Uptake and Transport of Naringenin and Its Antioxidant Effects in Human Intestinal Epithelial Caco-2 Cells

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Naringenin, a flavanone, has been reported for a wide range of pharmacological activities. However, there are few reports on the absorption, transport and antioxidant effects of naringenin. The study was to explore the uptake, transport and antioxidant effects of naringenin in vitro. Cell transmembrane resistance, lucifer yellow transmission rate, and alkaline phosphatase activity were used to evaluate the successful construction of cell model. The results showed that the absorption and transport of naringenin by Caco-2 cells were time- and concentration-dependent. Different temperatures (37 and 4°C) had a significant effect on the uptake and transport of naringenin. Verapamil, potent inhibitor of P-glycoprotein, significantly inhibit naringenin transport in Caco-2 cells. The results revealed that naringenin was a moderately absorbed biological macromolecule and can penetrate Caco-2 cells, mainly mediated by the active transport pathway involved in P-glycoprotein. At the same time, naringenin pretreatment could significantly increase the viability of H2O2-induced Caco-2 cells. Twenty four differential metabolites were identified based on cellular metabolite analysis, mainly including alanine, aspartate and glutamate metabolism, histidine metabolism, taurine and hypotaurine metabolism, pyruvate metabolism, purine metabolism, arginine biosynthesis, citrate cycle, riboflavin metabolism, and D-glutamine and D-glutamate metabolism. We concluded that the transport of naringenin by Caco-2 cells is mainly involved in active transport mediated by P-glycoprotein and naringenin may play an important role in oxidative stress-induced intestinal diseases.

Keywords: naringenin, uptake, transport, anti-oxidation, metabolomics

INTRODUCTION

The gut is the primary site for nutrient absorption in all animals and humans (1). Many single traditional Chinese medicine (TCM), extracts of TCM, and some TCM monomers enter the blood through the intestinal tract after oral administration (2–4). Many parameters affect the intestinal absorption of substances and their bioavailability, such as transit and absorption time (5). The cell models in vitro have many advantages, such as good reproducibility, low cost, and short cycle. Therefore, research models in vitro are often used for drug transport and absorption studies. More and more cell models are used to explore the transport and absorption of flavonoids (6–8). Among them, Caco-2 cell model has a good correlation with in vivo research. It is also possible to explore
the transit time and absorption capacity of the drug ingested from the AP side and the BL side. The transport and absorption mechanism of drug molecules via the intestines of humans will be further explored (9, 10). By calculating the Papp value, the absorption information at the cellular level can be determined (11).

Naringenin is a flavonoid compound that mainly found in grapes and oranges (12–14). Studies have shown that naringenin has anti-inflammatory, anti-oxidant, anti-diabetic, anti-cardiovascular disease, and anti-fibrosis pharmacological activities (15–20). Zhao et al. found that naringenin protects human umbilical vein endothelial cells from oxidative damage induced by palmitate via reducing autophagy flux (21). Li et al. also found that naringenin can stimulate skeletal muscle cells to take up glucose and increase insulin sensitivity via AMPK signaling pathway (14).

In this study, the model of Caco-2 cells was constructed. The transport, uptake, and antioxidant effects of naringenin in the monolayer culture model were investigated. This study provided evidence for the absorption, transport and antioxidant effects of naringenin in the gut.

MATERIALS AND METHODS

Chemicals

Caco-2 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Transwell™ cell culture dish (12 mm membrane diameter) were obtained from Corning Costar Corp. (Cambridge, MA). Fetal bovine serum, MEM glucose medium, and cell culture flask were from Gibco (Grand Island, NY, USA). CCK-8 kit was obtained from Beyotime (Shanghai, China). Lucifer yellow (LY) was from Solarbio (Beijing, China). Alkaline Phosphatase Kit was from MiBio (Shanghai, China). Gradedacetonitrile and formic acid (>99%, for LC-MS) were obtained from Thermo fisher scientific (Waltham, MA, USA).

Cell Culture

Caco-2 cells were routinely maintained in 20% fetal bovine serum, 1% gluta-max, 1% sodium pyruvate, 1% non-essential amino acids, 77% modified eagle medium supplemented at 37°C under humidified atmospheric conditions containing 5% CO₂.

Cell Viability

Cell viability was determined via CCK-8 assay (22).

Establishment of Caco-2 Cells Model

The Caco-2 cell model was established as previously published (23). Different concentrations of naringenin (9.375, 18.75, 37.5, 75 and 150 μM) had no effect on the activity of cells for 24 h (Figure 1). As shown in Figure 2A, when the Caco-2 cells were cultured for 21 days, the Caco-2 cells were tightly connected without gaps. From Figure 2B, the TEER value reached 602 Ω•cm² on the 21st day. The results indicated that the monolayer membrane of Caco-2 cells had good integrity.

Lucifer Yellow Transmission Rate Experiment

After treatments, lucifer yellow transmission rate was assessed using the corresponding commercial kit according to the manufacturer’s protocols (24). From Figure 2C, the linear regression equation of lucifer yellow absorbance was

\[ y = 0.0556x + 0.0427 \quad (R^2 = 0.9997) \]

The permeation rate of lucifer yellow with cells was determined as 0.42 (±0.15) \times 10^{-6} \text{ cm/s}. The results showed that Caco-2 cells were fully differentiated and morphologically intact after being cultured for 21 days.

Alkaline Phosphatase Activity Assay

Alkaline phosphatase kit was used to detect the enzyme activity of AP and BL side (23). It could be seen from Figure 2D that the alkaline phosphatase activity of the AP side was significantly higher than that on the BL side. This model could be used as an in vitro cellular model for subsequent transport and uptake experiments.

Analytical Methods

Sample Extraction

In total 200 μL methanol was added to the 200 μL sample and centrifuged at 14,000 g for 10 min. The supernatant was collected and evaporated to dryness, reconstituted in methanol, and analyzed by HPLC.

Liquid Chromatography Analysis

Naringenin chromatographic analysis was performed on a Sursil ODS-B column (250 × 4.6 mm, 5 mm particle size). The mobile phase consisted of an acid solution containing a 0.2% phosphoric acid and methanol (40:60, v/v) at a flow rate of 0.4 mL/min. Naringenin elution was recorded at a constant wavelength of 282 nm.
Transport Assay of Caco-2 Cells
Different concentrations of naringenin solutions (9.375, 18.75, 37.5, 75, and 150 µM) were added on the Caco-2 cells. In total 100 µL of sample solution was collected from the BL side at different times (15, 30, 45, 60, 90, and 120 min) and then 100 µL of HBSS was added.

Caco-2 Cells Uptake Experiment
Different concentrations of naringenin solution (9.375, 18.75, 37.5, 75, and 150 µM) were added on the Caco-2 cells. The cells were collected at different time periods (15, 30, 45, 60, 90, and 120 min), respectively.

Metabonomic Analysis
Cellular Metabolite Extraction
After incubation, the Caco-2 cells were collected. In total 80% ice methanol was added to the Caco-2 cells, incubated at low temperature for 5 min, and the Caco-2 cells were scraped from the cell culture plate. Samples were lysed by three freeze-thaw cycles and pelleted by centrifugation at 14,000 × g for 10 min at 4°C. Twenty µl of supernatant from each sample was taken and mixed well to prepare quality control (QC) samples. The samples were then filtrated through 0.2 µm filters into sample vials.

UHPLC-QE Orbitrap/MS/MS Conditions
LC-MS/MS analyses were performed using a HPLC system with a HSS T3 column coupled to Q Exactive (Orbitrap MS, Thermo). The mobile phase A was 0.1% formic acid in water and the mobile phase B was acetonitrile. The elution gradient was set as follows: 0 min, 2% B; 1 min, 2% B; 18 min, 100% B; 22 min, 100% B; 25 min, 2% B. The flow rate was 0.3 mL/min. The injection volume was 2 µL. The QE mass spectrometer was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. ESI source conditions were set as follows: Aux gas flow rate as 16 Arb, Full ms resolution as 70,000, Collision energy as 25 eV in NCE model, MS/MS resolution as 17,500, and spray voltage as −3.0 kV (negative) or 3.6 kV (positive), respectively.
Qualitative Analysis of Metabolites

The raw data were converted into “Analysis Base File” (ABF) format files by ABF Converter software. The peak detection, deconvolution and peak alignment in data processing were performed using the MSDIAL 2.2.62 software. The data obtained are imported into SIMCA (version 14.1). We performed PCA and OPLS-DA on the data in SIMCA The Human Metabolome Database (HMDB) was used to search for accurate mass values of differential metabolites. Cluster analysis and pathway analysis of differential metabolites were performed using MetaboAnalyst 5.0.

Statistical Analysis

SAS 9.2 (SAS Institute Inc., NC, USA) was used for statistical analysis. All data are presented as means ± SD. Statistical significance was considered at \( p < 0.05 \).

RESULTS

Transport Experiment Results

The Transport Results of Naringenin in Caco-2 Cells at Different Times

Under the condition of 37°C, the naringenin transport volume on both sides of Caco-2 cells gradually increased with the increase of time, and the transport volume reached the maximum at 120 min but did not reach the saturation state (Figure 3A).

The Transport Results of Naringenin in Caco-2 Cells at Different Temperatures

37 and 4°C were selected to study the effect of temperature on the transport of naringenin. The results showed that compared to 37°C, the transport volume in Caco-2 cells was significantly reduced at 4°C. This indicated that temperature has a significant effect on its transport capacity (Figure 3B).

Transport of Naringenin on Caco-2 Cells

Under the condition of 37°C, the results showed that the transport amount of naringenin in Caco-2 cells gradually increased with the increase of the concentration (Figures 4A,B). The naringenin transport rate on the BL-AP side was 10.09%, and the naringenin transport rate on the AP-BL side was 4.89%, and the BL-AP side was significantly higher than the AP-BL side (Figure 4C).

Papp of Transport of Naringenin

On the AP-BL side, the Papp values of naringenin at different concentrations increased with time and concentration during transport (Figures 5A,B). During the transportation of different concentrations of naringenin on the AP-BL side, the Papp value of 150 µM was the maximum value at 30 min. Interestingly, on the BL-AP side, the Papp values of different concentration groups gradually decreased with time. Similarly, the Papp values of the same concentration and different time groups also decreased gradually. The results indicated that there may be active transport of naringenin in Caco-2 cells.

Uptake Result

The Absorption of Naringenin by Caco-2 Cells at Different Times

Under the condition of 37°C, naringenin was constant in Caco-2 monolayer cells (Figure 6A). The results suggested that Caco-2 cells have a time-dependent uptake of naringenin.

The Absorption of Naringenin by Caco-2 at Different Temperatures

As shown in Figure 6B, compared with 37°C, the intake of naringenin at 4°C was significantly reduced. It was possible that low temperature affects the fluidity of cell membranes. As shown in Figure 6C under the condition of 37°C and 120 min, the uptake rate of naringenin by Caco-2 monolayer cells decreased...
with the increase of the concentration, and finally tended to be saturated.

**The Impact of Verapamil on Naringenin Transport and Uptake**

To demonstrate the potential role of P-glycoprotein in the transport and uptake of naringenin across the Caco-2 cell monolayer, verapamil and ABCB1 shRNA were used to interfere with the transport and uptake of naringenin by Caco-2 (25, 26). After the application in the apical chamber prior to naringenin administration, Verapamil significantly lowered the transport of naringenin (Figures 7A,B). Compared with the inhibitor (100 µM Verapamil) group, Caco-2’s intake of naringenin was significantly reduced in the control group (Figure 7C).
results showed that the transport of naringenin by Caco-2 cells depended on P-glycoprotein.

**Naringenin Protects the Cell Viability of H$_2$O$_2$-Stimulated Caco-2 Cells**

Naringenin (9.375, 18.75, 37.5, 75, and 150 µM) had no significant effect on the activity of Caco-2 cells at 24 h in Figure 1. As shown in Figure 8A, the cell viability significantly decreased. The cell viability was reduced at 48.6% when 500 µM H$_2$O$_2$ was added for 3 h in the medium compared with the control group (Figure 8A). As shown in Figure 1B, when Caco-2 cells were pre-treated with different concentrations of naringenin for 24 h and then incubated with with 500 µM H$_2$O$_2$ for 3 h, naringenin showed a dose-dependent recovery of the cell viability. Therefore, 150 µM of naringenin was used in the subsequent experiments (Figure 8B).

**Metabolomics Analysis of Naringenin Effect on H$_2$O$_2$-Induced Caco-2 Cells**

In this study, unsupervised PCA was performed on four groups of data (control group, H$_2$O$_2$ group, naringenin group, and QC group). The four groups showed clear separation in both positive and negative ion modes in the PCA plots (Figures 9A,B,G,H). The OPLS-DA model was constructed to further investigate and analyze the separation of the H$_2$O$_2$ group and other
groups (control group and naringenin group). The results showed a clear separation of the H$_2$O$_2$ group from the other groups in the positive or negative mode in the OPLS-DA score plot (Figures 9C,E,I,K). The values of R$^2$X, R$^2$Y, and Q$^2$ indicated that the model was stable and had good predictive power (Figures 9D,F,J,L).

Twenty four metabolites were identified as differential metabolites in Table 1, including sulfonic acid, pantothenic acid, methyltyrosine, acetyl-L-aspartic, glucuronic acid, cysteic acid, taurine, L-Histidine, L-glutamic acid, lactic acid, oxoproline, inosine, hypoxanthine, histidine, guanosine, guanine, pantothenic acid, citramalic acid. After pre-treatment with naringenin, the levels of these differential metabolites were normalized due to up- or down-regulation.

Metabolic Pathway Analysis
Differential metabolites were imported into MetaboAnalyst 5.0 for relevant metabolic pathway analysis and KEGG enrichment analysis. There are 19 main metabolic pathways: purine metabolism, malate-aspartate shuttle, glutathione metabolism, glycerol phosphate shuttle, aspartate metabolism, taurine and hypotaurine metabolism, methylhistidine metabolism, alanine metabolism, warburg effect, mitochondrial electron transport chain, amino sugar metabolism, beta-alanine metabolism, gluconeogenesis, arginine and proline metabolism, de novo triacylglycerol biosynthesis, cysteine metabolism, histidine metabolism, lysine degradation (Figure 10). The influence of the path is mainly concentrated in alanine, aspartate and glutamate metabolism, histidine metabolism, taurine and hypotaurine metabolism, pyruvate metabolism, purine metabolism, arginine biosynthesis, citrate cycle, riboflavin metabolism, and D-glutamine and D-glutamate metabolism. H$_2$O$_2$-induced Caco-2 cells are mainly reflected in redox reactions, amino acid synthesis and metabolism, and energy metabolism (Figure 11). The results indicated that H$_2$O$_2$-induced oxidative damage could cause metabolic disturbances in Caco-2 cells, and naringenin pretreatment could effectively regulate this imbalance.

DISCUSSION
After oral administration of flavonoids in plant foods, their biological effects may be much weaker than in vitro studies (27, 28). In the past 20 years, this conclusion has been accepted by researchers. The pharmacokinetic characteristics have also been elucidated, including absorption, metabolism, disposal and elimination (29). In addition, studies have shown that flavonoids can only be absorbed by the body after the glycoside portion has been removed (30, 31). However, the transport and uptake of naringenin in this study could be observed in the Caco-2 cell model. The study showed that the transport of naringenin can be mediated by P-glycoprotein. However, some studies also have shown that the transport of naringenin does not depend on P-glycoprotein, but on the entry of MRPI carriers into cells (32). In this study, naringenin absorption was polarized with Papp$_{BA}$ superior to Papp$_{AB}$, and naringenin is transported by active efflux protein carriers. Verapamil could significantly inhibit the transport of naringenin, and it showed that naringenin transport was most likely dependent on P-glycoprotein. Previous research had shown that naringenin can inhibit P-glycoprotein mediated efflux of vincristine in the blood-brain barrier (33, 34). Moreover, naringenin inhibit drug efflux by directly interacting with various sites of P-glycoprotein (35, 36).

The results of this experiment indicated that the Papp of naringenin in the Caco-2 cell monolayer was between $1.0 \times 10^{-6}$ to $1.0 \times 10^{-5}$ cm$\cdot$s$^{-1}$. This result can well predict the absorption mechanism of naringenin in the gut. If the
### TABLE 1 | Differential metabolites in Caco-2 cell.

| No | RT      | VIP | Formal                  | Metabolites                      | SM    | m/z   | Fold change |
|----|---------|-----|-------------------------|----------------------------------|-------|-------|-------------|
| 1  | 1.881573| 2.18| C_{6}H_{7}N_{4}O_{2}   | Xanthine                         | ESI+  | 151.0254 | 0.32        |
| 2  | 1.832831| 3.31| C_{3}H_{6}O_{3}P         | Glycerol-3-phosphate              | ESI+  | 171.0078 | 1.88        |
| 3  | 8.151354| 1.36| C_{1}H_{3}O_{6}N_{6}       | Riboflavin                        | ESI+  | 375.1308 | 1.11        |
| 4  | 2.945925| 1.23| C_{6}H_{7}NO_{3}          | Pyroglutamic acid                 | ESI+  | 128.0343 | 0.89        |
| 5  | 1.896282| 2.19| C_{20}H_{16}O_{9}S         | Pyrogallin                        | ESI+  | 203.0369 | 1.09        |
| 6  | 4.815042| 2.21| C_{2}H_{12}O_{5}P         | Phosphoric acid                   | ESI+  | 96.9839  | 0.88        |
| 7  | 3.336667| 1.09| C_{2}H_{8}O_{4}S          | Sulfonic acid                     | ESI+  | 273.0383 | 0.41        |
| 8  | 3.337222| 2.11| C_{2}H_{7}NO_{5}           | Pantothenic acid                  | ESI+  | 218.1032 | 0.55        |
| 9  | 14.40785| 2.97| C_{3}H_{5}O_{3}            | Methyl tyrosine                   | ESI+  | 194.0817 | 0.45        |
| 10 | 3.332992| 5.21| C_{14}H_{12}N_{4}O_{5}     | Acetyl-L-Aspartic acid            | ESI+  | 174.0397 | 0.86        |
| 11 | 1.88385 | 3.21| C_{6}H_{10}O_{4}           | Glucuronic acid                   | ESI+  | 193.0349 | 0.65        |
| 12 | 10.86779| 1.99| C_{2}H_{12}NO_{5}S         | Cysteic acid                      | ESI+  | 167.9959 | 0.58        |
| 13 | 3.515183| 1.09| NH_{2}CH_{2}CH_{3}SO_{3}H | Taurine                           | ESI+  | 124.0662 | 1.92        |
| 14 | 1.36195 | 2.51| C_{6}H_{7}N_{2}O_{2}       | L-Histidine                       | ESI+  | 154.0616 | 0.77        |
| 15 | 3.1647  | 2.51| C_{3}H_{7}NO_{4}           | L-Glutamic acid                   | ESI+  | 146.0449 | 0.68        |
| 16 | 1.214575| 3.08| C_{2}H_{12}O_{3}           | Lactic acid                       | ESI+  | 89.02312 | 0.59        |
| 17 | 1.634986| 1.71| C_{5}H_{7}NO_{3}           | Oxoproline                        | ESI+  | 128.0344 | 0.82        |
| 18 | 1.53901 | 1.41| C_{10}H_{12}N_{4}O_{5}     | Inosine                           | ESI+  | 267.0735 | 0.78        |
| 19 | 1.538802| 3.77| C_{6}H_{12}N_{2}O          | Hypoxanthine                      | ESI-  | 135.0304 | 0.77        |
| 20 | 1.319585| 5.06| C_{6}H_{7}N_{2}O_{2}       | Histidine                         | ESI-  | 154.0615 | 0.77        |
| 21 | 1.888793| 1.17| C_{20}H_{16}O_{9}S         | Guanine                           | ESI-  | 282.0847 | 3.27        |
| 22 | 1.633602| 2.86| C_{2}H_{12}N_{3}O           | Guanine                           | ESI-  | 150.0412 | 0.67        |
| 23 | 2.889338| 3.90| C_{9}H_{17}NO_{5}           | Pantothenic acid                  | ESI-  | 218.1031 | 1.27        |
| 24 | 1.885903| 2.18| C_{3}H_{5}O_{3}            | Citramalic acid                   | ESI-  | 147.0291 | 0.78        |

The * symbol indicates the value of p < 0.05 compared with the H_{2}O_{2} group.

![FIGURE 10](image-url) | The results of enrichment and path impact of differential metabolites in Caco-2 cell.
macromolecule requires a paracellular pathway, the gap between cells can be opened (37). In this study, TEER did not decrease significantly, indicating that naringenin rarely crosses Caco-2 cells via paracellular pathways.

Flavonoids are a large group of plant-derived compounds, including quercetin and naringenin (38, 39). Quercetin and naringenin have similar physical and chemical properties. They are weakly acidic and have low water solubility. There have been many studies on the transport and absorption of quercetin. Studies have shown that quercetin glycosides might be actively absorbed in the human intestine via unspecified hexose transporters (40). The results confirmed the transport of naringenin in modeled Caco-2 cells and support the involvement of P-glycoprotein in this process.

A metabolomic approach based on LC-MS technology is a useful technique for evaluating the production of metabolites in cells under oxidative stress. Studies have shown that there are many metabolites of naringenin including apigenin, hesperetin, hippuric acid, 4-hydroxybenzoic acid and 3-(4′-hydroxyphenyl) propionic acid. These polyphenolic compounds play important roles in antioxidant, anti-inflammatory and anti-apoptotic roles (41, 42). In this study, we identified some metabolites of naringenin, such as apigenin and hesperetin. Apigenin has anti-inflammatory, antioxidant and anticancer properties. As a natural compound, apigenin may be an ideal and safe antitumor agent. Studies have shown that apigenin has good antitumor activity both in vitro and in vivo (43, 44). Hesperetin, a member of the flavonoid flavonoids, has been extensively studied for its anticancer, antioxidant, and anti-inflammatory properties. Hesperetin blocks neuroinflammation in microglia by regulating the expression of proteins associated with oxidative stress, inflammatory responses and apoptosis.

In the present study, compared with control group, 24 differential metabolites were identified in Caco-2 cells pretreated with naringenin in the absence of H$_2$O$_2$ compared to the control group. These differential metabolites are involved in amino acid synthesis and metabolism, redox reactions, energy metabolism and cofactor metabolism. The levels of xanthine and hypoxanthine were significantly increased in the H$_2$O$_2$ group. The increased purines (xanthine and hypoxanthine) are often used as markers of oxidative stress. However, in this case, it is also possible that they represent loss of ATP as part of tissue loss and turnover (45, 46). The production of lactate and the reversible conversion of dihydroxyacetone...
phosphate to glycerol 3-phosphate catalyzed by glycerol-3-phosphate dehydrogenase involve the redox reactions of NADH and NAD\(^+\) \((47, 48)\). After pretreating Caco-2 cells with naringenin, the levels of riboflavin were also markedly increased. As a natural antioxidant, riboflavin protects the body from oxidative stress \((49)\). The levels of pyroglutamic acid and glutamic acid were significantly increased in the \(\text{H}_2\text{O}_2\) group. Naringenin significantly reduced the elevation of sulfonic acid levels in Caco-2 cells. Studies have shown that under conditions of oxidative stress, \(\text{H}_2\text{O}_2\) causes irreversible sulfinic and sulfonic acid modifications, which often lead to inactivation of antioxidant enzymes \((50)\). Research has shown that rats deficient in pantothenic acid exhibit duodenitis and duodenal ulcers \((51)\). N-acetyl-L-aspartic acid, cysteine and taurine are also good antioxidants \((52)\). Hypoxanthine have been defined as biomarkers of hypoxia, hypoxemia, and ischemic brain injury \((53, 54)\). Inosine is a natural analog of adenosine and binds adenosine receptors A3 \((55, 56)\), thus decelerating inflammation. Histidine is an essential amino acid, and histidine supplementation inhibits inflammatory processes \((57)\). Naringenin significantly increased histidine content in Caco-2 cells. Furthermore, in vitro experiments, histidine supplementation reduced the expression of IL-6 and TNF-\(\alpha\) in adipocytes \((58)\). Cellular metabolomics suggested that the underlying mechanism of naringenin's anti-oxidative stress effect may be related to increased anti-oxidative stress activity and decreased inflammatory response by improving metabolic changes.

**CONCLUSION**

Naringenin can penetrate Caco-2 cells, mainly mediated by the active transport pathway involved in P-glycoprotein. By carefully examining the cellular absorption, and transport efficiency of naringenin and their antioxidant effect together, we can reasonably conclude that naringenin may play a more important role in preventing oxidative stress-induced intestinal diseases.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

**AUTHOR CONTRIBUTIONS**

J-YL conceived and designed experiments. Z-DZ and QT performed the experiments and analyzed the data. X-WL synthesized and purified AEE. S-HL, Y-JY, ZQ, and L-XB supplied reagents. All authors contributed to the article and approved the submitted version.

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