

APBA (98%), ARS (≥ 80%), and 2, 2′-azobisisobutyronitrile (AIBN, 98%) were bought from Heowns Biochem Technologies LLC (Tianjin, China). AIBN was recrystallized twice from ethanol and dried in a vacuum before being used. PEGMA (Mn ≈ 300) was bought from Innochem Co., Ltd. (Beijing, China) and filtered through an alumina column before use. 3-[4,5-, 4-Cyanopentanoic acid dithiobenzoate (CPADB, 97%) was purchased from Bidepharm Co., Ltd. (Shanghai, China). Baicalin (90%), baicalein (98%), gallic acid (98.5%), and protocatechuic acid (≥ 97%) were purchased from Shanghaiyuanye Bio-Technology Co., Ltd. (Shanghai, China). Other reagents were of analytical grade and used as received.

**Synthesis of AAPBA**

AAPBA was prepared using the modified method described by Lee et al. (2004). APBA (5.0 g, 3.22 × 10⁻² mol), NaHCO₃ (5.0 g, 6.0 × 10⁻² mol), THF (15 mL), and H₂O (30 mL) were added to a round bottom flask in an ice bath. Acryloyl chloride (5.0 mL, 5.0 × 10⁻² mol) was added dropwise over 0.5 h, then the mixture was reacted for 2 h at 25 °C. Ethyl acetate was added to extract production three times and then the organic layer was concentrated under vacuum. The resulting solid residue was dissolved in distilled water (80 °C), and the insoluble residues were filtered off. Filtrate was kept at 4 °C overnight, then AAPBA was obtained as crystals.

**Synthesis of PAAPBA**

PAAPBA was synthesized through reversible addition-fragmentation chain transfer (RAFT) polymerization using AAPBA as a monomer, AIBN as initiator, and CPADB as RAFT agent. AAPBA (955 mg, 5 mmol), CPADB (6.9 mg, 0.1 mmol), and AIBN (3.28 mg, 0.02 mmol) were dissolved in DMF (1.5 mL). The mixture was reacted at 70 °C for 24 h after purging with argon for 30 min. The tube was doused into an ice bath for 5 min to terminate the reaction. PAAPBA was obtained after precipitation into ethyl acetate, washed, and dried.

**Synthesis of PPEGMA-b-PAAPBA**

PPEGMA-b-PAAPBA was synthesized through the RAFT method using PEGMA as a monomer, AIBN as initiator, and PAAPBA as macro-RAFT agent with [PEGMA]/[PAAPBA-RAFT]/[AIBN] = 50:1:0.2 ratio. PEGMA (0.55 mL, 5 mmol), PAAPBA-RAFT (955 mg, 0.1 mmol), and AIBN (3.28 mg, 0.02 mmol) were dissolved in DMF (2.0 mL). The mixture was reacted at 70 °C for 48 h after purging with argon for 30 min. The tube was doused into an ice bath for 5 min to terminate the reaction. PPEGMA-b-PAAPBA was obtained through precipitation into ethyl acetate, washed, and dried. The copolymer was simply named as PEₜPB.
Fig. 1  Binding mechanism between phenylboronic acid and a diol

**Synthesis of PPEGMA-\(r\)-PAAPBA**

PPEGMA-\(r\)-PAAPBA was synthesized through the RAFT method using PEGMA and AAPBA as monomers, AIBN as initiator, and CPADB as RAFT agent with \([\text{PEGMA}]/[\text{AAPBA}]/[\text{CPADB}]/[\text{AIBN}] = 50:50:1:0.2\) ratio. PEGMA (0.55 mL, 5 mmol), AAPBA (955 mg, 5 mmol), CPADB (6.9 mg, 0.1 mmol), and AIBN (3.28 mg, 0.02 mmol) were dissolved in DMF (2.0 mL). The mixture was reacted at 70 °C for 48 h after purging with argon for 30 min. The tube was doused into an ice bath for 5 min to terminate the reaction. PPEGMA-\(r\)-PAAPBA was obtained through precipitation into ethyl acetate three times, washed, and dried. The copolymer was simply named as PE\(r\)PB.

**Preparation of polymer nanoparticles**

Polymer nanoparticles were prepared using nanoprecipitation method. Briefly, 10 mg of polymers (PAAPBA, PE\(b\)PB, and PE\(r\)PB, respectively) was dissolved in DMSO (1 mL). Deionized H\(_2\)O (10 mL) was added dropwise under ultrasound. Then, the solution was transferred to a dialysis tube (MWCO 3500) and dialyzed against deionized H\(_2\)O for 24 h. The organic solvent was removed by replacing deionized H\(_2\)O every 3 h. Finally, three nanoparticles were obtained after freeze-drying the suspension, and they were simply named as NPS\(_{PAAPBA}\), NPS\(_{PEbPB}\), and NPS\(_{PErPB}\), respectively.

**Study of the interaction between PBA derivatives and ARS**

APBA, AAPBA, NPS\(_{PAAPBA}\), NPS\(_{PEbPB}\), and NPS\(_{PErPB}\), respectively, were diluted to different concentrations from 0.01 to 1.25 mmol/L. ARS (0.15 mmol/L) was added to the above solution with the same volume. The interaction...
between PBA derivatives and ARS was measured with UV and fluorescent spectrophotometer, respectively.

**The interaction between PBA derivatives and active ingredients**

PBA derivatives, ARS and four active ingredients molecules (baicalin, baicalein, gallic acid, and protocatechuic acid) were added to a series of tubes. The final concentrations of PBA derivatives and ARS were 1 mmol/mL and 0.1 mmol/mL. Four active ingredients were diluted to obtain the final concentrations from 0.0 to 9.0 mmol/L in tubes, respectively. The interaction between PBA derivative and active ingredients was measured by a fluorescent spectrophotometer.

**Cellular uptake**

To evaluate cellular internalization, NIH3T3 and A549 cells, respectively, were seeded in 24-well plates at a density of $5 \times 10^4$ cells/well and incubated overnight. Then cells were co-incubated with baicalin, PE$p$B@baicalin, and PE$r$PB@baicalin, respectively. After 6 h, cells were washed three times with PBS buffer, fixed with 4% paraformaldehyde, and sequentially stained with DAPI. The cellular uptake was determined by fluorescent microscopy.

**Results and discussion**

**Preparation and characterization of PBA derivatives**

we prepared AAPBA and three polymers (PAAPBA, PE$p$B, and PE$r$PB) through RAFT polymerization using CPADB as chain transfer agent, and AAPBA/PEGMA as monomers (Fig. 2). The structures of AAPBA and three polymers were evaluated by $^1$H NMR as shown in Fig. 3. In Fig. 3A of AAPBA spectrum, peaks at 5.7–6.5 ppm were assigned to protons on the double bond, and signals at 7.3 ~ 7.9 ppm
were assigned to protons on the benzene ring. Compared with the spectrum of AAPBA, signals of the double bond in PAAPBA (Fig. 3B) completely disappeared, and peaks of phenyl group at 6.8 ~ 7.9 ppm were retained, confirming the successful preparation of PAAPBA. Simultaneously, the new resonance signals in PAAPBA at 0.8 ~ 2.4 assigned to protons from the main chain appeared. After copolymerization of PAAPBA and PEGMA, the new resonance signals appeared at 3.2 ~ 3.7 ppm assigned to protons from methylene in side chains of PEGMA, indicating PEbPB was synthesized successfully (Fig. 3C). In Fig. 3D, there were...
Fig. 5 The interaction between different PBA derivatives and ARS measured by fluorescent spectrophotometer. A, B The fluorescent spectra of APBA-ARS complex and C the peak values of fluorescent spectra at different APBA concentrations; D, E The fluorescent spectra of AAPBA-ARS complex and F the peak values of fluorescent spectra at different AAPBA concentrations; G, H The fluorescent spectra of PAAPBA-ARS complex and I the peak values of fluorescent spectra at different AAPBA concentrations; J, K The fluorescent spectra of PEbPB-ARS complex and L the peak values of fluorescent spectra at different PEbPB concentrations; M, N The fluorescent spectra of PErPB-ARS complex and O the peak values of fluorescent spectra at different PErPB concentrations.

Fig. 6 Four active ingredients of traditional Chinese medicine

Fig. 7 A The ternary competitive system of PBA derivatives, ARS and diphenol. B–E $^{11}$B NMR spectra of APBA and APBA-four compounds. F–I $^{11}$B NMR spectra of PErPB and PErPB-four compounds.
similar signals in the spectrum of PePb to that of PebPb, indicating that PePb were successfully prepared.

The interaction between PBA derivatives and ARS

PBA derivatives can combine with ARS reversibly (Fig. 4A) (Springsteen et al. 2001). The bonding of ARS with boronic acid groups can cause changes in fluorescence intensity, which can be used as a universally applicable indicator to analyze the interaction between boronic acid and diol/diphenol molecules (Li et al. 2014; Takahashi et al. 2018; Gen-nari et al. 2017). Compared with the weak fluorescence of ARS, the ARS-boronic acid compound showed strong fluorescence emission. When diol/diphenol molecules were added, the fluorescent intensity weakens due to the original ARS-boronic acid balance was broken.

The interaction between five PBA derivatives and ARS is studied by UV (Fig. 4) and fluorescent spectrophotometer (Fig. 5). In Fig. 4B and S1, UV spectra of APBA, ARS and APBA-ARS complex were measured. Compared with APBA and ARS, the absorption of APBA-ARS complex obviously increased at about 450 nm. The absorption at about 450 nm was increasing over the increasing concentrations of APBA (from 0.010 to 1.250 mmol/L) (Fig. 4C). These results indicated that APBA and ARS interacted and formed APBA-ARS compound. In Figs. 4D, E, there were similar phenomenon and similar peak types to APBA owing to the similar structure between AAPBA and APBA. These results indicated that AAPBA could interact with ARS and form AAPBA-ARS compound. For three polymers of PAAPBA (Figs. 4F, G), PEBP (Figs. 4H, I), and PePb (Figs. 4J, K), there were similar peak location and types among the three polymers, but different from that of APBA and AAPBA. These differences may be ascribed to the obvious different structures of three polymers to APBA and AAPBA. Compared with the spectra of polymers and ARS, the spectra of polymer-ARS compounds showed obvious absorption at 500–600 nm, and the absorption intensity of polymer-ARS compound obviously increased with the increasing concentrations of three polymers (the concentrations of AAPBA in the polymer are from 0.010 to 1.250 mmol/L). Simultaneously, the peak type changed from a single peak to double peak over the increasing concentrations of polymers. To dissolve polymers, PAAPBA, AAPBA, and PAAPA were dissolved in an alkaline solution. In alkaline solution, UV absorption spectrum of ARS was the double peak (Figure S2). With the increasing concentrations of polymers, the concentration of alkaline solution increased, which led to the UV absorption spectrum changing from a single peak to double peak. Above all, these results indicated that three polymers could interact with ARS and form polymer-ARS compounds.

Figure 5 showed the fluorescent spectra of APBA, AAPBA, PAAPBA, PEBP, and PePb. It was observed that all the fluorescent intensity increased with the increasing PBA derivatives concentrations with similar peak location at about 600 nm, indicating that five PBA derivatives can interact with ARS. For the fluorescent peak results, APBA and AAPBA showed similar phenomenon and three polymers showed similar phenomenon, which maybe resulted from their different structures. For APBA and AAPBA, the molecules existed alone in the solution and could freely combine with ARS. For PAAPBA, PEBP, and PePb, PBA molecules existed in the chain of polymers and its motion was limited by the polymer chain. Notably, there was a different pattern of fluorescent spectra and peak values at low and high concentrations of three polymers. According to Flory-Krigbaum dilute solution theory, molecular chains are far apart and independent of each other. In the dilute polymer solution, polymer monomolecular chain coils exist in isolation, and there is no overlap between each other. In a concentrated solution, polymer chains are close to each other or even penetrates each other, and physical cross-linking (entanglement) or interaction occurs between the molecular chains. Therefore, at lower concentrations of three polymers, polymers existed as a free chain. At higher concentrations, the polymer formed into nanoparticle, and PBA molecules gathered into the core of nanoparticles. Above all, these results indicated that five PBA derivatives were capable of combining with ARS.

The interaction between PBA derivatives and active ingredients

In recent years, active ingredients of traditional Chinese medicine have attracted much attention on the prevention and treatment of diseases (Liang et al. 2017; Kosuru et al. 2018; Sun et al. 2020). Many active ingredients own diphenol structures, which lays the foundation for complex PBA derivatives. The unique chemistry of PBA derivatives with diphenol compounds provides a way to design PBA-based drug delivery system. Herein, four common and important pharmacological active ingredients, namely baicalin (Fig. 6A), baicalein (Fig. 6B), gallic acid (Fig. 6C),...
and protocatechuic acid (Fig. 6D), were used to study the interaction with PBA derivatives. All four active ingredients show many important pharmacological effects. Baicalein has been used in the clinic for the treatment of pneumonia, cerebral ischemia, hepatitis, infection and tumors due to its antibacterial, anti-inflammatory, antihypertensive, anti-allergic properties. (Liang et al. 2017) Gallic acid has good anti-inflammatory and antioxidant effects and good pharmacological effects on cardiovascular system, nervous system diseases, diabetes, liver fibrosis, tumors, etc. (Khan et al. 2018; Bai et al. 2021; Choubey et al. 2018). Protocatechuic acid is a natural phenolic acid, which widely exists in our daily diet and herbs. Protocatechuic acid plays important roles in antioxidant, anti-inflammatory, anti-tumor, antiviral, and neuroprotective effects, protection from metabolic syndrome, and preservation of liver, kidneys, and reproductive functions (Krzysztoforska et al. 2019; Kakkar and Bais 2014; Song et al. 2020).

The interaction between PBA derivatives and ARS was in dynamic equilibrium (Springsteen et al. 2001). When diol/diphenol molecules were added to PBA-ARS system, the fluorescent intensity weakened due to the original ARS-boronic acid balance being broken (Fig. 7A). To verify the binding action between phenylboronic acid and four active ingredients, APBA and PErPB were chosen to measure the relative contents of free Boronic and boronate complex using $^1$B NMR spectra. As shown in Fig. 7, all the $^1$B NMR spectra consist of two peaks: free boronic peak and boronate complexed peaks. Compared with APBA, the boronate complexed peak intensity was higher for APBA-four compounds (Fig. 7B–E). The $^1$B NMR spectra of PErPB and PErPB-four compounds showed similar phenomenon to APBA (Fig. 7F–I). These results indicated that there was more boric acid ester formed after four compounds were added into APBA and PErPB, respectively. Based on these results, we guessed that APBA, AAPBA, PAAPBA, PEbPB, and PErPB could bind with baicalein, baicaline, gallic acid, and protocatechuic acid. To study the interaction between PBA derivatives and baicalein, fluorescent spectra were measured using a fluorescent spectrophotometer and results were shown in Fig. 8. In Figs. 8A,D,G,J, M, no fluorescence was found for the mixture of PBA derivatives and baicalein, or the mixture of ARS and baicalein. Figures 8B,E,H,K, N showed the fluorescent spectra of PBA derivatives-ARS-baicalein mixture with the increasing concentrations of baicalein. In Figs. 8B, fluorescent intensity slightly decreased at lower concentrations of baicalein, and obviously decreased at higher concentrations of baicalein. And the peak values of fluorescent spectra showed a completely different pattern at the low and high concentrations. This phenomenon may be ascribed to more baicalein leading to a stronger interaction with APBA. For AAPBA (Figs. 8E, F), PAAPBA (Figs. 8H, I), PEbPB (Figs. 8K, L), and PErPB (Figs. 8N, O), the fluorescent intensity obviously decreased (from hundreds to dozens) after baicalein was added. The results may be due to the stronger interaction between baicalein and PBA derivatives. The interaction between PBA derivatives and ARS led to high fluorescent intensity. After baicalein was added, the interaction between PBA derivatives and ARS was destroyed and replaced. Owing to the concentration of baicalein was much high than ARS, most PBA derivatives bound with baicalein leading to the fluorescent intensity decreased from hundreds to dozen. These results indicated that there was obvious interaction between baicalein and five PBA derivatives.

To study the interaction between PBA derivatives and baicalein, fluorescent spectra were measured using a fluorescent spectrophotometer and results were shown in Fig. 9. In Figs. 9A,D,G,J, M, no obvious fluorescence was found for the mixture of PBA derivatives and baicalein, or the mixture of ARS and baicalein. Figures 9B,E,H,K, N showed the fluorescent spectra of PBA derivatives-ARS-baicalein mixture with the changing concentrations of baicalein. In Figs. 9B, C, fluorescent intensity decreased regularly over the increasing concentrations of baicalein. In Figs. 9E, F, fluorescent intensity slightly decreased at lower concentrations of baicalein (from 450 to 340), and obviously decreased at higher concentrations of baicalein (from 340 to 130). This phenomenon may be ascribed to more baicalein leading to the stronger interaction between baicalein and AAPBA. For PAAPBA (Figs. 9H, I), PEbPB (Figs. 9K, L), and PErPB (Figs. 9N, O), the fluorescent intensity obviously decreased (from hundreds to dozens) after baicalein was added, indicating that strong interaction between four PBA derivatives and baicalein.

To study the interaction between PBA derivatives and gallic acid, fluorescent spectra were measured using a fluorescent spectrophotometer and results were shown in Fig. 10. In Figs. 10A,D,G,J, M, no fluorescence was found for the mixture of PBA derivatives and gallic acid, or the mixture of ARS and gallic acid. Figures 10B,E,H,K, N showed the fluorescent spectra of PBA derivatives-ARS-gallic acid mixture with the changing concentrations of gallic acid. In Figs. 10B, C, the fluorescent intensity decreased regularly.
over the increasing concentrations of gallic acid. For AAPBA (Figs. 10E, F), PAAPBA (Figs. 10H, I), PE\textsubscript{b}PB (Figs. 10K, L), and PE\textsubscript{r}PB (Figs. 10N, O), the fluorescent intensity obviously decreased after gallic acid was added, indicating the strong interaction between these four PBA derivatives and gallic acid.

To study the interaction between PBA derivatives and protocatechuic acid, fluorescent spectra were measured using a fluorescent spectrophotometer and results were shown in Fig. 11. In Figs. 11A, D, G, J, M, no fluorescence was found for the mixture of PBA derivatives and protocatechuic acid, or the mixture of ARS and protocatechuic acid. Figures 11B, E, H, K, N showed the fluorescent spectra of PBA derivatives-ARS-protocatechuic acid mixture with the changing concentrations of protocatechuic acid. For APBA (Figs. 11B, C), AAPBA (Figs. 11E, F), PAAPBA (Figs. 11H, I), and PE\textsubscript{r}PB (Figs. 11N, O), the fluorescent intensity decreased regularly over the increasing concentration of protocatechuic acid. In Figs. 11K, L, the fluorescent intensity obviously decreased after protocatechuic acid was added, indicating the strong interaction between PE\textsubscript{r}PB and protocatechuic acid. In comparison, there were different changing regular of fluorescent spectra and fluorescent peak values for all the active ingredients with PBA derivatives. This phenomenon may be ascribed to the different structures of four active ingredients and five PBA derivatives. Generally, three polymers showed stronger interaction with four active ingredients than APBA and AAPBA.

**Cellular uptake**

Drug delivery systems have received much attention in recent years, especially for cancer treatment. Drug delivery system is an interface between the patient and the drug. In addition to improving the pharmacokinetics of the loaded poorly soluble hydrophobic drugs, the drug delivery system allowed cancer-specific drug delivery by inherent passive targeting phenomena and adopted active targeting strategies. Therefore, drug delivery system can improve the efficacy and safety of drugs by controlling the rate, time, and place of release of drugs in the body (Kumari et al. 2016). To explore the drug delivery capacity of phenylboronic acid derivatives, PE\textsubscript{b}PB and PE\textsubscript{r}PB were chosen to act as the carriers. Baicalin with green fluorescence was chosen as a drug model. Baicalin was loaded into PE\textsubscript{b}PB and PE\textsubscript{r}PB nanoparticles, respectively. Then these drug-loaded nanoparticles were co-incubated with NIH3T3 and A549 cells for 6 h, respectively. The cellular uptake of baicalin were shown in Fig. 12. For NIH3T3 and A549 cells, baicalin showed stronger green fluorescence in PE\textsubscript{b}PB@baicalin and PE\textsubscript{r}PB@baicalin groups than that in baicalin groups, implying that PE\textsubscript{b}PB and PE\textsubscript{r}PB could enhance cellular uptake of baicalin. In addition, there was stronger green fluorescence for A549 than NIH3T3, and there were many green fluorescent dots around HUVEs cells. The results implied that PE\textsubscript{b}PB and PE\textsubscript{r}PB could decrease cellular uptake of baicalin in NIH3T3 and enhance cellular uptake of baicalin in A549 cells. The phenomenon may be beneficial to design drug delivery system to treat cancer disease.

**Conclusion**

Herein, we used five PBA derivatives of APBA, AAPBA, PAAPBA, PE\textsubscript{b}PB, and PE\textsubscript{r}PB to study the interaction with active ingredients of traditional Chinese medicine. The chosen active ingredients with important pharmacological effect were baicalin, baicalein, gallic acid, and protocatechuic acid with diphenol structure. The interactions between five PBA derivatives and four active ingredients were explored by a fluorescent spectrophotometer. The fluorescent spectra showed that five PBA derivatives can combine with four active ingredients, testified by the decreasing fluorescent intensity over the increasing concentrations of four active ingredients. Three polymers of PAAPBA, PE\textsubscript{b}PB, and PE\textsubscript{r}PB showed stronger interaction with four active ingredients than APBA and AAPBA. After adding four active ingredients, the fluorescent intensity of three polymers showed a sharp decrease compared with that of the PBA derivatives-ARS compound. Simultaneously, PE\textsubscript{b}PB and PE\textsubscript{r}PB could act as carriers and load baicalin to obtain PE\textsubscript{b}PB@baicalin and PE\textsubscript{r}PB@baicalin, and they all could decrease cellular uptake of baicalin in NIH3T3 cells and enhance cellular uptake of baicalin in A549 cells. This research provided new strategies for the wider application of active ingredients in the prevention and therapy of diseases by building drug delivery systems based on the interaction with PBA derivatives.
Fig. 11 The interaction between different PBA derivatives, ARS and protocatechuic acid measured by fluorescent spectrophotometer. A, B The fluorescent spectra of APBA-ARS-protocatechuic acid mixture and C the peak values of fluorescent spectra at different APBA concentrations; D, E The fluorescent spectra of AAPBA-ARS-protocatechuic acid mixture and F the peak values of fluorescent spectra at different AAPBA concentrations; G, H The fluorescent spectra of PAAPBA-ARS-protocatechuic acid mixture and I the peak values of fluorescent spectra at different AAPBA concentrations at different AAPBA concentrations; J, K The fluorescent spectra of PE/PB-ARS-protocatechuic acid mixture and L the peak values of fluorescent spectra at different PE/PB concentrations; M, N The fluorescent spectra of PE/PB-ARS-protocatechuic acid and O the peak values of fluorescent spectra at different PE/PB concentrations.

Fig. 12 Cellular uptake of baicalin, PE/PB@baicalin and PErPB@baicalin against A NIH3T3 and B A549 cells.
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References

Bai J, Zhang Y, Tang C, Hou Y, Ai X, Chen X et al (2021) Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases. Biomed Pharmacother 133:110985

Bhavya P, Melavanki R, Narayanappa CK, Kusranur R, Meghana U, Suma B (2020) Binding interaction between boronic acid derivatives with monosaccharides: effect of structural change of monosaccharides upon binding using S-V plots. Macromol Symp 392:4

Bie B, Sun J, Guo Y, Li J, Jiang W, Yang J et al (2017) Baicalin: a review of its anti-cancer effects and mechanisms in Hepatocellular Carcinoma. Biomed Pharmacother 93:1285–1291

Choubey S, Goyal S, Varughese LR, Kumar V, Sharma AK, Beniwal V (2018) Probing gallic acid for its broad spectrum applications. Mini Rev Med Chem 18:1283–1293

Gennari A, Gujral C, Hohn E, Lallana E, Cellesi F, Tirelli N (2017) Revisiting boronate/diol complexation as a double stimulus-responsive bioconjugation. Bioconjug Chem 28:1391–1402

Govindaraj D, Rajan M, Munusamy MA, Alarfaj AA, Sadasivuni KK, Kumar SS (2017) The synthesis, characterization and in vivo study of mineral substituted hydroxyapatite for prospective bone tissue rejuvenation applications. Nanomed Nanotechnol Biol Med 13:2661–2669

Kakkar S, Bais S (2014) A review on protocatechuic Acid and its pharmacological potential. ISRN Pharmacol, 2014:952943

Khan BA, Mahmood T, Menaa F, Shahzad Y, Yousaf AM, Hussain T et al (2018) New perspectives on the efficacy of gallic acid in cosmeceuticals & nanocosmeceuticals. Curr Pharm Des 24:5181–5187

Kosuru RY, Roy A, Das SK, Bera S (2018) Gallic acid and gallates in human health and disease: do mitochondria hold the key to success? Mol Nutr Food Res. https://doi.org/10.1002/mnfr.201700699

Krzysztoforska K, Mirowska-Guzel D, Widy-Tyszkieiwicz E (2019) Pharmacological effects of protocatechuic acid and its therapeutic potential in neurodegenerative diseases: Review on the basis of in vitro and in vivo studies in rodents and humans. Nutr Neurosci 22:72–82

Kumari P, Ghosh B, Biswas S (2016) Nanocarriers for cancer-targeted drug delivery. J Drug Target 24:179–191

Lalani SS, Anasir MI, Poh CL (2020) Antiviral activity of silymarin in comparison with baicalein against EV-A71. BMC Complement Med Ther 20:97

Lan T, Guo Q (2019) Phenylboronic acid-decorated polymeric nanomaterials for advanced bio-application. Nanotechnol Rev 8:548–561

Lee MC, Kabilan S, Hussain A, Yang X, Lowe CR (2004) Glucose-sensitive holographic sensors for monitoring bacterial growth. Anal Chem 76:5748–5755

Li Y, Xiao W, Xiao K, Berti L, Luo J, Tseng HP et al (2012) Well-defined, reversible boronate crosslinked nanocarriers for targeted drug delivery in response to acidic pH values and cis-diols. Angew Chem Int Ed Engl 51:2864–2869

Li S, Hu K, Cao W, Sun Y, Sheng W, Li F et al (2014) pH-responsive biocompatible fluorescent polymer nanoparticles based on phenylboronic acid for intracellular imaging and drug delivery. Nanoscale 6:13701–13709

Liang W, Huang X, Chen W (2017) The effects of baicalin and baicalein on cerebral ischemia: a review. Aging Dis 8:850–867

Liu SH, Chuang WC, Lam W, Jiang Z, Cheng YC (2015) Safety surveillance of traditional Chinese medicine: current and future. Drug Saf 38:117–128

Lorand JP, Edwards JO (1959) Polyol complexes and structure of the benzeneboronate ion. J Org Chem 24:769–774

Melavanki R, Kusuranur R, Sadasivuni KK, Singh D, Patil NR (2020) Investigation of interaction between boronic acids and sugar: effect of structural change of sugars on binding affinity using steady state and time resolved fluorescence spectroscopy and molecular docking. Heliyon. 6:e05081

Nahin RL, Barnes PM, Stussman BJ, Bloom B (2007) Costs of complementary and alternative medicine (CAM) and frequency of visits to CAM practitioners: United States. Natl Health Stat Rep 2009:1–14

Ni N, Laughlin S, Wang Y, You F, Zheng Y, Wang B (2012) Probing the general time scale question of boronic acid binding with sugars in aqueous solution at physiological pH. Bioorg Med Chem 20:2957–2961

Paliere E, Hélary C, Kraft JM, Génouis I, Masse S, Laurent G et al (2021) Baicalein-modified hydroxyapatite nanoparticles and coatings with antibacterial and antioxidant properties. Mater Sci Eng C Mater Biol Appl 118:111537

Sadasivuni KK, Saiter A, Gautier N (2013) Effect of molecular interactions on the performance of poly(3-sobutylene-co-isoprene)g graphene and clay nanocomposites. Colloid Polymer Sci 291:1729–1740

Song J, He Y, Luo C, Feng B, Zhang D (2020) New progress in the pharmacology of protocatechuic acid: a compound ingested in daily foods and herbs frequently and heavily. Pharmacolet Res 161:105109

Springsteen G, Alizarin WB, Red S (2001) as a general optical reporter for studying the binding of boronic acids with sugars in comparison with baicalein against EV-A71. BMC Complement Med Ther 20:97

Saiter A, Gautier N (2014)1Y210, and No. ZK[2021]249).

Takayama S, Iwasaki K (2017) Systematic review of traditional Chinese medicines for geriatrics. Geriatr Gerontol Int 17:679–688

Takahashi S, Suzuki O, Ijima T, Minaki D, Anzai JI (2018) Voltammetric and time resolved fluorescence spectroscopy and molecular docking of structural change of sugars on binding affinity using steady state and time resolved fluorescence spectroscopy and molecular docking. Heliyon. 6:e05081

Vetriu 3-mediated decatcylylation of acetyl-CoA synthetase family member 3 by protecatechuc acid attenuates non-alcoholic fatty liver disease. Br J Pharmacol 177:4166–4180

Takayama S, Iwasaki K (2017) Systematic review of traditional Chinese medicine for geriatrics. Geriatr Gerontol Int 17:679–688

Van M, Duin, Peters JA et al (1984) Studies on borate esters 1: The pH dependence of the stability of esters of boric acid and borate in aqueous medium as studied by 11B NMR. Tetrahedron 40:2901–2911

Wang ZY, Liu JG, Li H, Yang HM (2016) Pharmacological effects of active components of Chinese herbal medicine in the treatment of Alzheimer’s disease: a review. Am J Chin Med 44:1525–1541
Yang M, Chen JL, Xu LW, Ji G (2013) Navigating traditional Chinese medicine network pharmacology and computational tools. Evid Based Complement Alternat Med. 2013:731969

Yu W, Ma M, Chen X, Min J, Li L, Zheng Y et al (2017) Traditional Chinese medicine and constitutional medicine in China, Japan and Korea: a comparative study. Am J Chin Med 45:1–12

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