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Identification of eupatilin and ginkgolide B as p38 ligands from medicinal herbs by surface plasmon resonance biosensor-based active ingredients recognition system

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ABSTRACT

Screening of bioactive ligands for a certain protein target from medicinal herbs is a highly important yet challenging task during drug discovery process. In this study, a surface plasmon resonance biosensor-based active ingredient recognition system (SPR-AIRS) was applied to screen p38 mitogen-activated protein kinase (p38) ligands from herbal extracts. After p38 protein was immobilized on a SPR chip and the suitability of SPR-AIRS was validated, thirty-four p38-related medicinal herbs were selected and pre-screened. Two medicinal herbs having high response signal with p38-immobilized chip, *Folium Ginkgo* and *Herba Artemisiae Scopariae*, were injected into SPR system for ligand fishing. Among them, two active compounds, eupatilin (EPT) and ginkgolide B (GKB), were identified as p38 ligands, and then the K_D values of EPT and GKB were measured as 21.68 ± 2.21 and 44.71 ± 1.80 μM, respectively. They can inhibit p38 activities significantly and bind to the ATP binding site on p38. Furthermore, EPT and GKB can inhibit cell proliferation (IC_{50} = 30.31 ± 6.84 and 42.97 ± 0.83 μM), induce apoptosis and G2/M cell cycle arrest against K562 cell line. This is the first time that EPT and GKB are reported as effective p38 binding ligands. These results prove that SPR-AIRS could be an effective method to screen active compounds acting on a specific protein from complex systems.

1. Introduction

Medicinal herbs not only have good efficacy towards diseases, but also could be regarded as important compound library for bioactive small molecules screening [1], which are drawn high attention in recent years. At present, more than 50% approved drugs are directly isolated or structure modified from natural products [2], so natural products are important sources of lead compounds discovery. Although each herb contains numerous ingredients, only a limited number of them can bind to a certain protein target and further display biological activity. Bioactive compound identification and analysis for herbal medicines are extremely difficult. The classical strategy for active compound identification is usually performed as follows: purified compounds were isolated from herbal extracts. Then phenotypic bioassays were operated to detect the activity of each compound one by one. Compounds with good activity are selected for further validation. Even though a handful of successful cases based on this strategy has been made and numerous active compounds were identified, the specific binding protein targets of compounds are still unrevealed, which hinders further research and development of natural ingredient from herbs.

Hence, researchers have developed some targeted active compounds screening technologies as advanced alternatives to the traditional strategy, such as ultrafiltration [3], cell membrane chromatography (CMC) [4], magnetic beads [5] and capillary electrophoresis (CE) [6]. The principle of these technologies is to detect the binding events between therapeutic targets and active ingredients in a complex mixtures background. Compounds which could bind to protein target will be selected for further analysis while non-binding compounds will be excluded. For example, Yang et al. have built an ultrafiltration high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS) approach to screen tyrosinase inhibitors from mulberry leaves [7]. Quercetin-3-O-(6-O-malonyl)-β-D-glucopyranoside and kaempferol-3-O-(6-O-malonyl)-β-D-glucopyranoside were identified as novel tyrosi-

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nase inhibitors. Han et al. have developed a two-dimensional α_{1A} CMC online HPLC-MS system to screen α_1A adrenoreceptor-targeted compounds from *Fructus Piperis* [8]. Piperine was identified as an active compound which could specifically bind to α_{1A} receptors. Li et al. have fabricated a magnetic hollow mesoporous silica spheres coated with titania to concentrate phosphorylated substrates from epidermal growth factor receptor (EGFR)-catalyzed reaction mixtures [9]. Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) was used to identify EGFR inhibitors. Li et al. reported a method to screen EGFR inhibitors from natural product extracts with CE in conjunction with HPLC-MS/MS [10]. With this method, quercetin and rutin were identified as potential EGFR ligands. These target-directed active compounds screening technology were all well-established and could screen lead compounds by evaluating binding affinities and kinetic parameters between ligands and protein targets, however, they also have some disadvantages which limit their application for ligand screening. Ultrafiltration-based strategies usually consume a large quantity of chemical reagents and proteins. CMC has a short life span and limited sensitivity. Magnetic beads-based strategies still confront some challenges, such as the effective desorption of the ligands from the target [11]. The sensitivity and reproducibility of CE is quite limited. Hence it prompts us to introduce novel technologies and develop methods to screen and identify bioactive components from complex mixture directly and specifically.

Surface plasmon resonance (SPR) biosensor is a technology to monitor the interaction between bio-molecules [12]. The past few years has witnessed an increasing interest in applying SPR biosensor during different processes of drug discovery, including lead compound discovery [13], pharmacokinetic study [14], ADME (adsorption, distribution, metabolism and execration) prediction [15] and target identification [16]. SPR is a robust and sensitive technique which has been successfully used in drug development and has already become a golden standard in compound-protein interaction determination field [17]. A major advantage of SPR is that it could detect the association and disassociation process between molecules in a real-time and label-free way [18]. This character makes SPR unique and suitable for ligand fishing. Another advantage of SPR is that it could detect the active compounds in complex mixture directly and specifically, which makes it applicable for active compound identification of medicinal herbals. Peng et al. have reported a SPR-HPLC-MS system to screen and identify human serum albumin ligands from *E. ulmoides* bark [19]. Totally, 22 active compounds were fished out. In a previous study, we combined this strategy with molecular docking, and molecular biology technology and developed a SPR-based active ingredient recognition system (SPR-AIRS) to screen targeted compounds from complex mixtures [20]. Then the features of SPR-AIRS, including specificity, limit of detection, linearity, saturability and robustness, were investigated, clearly demonstrating the effectiveness and strength of this approach for screening active compounds from complex systems [21].

In this study, SPR-AIRS was applied to screen p38 ligands from herbal extracts. p38 is an important drug target, which plays a key role in cell proliferation, apoptosis and tumorigenesis [22]. Firstly, recombinant p38 protein was immobilized on SPR chip, the activity and feasibility of the chip was validated by positive inhibitor SB203580. Then thirty-four p38-related medicinal herbs were selected and pre-screened. Two medicinal herbs having high response signal with p38-immobilized chip, *Folium Ginkgo* and *Herba Artemisiae Scopariae*, were injected into SPR system for ligand fishing. Subsequently, the recovered compounds were analyzed by UPLC-Q-TOF-MS to confirm their structures. Candidates were identified through the comparison between herbal extracts and recovered samples. Totally, two active compounds, eupatilin (EPT) and ginkgolide B (GKB), were identified by SPR-AIRS. Affinity constants of these compounds were 21.68 ± 2.21 and 44.71 ± 1.80 μM, respectively, determined by SPR assays. Enzyme linked immunosorbent assay (ELISA) revealed that EPT and GKB could inhibit the cellular activities of p38 significantly. Molecular docking showed that EPT and GKB could bind to the ATP binding site of p38. Furthermore, EPT and GKB could significantly inhibit proliferation, induce apoptosis and G2/M cell cycle arrest against K562 cell line. This is the first time that EPT and GKB are reported as effective p38 binding ligands. These results prove that SPR-AIRS could be an effective method to screen active compounds acting on a specific protein from complex systems.

2. Methods

2.1. Materials and reagents

Herbal medicines were bought from Changhai Hospital (Shanghai, China). Herbs were identified by Lianna Sun (Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, China). Standard compounds (SB 203,580, EPT and GKB, purity >99.9%) were purchased from Meilunbio (Dalian, China). Methanol, acetonitrile and formic acid (Sigma Aldrich, Missouri, United States) were all HPLC-grade. Materials and reagents used for SPR assays (including CM5 chips, EDC, NHS and ethanolamine) were purchased from GE Healthcare (Shanghai, China). Recombinant p38 protein and the ELISA kit for p38 activity were purchased from Abcam (Cambridge, United Kingdom). CCK-8 kits for cell proliferation were purchased from Beyotime (Shanghai, China). Annexin V-FITC apoptosis detection kits were purchased from Donjindo (Kumamoto, Japan). Cell cycle staining kits were purchased from MultiSciences (Hangzhou, China).

2.2. Immobilization of p38 on SPR sensor

A Biacore T200 system (GE Healthcare, Sweden) was applied to perform SPR assays. Flow cell 1 was defined as reference cell while flow cell 2, 3 and 4 were defined as detection cells. Recombinant p38 was diluted in10mM sodium acetate (pH = 4.0, 40 μg/mL) and then immobilized on three detection cells by EDC/NHS crosslinking reaction [23].

2.3. Recovery of p38-bound ingredients

The protocol of recovering p38-bound ingredients was performed according to our previous study [20]. The process of ligand recovery starts with the association active components with proteins when the samples were injected into the SPR system for 60 s at 5 μL/min. Then the system was washed with 0.5% formic acid to remove the retained sample solution. Subsequently, 2 μL recovery buffer was injected into the flow cells and incubated for 20 s to dissociate components bound to the proteins. Finally, the flow direction over the sensor surface was reversed and the sample containing p38-bound ingredients was deposited in 10 μL ammonium bicarbonate (50 mM). Phosphate buffer saline (PBS) with 5% dimethyl sulfoxide (DMSO) was selected as running buffer. Recovery procedure was replicated for 5 times in a cycle.

2.4. Experimental condition for UPLC-QTOF-MS analysis

An Agilent 1290series UPLC system (Agilent Corp., USA) was used to analyze herbal extracts and SPR-recovered samples. A Waters XSelect® HSS T3 2.5 μM column (2.1 × 100 mm) was applied for chromatographic separation at 25 °C. The injection volume is 5 μL. The mobile phase is composed of 0.1% aqueous formic acid (v/v) (A) and acetonitrile (B), using a gradient elution. Elution

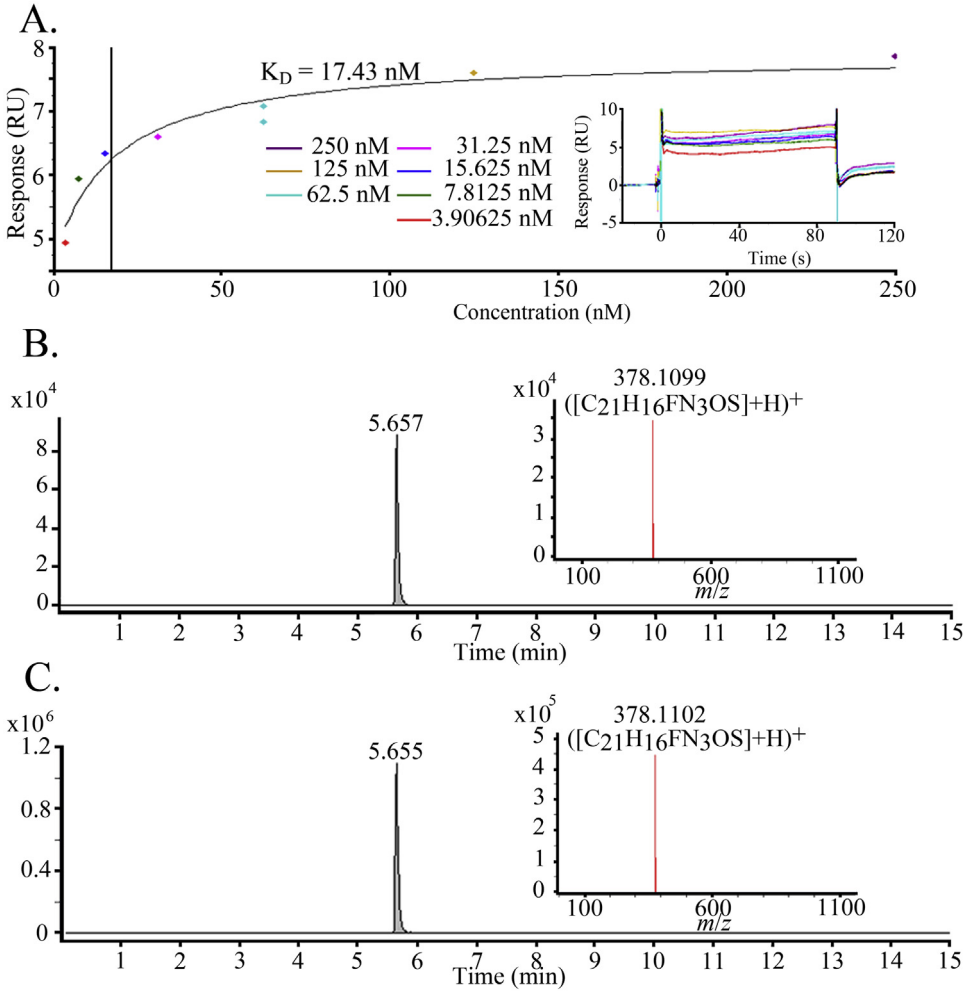

Fig. 1. (A) SPR affinity assay result of SB 203580 towards p38 (K_D = 17.43 nM). (B) EIC of SB 203580 recovery sample. (C) EIC of SB 203580 standard solution.

procedure is 10% B at 0–1 min, 10–90%B at 1–7 min, and 90%B at 7–15 min. The flow rate was kept at 3.5 mL/min, and a post-column split was used to maintain a flow rate of 0.4 mL/min into the mass spectrometer source to obtain good nebulization efficiency. An Agilent 6538 UHD Accurate-Mass QTOF/MS (Agilent Corp., USA) was used for molecular signal detection. The analysis was performed using a full-scan mode. Mass range was set from 100 to 1000 in positive ion mode. The settings of ESI source were as follows: drying gas (N_2) flow rate, 10 L/min; drying gas temperature, 350 °C; nebulizer, 35 psig; capillary voltage, 4000 V; fragmentor voltage, 120 V; skimmer voltage, 60 V; octopole RF, 250 V. All the data were processed by Agilent MassHunter Software ver. B.06.00 (Agilent Technologies). Tuning mix (G1969-85000, Agilent Corp., USA) was used for lock mass calibration in the assay.

2.5. Preparation and pre-screening of herbal extracts

Herbs were smashed into powders and then sieved through a 40-mesh sieve. Every 1 g powders were extracted by supersonic extraction with 10 mL ethanol: H_2O (75: 25) for 60 min. The extracts (100 mg/mL) were centrifuged at 5000×g for 5 min. and then filtered through a 0.22-μm filter. The samples were stored at 4 °C until further analysis.

Prepared herbal extract was diluted in PBS and centrifuged under 5000×g for 3 min. before injection. Then the extract (100 ng/mL) was injected into SPR system for 60 s dissociation. PBS was injected between two herbal samples to clean remaining compounds in the system.

2.6. Ligands recovery and identification

Herbal extract samples were recovered for 20 cycles. Recovered samples were dried by nitrogen gas and dissolved in methanol. Samples were centrifuged at 5000 *g* for 3 min. and then injected into UPLC-QTOF-MS system for further analysis. Herbal extracts were diluted in methanol and then injected into UPLC-QTOF-MS. Chemical information about constituents in herbal medicines were taken from Chemistry database of Shanghai Institute of Organic Chemistry, Chinese Academy of Science. According to the parameters provided by the Agilent Qualitative Analysis software, such as mass accuracy, isotope abundance match, spacing match and double bond equivalent index, components were confirmed due to the fine matching of these parameters.

2.7. Affinity validation

Standard compounds were diluted in PBS with 5% DMSO at concentrations ranging from 1 to 128 μM. Analytes were injected at a flow rate of 30 μL/min. The association were 90 s and dissociation times were 180 s. The affinity fitting was performed with Biacore T200 evaluation software by global fitting using a steady-state affinity model to obtain the affinity constant. The K_D values of

each compounds were detected three times and expressed in the format of mean ± S.D.

2.8. Competitive assay

Competitive assay was performed according to the protocol [24]. Firstly, 20 nM SB 203580 or 20 μM EPT solution was injected into SPR system, respectively. Then SB 203580 and EPT were mixed together, the final concentrations were 20 nM and 20, respectively. Mixed solutions were then injected into SPR system. The contact time was 60 s and the dissociation time was 120 s. Competition between EPT and SB 203580 was analyzed by comparing the response signal between single compound and mixed solution. Competitive assay of GKB and SB 203580 was performed as the same approach.

2.9. Molecular docking

The crystal structure of p38 was downloaded from Protein Data Bank (PDB ID: 1 A9U) [25]. Water molecules were removed from the structure. The docking site of original ligand (SB 203580) was selected with a 10 Å radius from the center of the binding pocket. Original ligand was removed from the structure. All chemical structures were designed and optimized using Discovery Studio v. 3.0 (DS 3.0, Accelrys Inc., San Diego, CA, USA). Molecular docking was performed by employing the Libdock module in the DS 3.0 package. Candidate compounds were docked on the defined binding site. Top hits were set as 100 and poster cluster radius was set as 0.5. The other options were set as default settings. Absolute energy and Libdock score were calculated to evaluate the best pose. The docking results were visualized in pymol.

2.10. Cell culture

K562 cell line was obtained from the Cell Bank of Shanghai Branch of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium containing 10% FBS, 100 U/ml penicillin and 100 μ g/ml streptomycin at 37 °C and in a humidified CO_2 (5%) atmosphere.

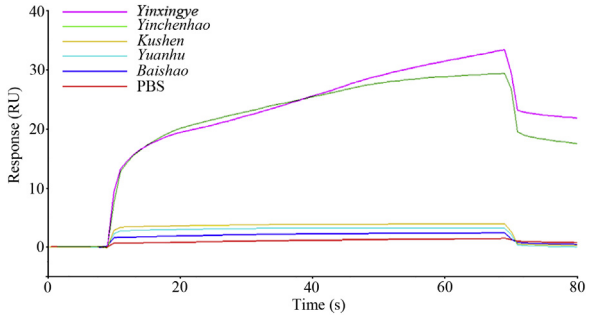

Fig. 2. The pre-screening results of five representative medicinal herbs.

2.11. p38 cellular activity assay

K562 cells (2×10^5 per well) were treated with different concentrations of EPT and GKB for 48 h. Cells were then collected and re-suspended in lysis buffer and subjected to repeated freeze/thaw cycles to fully lyse the cells. The p38 enzymatic activities were measured with a p38 MAP kinase Elisa kit (Abcam) following the manufacturer's protocol.

2.12. Cell proliferation assay

CCK-8 assays were performed according to the manufacturer's instructions. K562 cells (2×10^4 per well) were treated with EPT and GKB at various concentrations for 48 h. Then, 20 μL of CCK-8 solution was added to each well and incubated at 37 °C for another 2 h. Bubbles were removed with a hair dryer prior to reading the absorbance at 450 nm on a microplate absorbance reader (Bio-RAD instruments, USA).

3. Results and discussion

3.1. Suitability of SPR-AIRS

Recombinant p38 protein was immobilized on three flow cells of the sensor chip with immobilization levels of 12,719.6, 13,291.3 and 13,596.4 response units (RU), respectively. SB 203580, a direct inhibitor for p38 [26], was selected to validate the specificity of

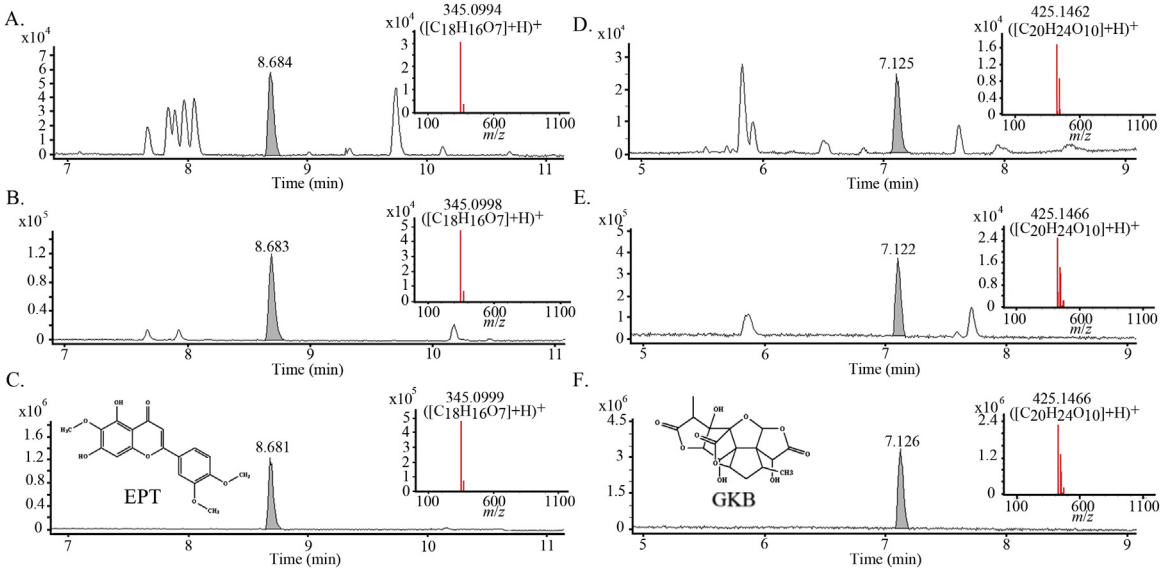

Fig. 3. Base peak chromatograms (BPC) of *Herba Artemisiae Scopariae* extract sample (A), *Herba Artemisiae Scopariae* recovery sample (B) and EPT standard solution sample (C). BPC of *Folium Ginkgo* extract sample (D), *Folium Ginkgo* recovery sample (E) and GKB standard solution sample (F).

Table 1
R_T and *m/z* of 2 candidate compounds in different samples.

Compound name	Chemical formula	Origin	*m/z*		
			herbal extract	Recovery sample	Standard sample
Eupatilin	$C_{18}H_{16}O_7$	*Herba Artemisiae Scopariae*	345.0994([M+H]$^+$)	345.0998([M+H]$^+$)	345.0999([M+H]$^+$)
Ginkgolide B	$C_{20}H_{24}O_{10}$	*Folium Ginkgo*	425.1462([M+H]$^+$)	425.1466([M+H]$^+$)	425.1467([M+H]$^+$)

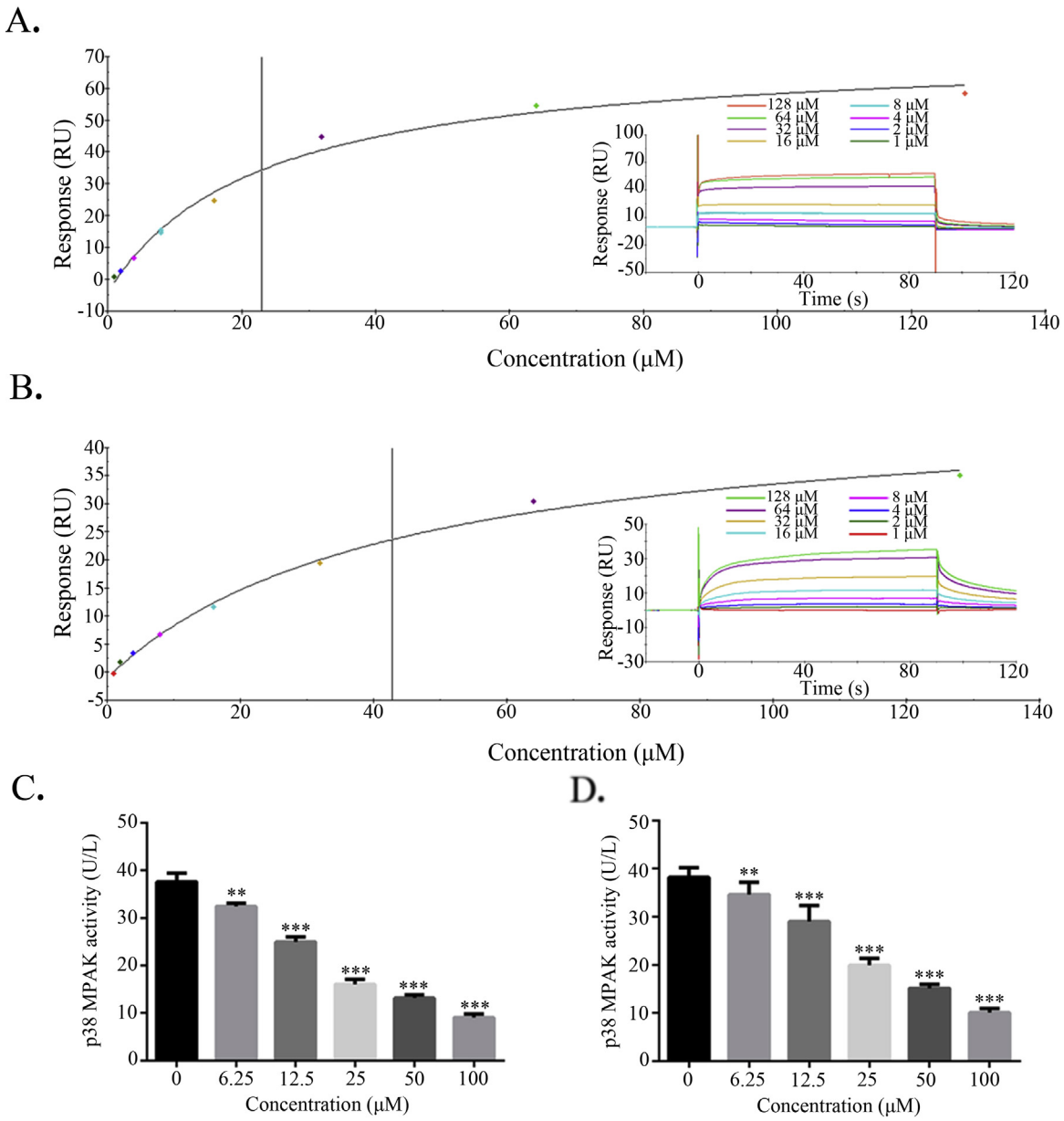

Fig. 4. SPR analysis of the interaction between EPT (A) and GKB (B) with p38. Measurement of the inhibition of p38 activity in K562 cells by EPT (C) and GKB (D). $^{**}p < 0.01$, $^{***}p < 0.001$ treatment group versus control group.

the chip. The activity of SPR sensor chip was characterized by testing the affinity between SB 203580 and p38. As a result, SB 203580 could bind to the p38-immobilized chip in a concentration-dependent way and the K_D of SB 203580 was 17.43 nM (Fig. 1A), which is consistent with the current research [27]. Subsequently, the recovery ability of SPR-AIRS was investigated by SB 203580 (100 nM). SB 3580 was recovered for 50 times by SPR. Extracted ion chromatograms (EIC) of SB 203580 recovery sample and standard solution (10 mM) were shown in Fig. 1B and C. It could be found that SB 203580 could be recovered and identified by SPR-AIRS (*m/z* = 378.1099). These results collectively validate the suitability of SPR-AIRS, which could be used to screen p38 ligands from medicinal herbs.

3.2. Screening of active ingredients from herbal extracts

Next, SPR-AIRS was applied to screen p38 ligands from herbal extract. After querying p38 related medicinal herbs in PubMed and China Knowledge Network database (CNKI), 34 medicinal herbs were selected in this study (Table S-1). The extraction sam-

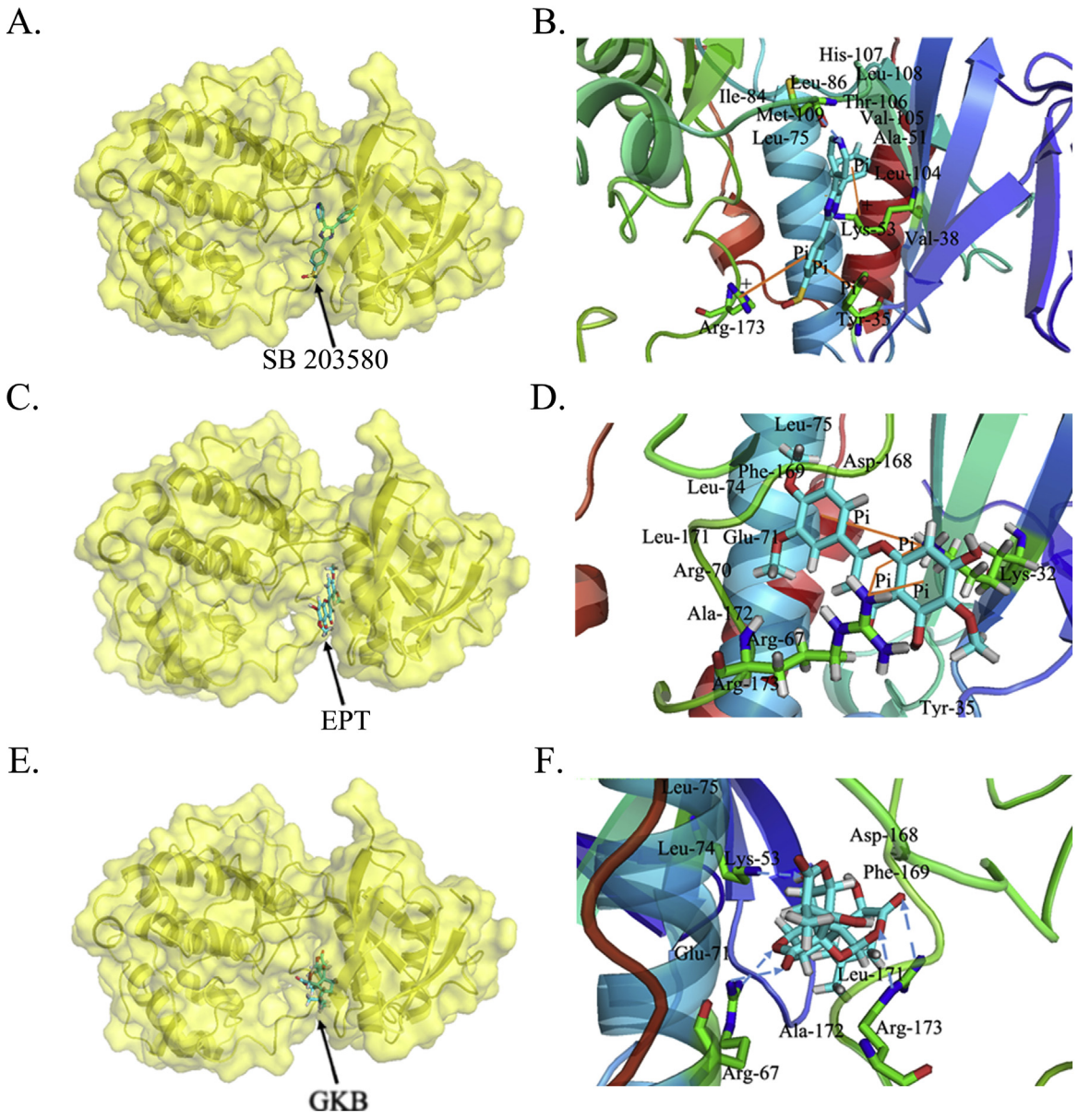

Fig. 5. Molecular docking results of p38 ATP binding site with SB 203580 (A and B), EPT (C and D) and GKB (E and F).

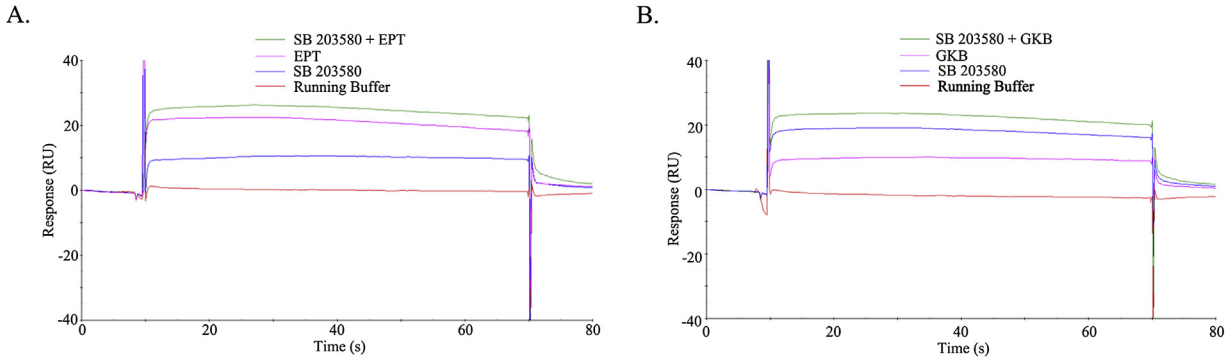

Fig. 6. Competitive assay for EPT (A) and GKB (B) on p38-immobilized chip. EPT and GKB could compete with SB 203580 on the same site on p38.

ples (100 ng/mL) were injected into SPR system, respectively. The results of five representative herbs are shown in Fig. 2, including *Folium Ginkgo* (called *Yinxingye* in Chinese), *Radix Paeoniae* Alba (Baishao), *Rhizoma Corydalis* (*Yuanhu*), *Herba Artemisiae Scopariae* (*Yinchenhao*) and *Radix Sophorae Flavescentis* (Kushen). Among the five herbs, the response signal of *Yinxinye* was the highest, which was over 30 RU. In addition, *Yinchenhao* ranked as the second position with slightly less than 30 RU. Based on the pre-screening

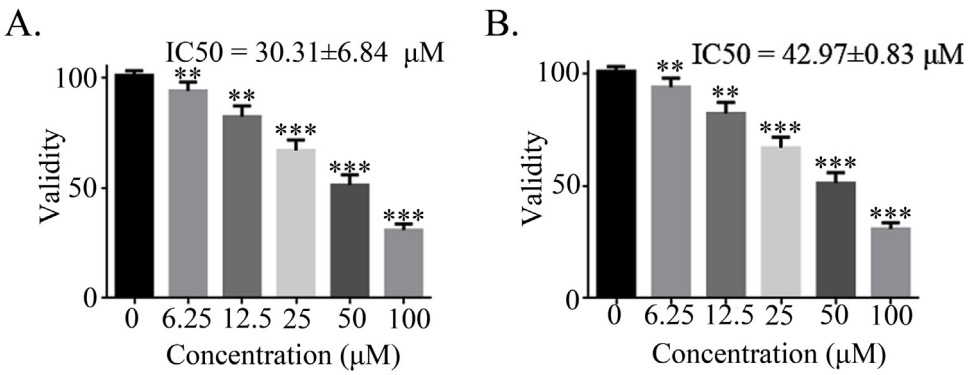

Fig. 7. Cell proliferation assay results of EPT (A) and GKB (B). $^{**}p < 0.01$, $^{***}p < 0.001$ treatment group versus control group.

Table 2
SPR assays results.

Compound name	Kd (μM)	R_{max}	Chi^2
Eupatilin	22.97	76.66	6.83
Ginkgolide B	42.81	49.37	1.15

Table 3
Molecular docking results.

Compound name	Absolute Energy	Libdock score
Eupatilin	119.215	91.0247
Ginkgolide B	87.295	93.6128

results, *Yinxinye* and *Yinchenhao* were selected for further ligand fishing and identification. The extracts were recovered for 20 times. An ion signal was identified both in *Yinchenhao* extract sample (R_t = 8.684 min, *m/z* =345.0994 ($[M+H]^+$) (Fig. 3A) and *Yinchenhao* recovery sample (R_t = 8.683 min, *m/z* =345.0998 ($[M+H]^+$) (Fig. 3B). Record in database suggested that this compound might be EPT. Subsequently, we injected the standard solution of EPT into UPLC-QTOF-MS under the same chromatographic condition. The R_t of EPT was 8.681 min and *m/z* was 345.0999 ($[M+H]^+$) (Fig. 3C). So EPT was identified as a candidate compound from *Yinxinye* extract sample. Besides, GKB was also identified as a candidate compound from *Yinxinye* extract (Fig. 3D–F). Rt and *m/z* of EPT and GKB in herbal extracts, recovery samples and standard solution are listed in Table 1.

3.3. Validation of p38 as a cellular target for EPT and GKB

In order to validate the interaction between candidates and p38, SPR affinity assays were performed three times for each compound. Typical SPR assay results for EPT and GKB are shown in Fig. 4A and B. The parameters were listed in Table 2. The other results could be found in Fig. S-1 and Table S-2. According to the results, EPT and GKB have good affinities with p38 (21.68 ± 2.21 and 44.71 ± 1.80 μM, respectively) and they can bind to p38 directly.

Next, we measured the activity changes of cellular p38 to further evaluate the interaction of candidates and p38 on K562 cell line, which is derived from chronic myeloid leukemia (CML) patients. CML is a malignant clonal disorder of hematopoietic stem cells, characterized by a reciprocal chromosomal translocation [28]. There is accumulating evidence suggesting that modulation of the p38 MAPK pathway plays an important role in the treatment of CML [29,30]. K562 cells were treated with EPT and GKB at different concentrations and lysed. The catalytic activity of p38 was determined by detecting the phosphorylation products of a p38 substrate peptide ATF-2 (Activating Transcription Factor 2) [31]. As shown in Fig. 4C and D, EPT and GKB inhibited the cellular p38 activity in a dose-dependent manner, validating the inhibition effect of EPT and GKB on p38 cellular activity.

To further evaluate the binding modes of compounds to the active site of p38, molecular docking was performed to calculate the above interactions by Libdock in DS 3.0. A canonical mechanism for cellular p38 activation is the phosphorylation of the activation loop. We performed docking of EPT and GKB with the crystal structure of p38 (PDB: 1 A9U); it is shown that EPT and GKB binds to p38 at a site overlapping with the SB203580 binding site (Fig. 5A and B). This model suggests that two compounds may compete with SB203580 for p38 binding, thereby impeding the activation of p38. The docking result of EPT and GKB is shown in Fig. 5C–F and Table 3. EPT and GKB have good Libdock scores with p38 and the absolute energy results indicated that the models are stable. According to the docking results, EPT (Fig. 5C) and GKB (Fig. 5E) could specifically bind to the ATP binding site of p38. EPT could form π-π interaction with Lys-32 and Arg-173 (Fig. 5D). GKB could form hydrogen bond with Lys-53, Arg-67 and Arg-173 (Fig. 5F).

Besides, competitive assays were performed to validate whether EPT and GKB could bind to the same site of SB 203580 on p38. In the assay, 20 nM SB 203580 solution was injected into SPR system and its response signal on p38 was about 10 RU. Then 20 μM EPT solution, which the concentration is near EPT's K_D, was injected into SPR system and its response signal was about 20 RU. Then SB 203580 and EPT were mixed together to the final concentrations of 20 nM and 20 μM, respectively. The mixed solution was injected into SPR system and its response signal on p38 was about 25 RU (Fig. 6A). Theoretically, the response unit of mixed solution could be equal to the sum of that of each single solution if the compounds bind to different sites, while the RU of mixed solution would be less than the sum of RU of each single solution if the compounds could compete with each other on the same ATP binding site. In this assay, the response signal of mixed solution was less than the sum of RU of each solution, which indicated that EPT and SB 203580 could bind to the same site on p38. As for GKB, we selected 40 μM as detect concentration. Similar trend was observed in the competitive assay (Fig. 6B). These results demonstrate that EPT and GKB could bind to the same site of SB 203580 on p38.

Collectively, the above experimental results demonstrate that p38 is a cellular target of EPT and GKB. EPT and GKB could inhibit the p38 cellular activity by binding to the ATP binding site.

3.4. Biological activity evaluation of EPT and GKB

Furthermore, we have also verified that EPT and GKB inhibited cell proliferation and apoptosis significantly after 48 h, as determined by CCK-8 kits and flow cytometry. As shown in Fig. 7A and B, EPT and GKB could both inhibit K562 cells proliferation and apoptosis in a dose-dependent manner. The IC_{50} of EPT and GKB are 30.31 ± 6.84 and 42.97 ± 0.83 μM, respectively. Besides, we have

also performed apoptosis and cell cycle assays (Fig. S-2). The results indicate that EPT and GKB could induce the apoptosis of K562 cells and induce the G2/M phase cell cycle arrest in K562 cells.

4. Conclusion

In this study, a SPR-based active ingredients recognition system was constructed and applied to screen p38 binding ligands from herbal extracts. Two candidate compounds (EPT and GKB) were discovered from complex extracts. SPR assays were performed and these two compounds showed good affinity with p38 and have common binding site with SB 203580 on p38. EPT and GKB inhibited the cellular p38 activity in a dose-dependent manner and were predicted to bind to the ATP binding site on p38 through molecular docking. Competitive assays demonstrate that EPT and GKB could bind to the same site of SB 203580 on p38. Pharmacological experiments reveal that these two ligands could inhibit cell proliferation, induce apoptosis and G2/M phase cell cycle arrest against K562 cell lines. This is the first time that EPT and GKB are reported to be effective p38 ligands. Herein, the SPR-based active ingredients recognition system is effective and feasible for screening active ingredients from herbal extracts and other complicated systems. We are digging deep on SPR-AIRS and it could be an alternative method to study the bioactive small molecules and target binding proteins in drug discovery.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2019.03.029.

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