



Original Article

Directional screening and identification of potential cytotoxic components from *Achnatherum inebrians* by a combination of surface plasmon resonance and chromatography

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ABSTRACT

Objective: To establish a method for directional screening of the cytotoxic components from the medicinal herb of *Achnatherum inebrians* by a combination of surface plasmon resonance (SPR) biosensor and chromatographic isolation technology.

Methods: Under the guidance of bioactive assessment based on binding abilities between objects and the α -Mannosidase (α -Man) target, the active components from different solvents extracts, different polar extraction parts and fractions were screened orderly and directionally using SPR. Components with a high binding ability to α -Man can be precisely oriented in a narrower fractions range and are easy to isolate. Three human cancer cells were used to evaluate the cytotoxic activity of component with the highest affinity to α -Man.

Results: Eight compounds were isolated and identified from *A. inebrians* for the first time. Deoxyvasicinone possessed the highest affinity to α -Man among them. Moreover, deoxyvasicinone showed good effects on inhibited proliferation of human hepatoma cells HepG2 ($IC_{50} = 5.7 \mu\text{mol/L}$), human breast cancer cells MCF7 ($IC_{50} = 7.21 \mu\text{mol/L}$) and human lung cancer cells HCC827 ($IC_{50} = 0.75 \mu\text{mol/L}$), respectively. In particular, its inhibitory effect on HCC827 was stronger than the positive drug gefitinib ($IC_{50} = 1.65 \mu\text{mol/L}$).

Conclusion: A comprehensive strategy of directional screening potential cytotoxic components from herb based on biomolecular interaction and chromatography was established. Deoxyvasicinone as an effective anti-cancer component was initially isolated from *A. inebrians*. It is expected that this screening strategy could provide new perspectives for rapid screening and identification of active components from natural plants with the complex matrix.

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1. Introduction

As a key enzyme in the process of *N*-terminal glycosylation of eukaryotic proteins, α -Mannosidase (α -Man) plays a crucial role in protein synthesis and fold (Herscovics, 1999). The abnormal function of α -Man can cause the metastasis and spread of cancer cells, the abnormal metabolism of mannose and virus infection. The occurrence of cancer diseases is often accompanied by an increase in its expression; α -Man has been regarded as an important target for screening anti-cancer active substances (Li et al., 2018; Srikanth & Chen, 2016).

As a genus of *Achnatherum* in Gramineae, *Achnatherum inebrians* (Hance) Keng is an important Chinese traditional medicinal herb, according to the theory of traditional medicine, and is widely used to treat mumps and joint pain (Zhang et al., 2019; Dong, Guo, Lan, Zhou & Shen, 2020). *A. inebrians* is also called 'mad grass' or 'drunk horse grass' because it grows recklessly in grasslands and leads to horses' and other herbivores' poisoning and death if it is eaten by mistake. Some studies show that the alkaloids presented in poisonous plants such as *A. inebrians* can inhibit the growth and metastasis of cancer cells and have excellent anti-cancer activity (Santos et al., 2011; Ma et al., 2018). Our preliminary study also found that the crude extracts of this herb can effectively inhibit α -Man activity (Zhang, 2018). Nevertheless, there are a few studies on its active substances (Yan, Li, & Mei, 2004; Yuan, Li, Mei, & Pei, 2021). Therefore, it is a significant work to screen potential anti-cancer active components from *A. inebrians*.

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The active substance screening from natural plants is very arduous. Only a few of substances have bioactivity among the up to hundreds or even thousands of components contained in each kind of plant. A conventional strategy is to separate compounds from plants extracts and then evaluate the activity of the purified compounds. The way is poor in goal-orientation, time-consuming and laborious, although it could comprehensively evaluate the activity of different kinds of components of plants extracts. To overcome these problems in conventional strategy, using an enzyme as a drug target provides a new opportunity due to the characteristics of strong goal-orientation, good selectivity, high throughput and so on (Xia et al., 2019).

A variety of screening methods have been developed using an enzyme as a drug-target based on the molecular level, such as enzyme microreactor (Tang & Kang, 2006; Zhao & Chen, 2014), cell membrane chromatography (Chen et al., 2014; Wang et al., 2009), molecular docking technology (Chen et al., 2017; Tang, Huang, Lee, & Chen, 2017) and surface plasmon resonance (SPR) biosensor technology. The enzyme reactor-CE is mainly used to screen enzyme inhibitors by monitoring the conversion of the enzyme-substrate to the product. Some reagents, including substrates and products of the enzyme, are required (Iqbal, Lqbal, & Müller, 2013). Cell membrane chromatography is used to discover active components that can bind to the target protein, but it has limitations in screening low abundance components in a complex matrix (Hou et al., 2014). Molecular docking technology can theoretically predict and evaluate active compounds by examining the interactions between target and compounds. But the molecular structures, both of the target and the screening object, must be determined (Peng, Zhang, Shi, & Peng, 2013). Compared with these methods, SPR biosensor technology provides a new approach. SPR can detect active components in complex matrix directly without the requirement of sample separation due to its unique advantages of being label-free, strong specificity, high sensitivity and real-time monitoring (Peng, Zhang, Shi, & Peng, 2013; Cappi et al., 2015). Moreover, the whole process of interactions between enzyme and screening object, including molecules association and dissociation, can be monitored in real-time, and the information of interactions can be obtained precisely (Boozer, Kim, Cong, Cong, & Londergan, 2006; Shen et al., 2011; Wang et al., 2017). Some screening methods based on the SPR technology have been established for screening active components (Han et al., 2018; Wang et al., 2018; Zhang, Shi, Guo, You, & Feng, 2013; Cao et al., 2016; Chen et al., 2018). For example, Zhang et al. developed a SPR-high performance liquid chromatography-tandem mass spectrometry system for screening human serum albumin ligands from medicinal herb (Zhang, Shi, Guo, You, & Feng, 2013). In addition, Cheng et al. established a SPR biosensor-based active ingredients recognition system for screening signal transducer and activator of transcription 3 ligands from medicinal herbs (Chen et al., 2018). However, these reported studies mainly screen active components from the total extract of medicinal herbs, which are preliminary and are not verified systematically. In fact, the active components in each kind of extract are significantly different due to the difference in such extraction solvents and polar parts even for the same type of medicinal herb.

To improve screening accuracy and reduce the leakage and error of screening, this study is to develop a method for comprehensively and systematically screening of potential cytotoxic components from *A. inebrians* taking α -Man as a target. The schematic illustration of the screening strategy is presented in Fig. 1.

Combining the reusability of an immobilised enzyme with the advantages of SPR in the study of interaction, α -Man was immobilised on the surface of the SPR chip as a target for screening active components. Based on the binding abilities between objects and α -Man, the bioactivity of different extracts was assessed and

the extract with the highest bioactivity was screened out and used for the next round of assessment. After orderly bioactive assessment to different types of extracts, including extraction solvents, polar parts and fractions, the components with high bioactivity to α -Man could be precisely oriented in a narrow range of fractions (Fr.) that were easy to be separated. This method can significantly improve the efficiency and accuracy of the active components screening. The components were then isolated and purified using the chromatographic method. The cytotoxic activities of compounds with the highest affinity to α -Man were finally verified with cancer cells *in vitro*.

2. Methods and materials

2.1. Materials and instruments

α -Man (from *Canavalia ensiformis* (Jack bean), product number: M 7257) was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). *A. inebrians*, the whole grass was collected from Mulei County, Xinjiang Province, and identified by Professor Wenli Chen from the Institute of Botany, Chinese Academy of Sciences. GLH sensor chip (catalogue number: 176–5013), amino coupling kit (including *N*-hydroxythiosuccinylsuccinimide (sulfo-NHS), *N*-ethyl-*N'*-(dimethylaminopropyl) carbodiimide (EDC), 1 mol/L ethanolamine hydrochloride at pH 8.5, respectively) were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Swainsonine of chromatographic reagent was purchased from Weiqi Boxin Biology Co., Ltd. (Wuhan, China). Four types of cell lines were purchased from the cell culture centre of the Chinese Academy of Medical Sciences (Beijing, China): mouse fibroblast cell line (L929), human hepatoma cancer cell line (HepG2), human breast cancer cell line (MCF7) and human lung cancer cell line (HCC827). Cell counting kit-8 (CCK-8) assay was purchased from Beyotime (Shanghai, China). DMEM, fetal bovine serum and trypsin-EDTA (Gibico, 11965–092) were purchased from Gibico (Thermo Fisher Scientific, Carlsbad, CA, USA). Acetonitrile and methanol of chromatographic reagents were obtained from Thermo Fisher Scientific (Burlington, MA, USA). Unless mentioned otherwise, all chemicals were analytical reagents and purchased from Beijing Chemical Reagent Company (Beijing, China). The ultrapure water was used in all experiments, and the solutions were filtered by a 0.22 μ m membrane. PBST buffer with pH of 7.4 composed of 10 mmol/L Na_3PO_4 , 150 mmol/L NaCl and 0.005% (volume percentage) Tween 20.

Biomolecule Interaction instrument (ProteOn XPR36, BioRad, USA), Microplate Spectrophotometer (680, BioRad, USA), CO_2 Incubator (371, Thermo, USA), pH Metre (PB-10, Sartorius, Germany), High-speed Centrifuge (Haonuosi Biology, China), XP205 Analytical Balance (Mettler Toledo, Switzerland), High-performance Liquid Chromatography (HPLC, LC-20AT, Shimadzu, Japan), Semi-preparative Liquid Chromatography (LC-6AD, Shimadzu, Japan), Ion Trap Time of Flight Liquid Mass Spectrometer (LCMS-IT-TOF, Shimadzu, Japan), Nuclear Magnetic Resonance Instrument (NMR, 600 MHz, Avenue III Brooke, Switzerland), Vacuum Freeze-drying Dryers (Beijing Oriental Technology Development Co., Ltd., Beijing, China).

2.2. Preparation of α -Man sensor chip

The α -Man sensor chip was prepared by immobilisation of α -Man on the surface of the GLH chip. The optimal pH and concentration of α -Man were first determined by preliminary adsorption experiment, and the process was as follows: at 25 $^{\circ}\text{C}$, 10 $\mu\text{g/mL}$ α -Man solutions were prepared with 10 mmol/L sodium acetate buffers at pH of 3.75, 4.0, 4.5, 5, 5.5, respectively, and then they were flowed through the surface of GLH chip at a flow rate of

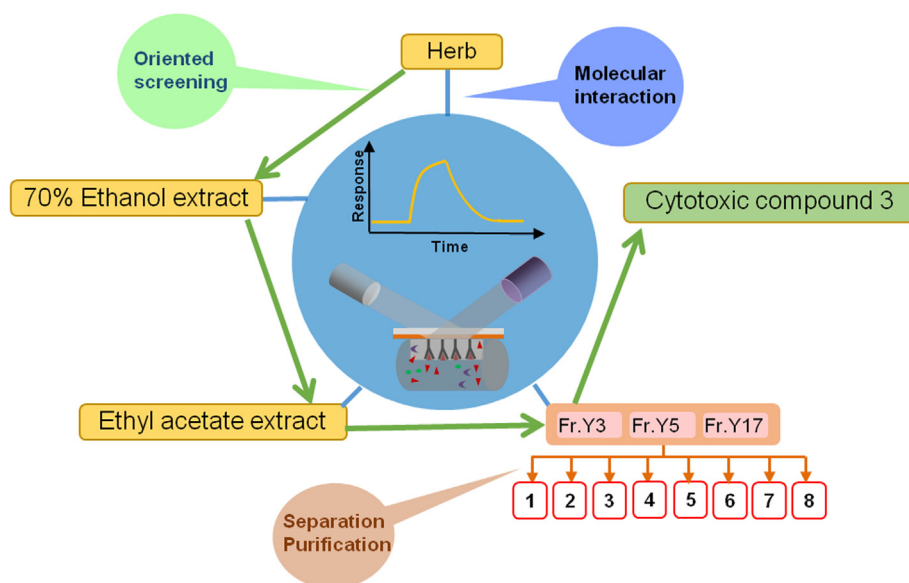


Fig. 1. Schematic illustration of directional screening and identification of potential cytotoxic components.

25 $\mu\text{L}/\text{min}$ for 120 s in parallel. Based on the value of the response signal (RU), the optimal buffer pH was selected. Afterwards, 5, 10, 15, 20 and 25 $\mu\text{g}/\text{mL}$ of α -Man solutions were prepared with the optimal pH of sodium acetate buffer and flowed through the chip in parallel at a flow rate of 25 $\mu\text{L}/\text{min}$ for 360 s. Finally, the optimal binding concentration was selected according to the value of RU.

Based on preliminary experiments, the α -Man chip was prepared as follows: 1) a 1:1 (volume percentage) mixture of 0.1 mol/L sulfo-NHS and 0.4 mol/L EDC was passed through the fifth channel (A5, binding channel) and the sixth channel (A6, reference channel) on the GLH chip in parallel at a flow rate of 25 $\mu\text{L}/\text{min}$ for 360 s; 2) a α -Man acetic acid buffer solution with the optimum condition was passed through the A5 channel at a rate of 25 $\mu\text{L}/\text{min}$. It reacted with the activated carboxyl groups on the chip surface for 360 s. At the same time, the operating buffer was passed through the A6 channel with the same condition; 3) The residual activated carboxyl groups were blocked by passing 1 mol/L ethanolamine hydrochloride through the A5 and A6 channels at a flow rate of 25 $\mu\text{L}/\text{min}$ for 360 s; 4) The A5 and A6 channels were flushed with operating buffer at a flow rate of 25 $\mu\text{L}/\text{min}$ for 360 s.

2.3. Screening active components from extraction solvents, polar extraction parts and fractions successively on guidance of bioactive assessment

2.3.1. Bioactive assessment of extracts with different extraction solvents

After being dried in the shade, the crude herb was crushed into powder in a grinder. The powder (400 g) was taken out and divided into four parts. Each part of them was soaked in 800 mL pure water, 30%, 70% and 95% ethanol aqueous solution, respectively, for 2 h. Then each part was extracted with an ultrasonic wave at the power of 4 kW for about 1 h. The extraction was repeated three times. After combining them together, the extract was filtered through a 0.45 μm membrane, vaporised and frozen to dry. Each part of dried powder about 0.1 g was dissolved in DMSO to prepare 50 mg/mL mother liquor. The mother liquors were diluted to 0.25 mg/mL by PBST buffer with 5% DMSO and stored for bioactivity assessment.

In the experiment of bioactivity assessment with SPR technology, four types of analyte were injected into the sensor chip in parallel at a flow rate of 25 $\mu\text{L}/\text{min}$ at 25 $^{\circ}\text{C}$ and interacted with α -Man. The 200 s association and 600 s dissociation time were used. All tests were repeated three times. A DMSO calibration curve was generated over a DMSO concentration range of 4.0% to 6.0%. The bioactivity of the analyte was assessed by the binding ability to α -Man.

2.3.2. Bioactive assessment of extracts with different polar extracts

The 4 kg powder was extracted with 70% ethanol aqueous solution three times with ratios of material to liquid of 1:8, 1:8 and 1:6 respectively by an ultrasonic wave at the power of 4 kW for 1 h. After combining them, the extract was filtered through a 0.45 μm membrane and vaporised to dry. Then, it was immersed in 5000 mL water and its pH was adjusted to 10 with ammonia water. The mixture was extracted with petroleum ether, ethyl acetate and saturated *n*-butanol successively three times, according to the ratio of material to liquid of 1:1. Combining them respectively, each of the extracts was filtered through a 0.45 μm membrane and vaporised to dry. The extracts of petroleum ether, ethyl acetate and *n*-butanol were obtained with the weight of 10.4 g, 45.0 g and 50.0 g.

Each of the extracts (0.5 g) was diluted with water, frozen to dry and dissolved in DMSO to prepare 50 mg/mL mother liquor. Next, the mother liquors were diluted to 0.25 mg/mL by PBST buffer with 5% DMSO for bioactivity assessment. The bioactivity assessment experiment was similar to the work, as shown in 2.3.1.

2.3.3. Bioactive assessment of fractions from ethyl acetate extract

30 g ethyl acetate extract was eluted with CH_2Cl_2 -MeOH (50:1, 30:1, 10:1) and CH_2Cl_2 -MeOH- H_2O (70:15:2, 70:26:6, 65:35:10) by 120 g silica gel column chromatography (100 – 200 mesh) successively. Eighteen fractions of Fr.Y 1–18 were collected, concentrated and frozen to dry, respectively. Each powder (0.1 g) was dissolved in DMSO to prepare 50 mg/mL mother liquor. The mother liquors were diluted to 0.25 mg/mL by PBST buffer with 5% DMSO for bioactivity assessment. The bioactivity assessment experiment was similar to the work, as shown in 2.3.1.

2.4. Isolation and determination of the compounds

Fr.Y3 (1.6 g) was eluted with MeCN-H₂O (15–19:85–81) and MeOH-H₂O (30–43:70–57) successively by semi-preparative liquid chromatography with MegresC₁₈ column (250 mm × 10 mm, 5 μm, Hanbon Sci. & Tech, Huai'an, Jiangsu, China), compounds **1** (7.1 mg), **2** (10.1 mg) and **3** (7.2 mg) were isolated. Fr.Y5 (0.6 g) was eluted with MeOH-H₂O volume percent (25:75) by semi-preparative liquid chromatography with C₁₈ column, compound **4** (4.7 mg) was isolated. Fr.Y17 (1.9 g) was eluted by MeOH-H₂O volume percent (20–22:80–78) successively by semi-preparative liquid chromatography with C₁₈ column, compounds **5** (3.3 mg), **6** (3.2 mg), **7** (4.0 mg) and **8** (4.5 mg) were isolated. After the purity of compounds prepared was detected by HPLC, their chemical structures were identified by MS and NMR.

2.5. Interactions analysis between compounds and α-Man

In interaction analysis between compounds and α-Man, each compound was tested at six concentrations using twofold dilution series (Table S1, in Supplementary Data). The six concentrations of each compound were injected simultaneously at a flow rate of 25 μL/min for 12 min association followed by 20 min dissociation. All tests were repeated three times. DMSO calibration curve was generated over a DMSO concentration range of 4.0% to 6.0%. The kinetic parameters, including association rate constant (k_a), dissociation rate constant (k_d) and equilibrium dissociation constant (K_D) of each compound with α-Man, were calculated by using protenon manager 3.1.0 software based on the 1:1 Langmuir binding model. The equilibrium parameters were also calculated by using equilibrium steady-state analysis.

2.6. Cell viability assay

The cell line was cultured at 37 °C in a 5% CO₂ humidified incubator, maintained in DMEM medium supplemented with 10% low-endotoxin fetal calf serum, and passaged by 0.25% trypsin-EDTA digestion. A total of 5×10^3 cells were seeded into 96 well plates and cultured for 24 h at 37 °C. Then, different concentrations of the compounds were added and cultured for 72 h at 37 °C. Five multiple holes were set for the parallel experiment at each concentration. Cells were then incubated with 10 μL CCK 8 for 4 h at 37 °C. The absorbance at a wavelength of 450 nm was measured with a spectrophotometer. Data are presented as mean ± standard deviation (SD) of five parallel experiments.

3. Results and discussion

3.1. Immobilisation and activity of α-Man on the chip

α-Man was immobilised generally on the chip using the amino coupling method. The binding amount and enzyme activity of the α-Man on the chip are the key to the effective identification of active components with different with low abundance in complex matrix. Therefore, it is very important to select the appropriate chip type and optimise the immobilisation conditions. The GLH chip was selected for immobilisation of α-Man for its surface contained an alginate-based polymer matrix layer with the highest binding capacity that efficiently activated the carboxylic group for amine coupling. It could provide an excellent hydrophilic environment for biological reactions but also increase the amount of immobilised protein, suitable for analysis of protein-small molecule ($M_w < 1000$) interaction (Turner, Tabul, Nimri, & Laboratories, 2008).

The amino coupling reaction can be carried out when the protein is quickly adsorbed on the surface of the sensor chip in the form of electrostatic adsorption. So, the adsorption of α-Man on the surface of the GLH chip was investigated. For the carboxyl group on the surface of the GLH chip is negatively charged at pH greater than 3.5, the protein in buffer solution with a positive charge is favourable for binding with the carboxyl group due to electrostatic action. Therefore, the pH of the buffer solution should be higher than 3.5 and lower than 6.2 that is the isoelectric point of α-Man. It can be seen in Fig. 2A that the value of RU gradually increased with the decrease of pH. However, the pH should not be too low, considering the carboxyl group reacts better with the uncharged amino group. Therefore, sodium acetate buffer solution with pH 4 was selected for dissolving α-Man. The concentration of α-Man can also affect its binding amount on-chip. As shown in Fig. 2B, the value of RU gradually increased with the increase in concentration and reached the maximum at the concentration of 20 μg/mL. Moreover, the adsorption rate was the fastest among them and reached equilibrium quickly (Fig. S1 in Supplementary Data). As a consequence, the concentration of 20 μg/mL was selected for the immobilisation concentration.

Fig. 2C showed the whole immobilisation process of α-Man on the chip surface using 20 μg/mL α-Man sodium acetate buffer solution with a pH 4. The immobilising curves fitted well with the characteristics of dynamic binding standard curves, suggesting that α-Man was uniformly immobilised on the surfaces of the chip. The final response signal of immobilised α-Man was about 18 000 RU, equated to the immobilisation concentration of 18 ng/mm. The high binding amount of α-Man on-chip was beneficial to detect small molecule active compounds with different abundance.

To validate the activity of the α-Man, swainsonine was selected as a positive compound, which is a direct inhibitor for α-Man. Swainsonine standard solution were injected into the sensor chip with running buffer PBST as the blank control. It can be found (in Fig. S2 in Supplementary Data) that the response signal of swainsonine increased gradually to 20 RU during the association process. While at the disassociation process, the response signal does not decrease and is very stable, indicating that swainsonine has a strong affinity to the α-Man. The SPR sensing curve of swainsonine is similar to that of antigen-antibody interaction, which demonstrated the immobilised α-Man had high activity.

3.2. Screening of active components from extraction solvents, polar extraction parts and fractions

To improve the accuracy of screening active components, it is necessary to evaluate the active compounds from different extraction solvents, polar extraction parts and fractions comprehensively and systematically. Therefore, on the guidance of bioactivity assessment, four types of extracts with different solvents were first screened. The response signal from 70% ethanol extract was the highest and more than 100 RU, as shown in Fig. 3A, indicating that 70% ethanol extract possesses more active components (Liu et al., 2009; Liu et al., 2011).

The binding abilities between four types of polar extraction parts from 70% ethanol extract and α-Man were shown in Fig. 3B. The response signal of all three types of polar extraction parts was more than 100 RU except for the water phase. The binding ability between ethyl acetate extract part and α-Man was the highest, and the binding rate was also the fastest (Fig. S3 in Supplementary Data).

The binding abilities among 18 fractions from ethyl acetate extract part and α-Man were shown in Fig. 3C. The response signal of most of them was over 100 RU, indicating that all of them possess high binding abilities with α-Man. Taken into account the binding rate with α-Man (Fig. S4, in Supplementary Data) and sep-

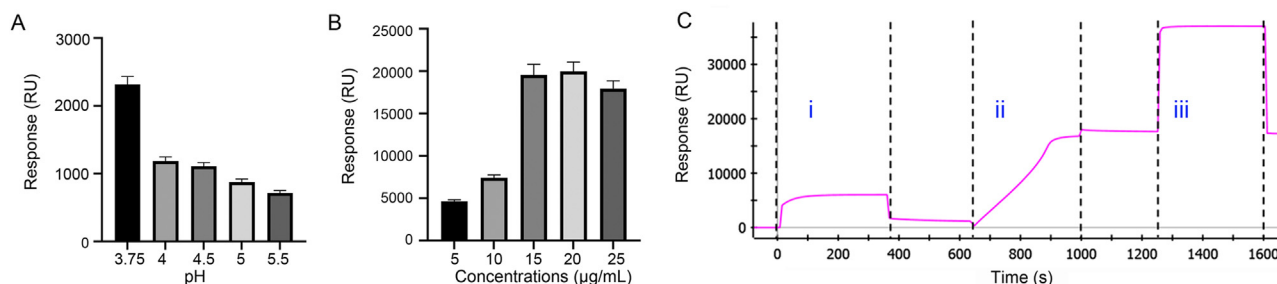


Fig. 2. Immobilisation of α -Man sensor chip. Adsorption capacity of α -Man on-chip surface with different pH values (A) and different concentrations (B). Kinetic immobilisation process of α -Man on the surface of GLH sensor chip (C: i: activating channel with sulfo-NHS/EDC; ii: binding of α -Man on channel; iii: blocking with ethanolamine hydrochloride).

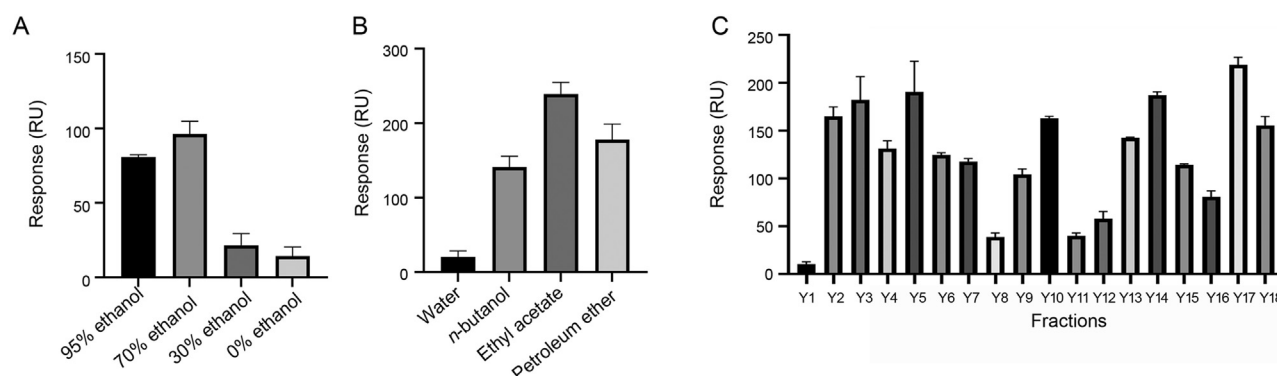


Fig. 3. Binding abilities between different kinds of extracts and α -Man. (A) Four kinds of extracts with different percentages of ethanol; (B) Four kinds of extracts with different polar extraction parts of 70% ethanol extracts; (C) eighteen kinds of fractions from ethyl acetate extract.

aration and purification conditions, Fr.Y3, Fr.Y5, Fr.Y17 were finally selected for isolation and purification.

3.3. Isolation and identification of active components

After Fr.Y3, Fr.Y5 and Fr.Y17 fractions were isolated by semi-preparative liquid chromatography, eight compounds were obtained, and their purities were detected by HPLC. The HPLC chromatograms of **1**, **2** and **3** showed them high purities (Fig. S5, in Supplementary Data). Eight compounds were identified by MS and NMR (Table S2, in Supplementary Data), and their structures were shown in Fig. 4. To the best of our knowledge, all these compounds were found from *A. inebrians* for the first time.

3.4. Interactions between compounds and α -Man by SPR

The results of kinetic analysis and thermodynamic parameters of interactions between α -Man and all compounds were shown in Table 1. Among them, the K_D value of compound **3** with

4.73×10^{-5} was the lowest among them, indicating the affinity of deoxyvasicinone to α -Man was the highest.

At the same time, the difference between deoxyvasicinone and vasicinone was only on the C-3 group, as shown in Fig. 5A and B. However, the association rate between deoxyvasicinone and α -Man was faster than the vasicinone and α -Man, and the dissociation rate was slower than that of vasicinone. So, the affinity of the deoxyvasicinone for α -Man was significantly greater than that of vasicinone. To verify the results, equilibrium steady-state analysis is used to evaluate the affinity between compounds and α -Man. It can be found from Fig. 5C and D that the concentrations curves fit well, and the affinity data obtained from equilibrium analysis were very close to kinetic data. Therefore, the results fully confirmed the reliability of this method.

SPR is the gold standard for detecting drug-target interactions. These results indicated that the candidate active component deoxyvasicinone could effectively bind to α -Man with high affinity, which may be a potential anti-cancer active component (Bravman et al., 2006; Chen et al., 2021).

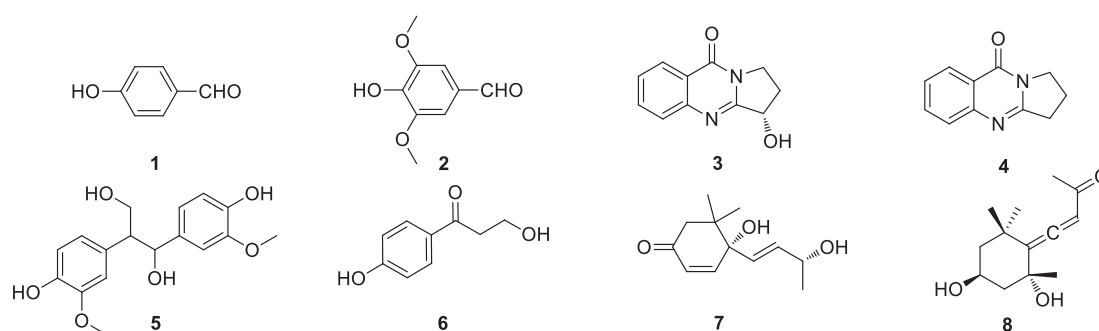


Fig. 4. Structures of compounds **1–8**.

Table 1
Kinetic parameters of interactions between compounds and α-Man.

No.	Compounds	k_a [L/(mol·s)]	k_d (/s)	K_D (mol/L)
1	<i>p</i> -Hydroxybenzaldehyde	1.54×10^0	2.11×10^{-3}	1.37×10^{-3}
2	3, 5-Dimethoxy-4-hydroxy-benzaldehyde	1.20×10^1	4.18×10^{-3}	3.47×10^{-4}
3	Deoxyvasicinone	7.33×10^1	3.47×10^{-3}	4.73×10^{-5}
4	Vasicinone	3.91×10^0	1.94×10^{-3}	4.96×10^{-3}
5	1, 2-Di-(4-hydroxy-3-methoxyphenyl)- propyl-1,3-diol	1.62×10^1	6.65×10^{-3}	4.10×10^{-4}
6	3-Hydroxy-1-(4-hydroxyphenyl) – 1-acetone	9.92×10^0	7.05×10^{-3}	7.10×10^{-4}
7	Vomifolios	4.76×10^0	4.41×10^{-3}	9.26×10^{-4}
8	3, 5-Dihydroxymegastigma-6, 7-dien-9-one	6.12×10^0	4.37×10^{-3}	7.14×10^{-4}

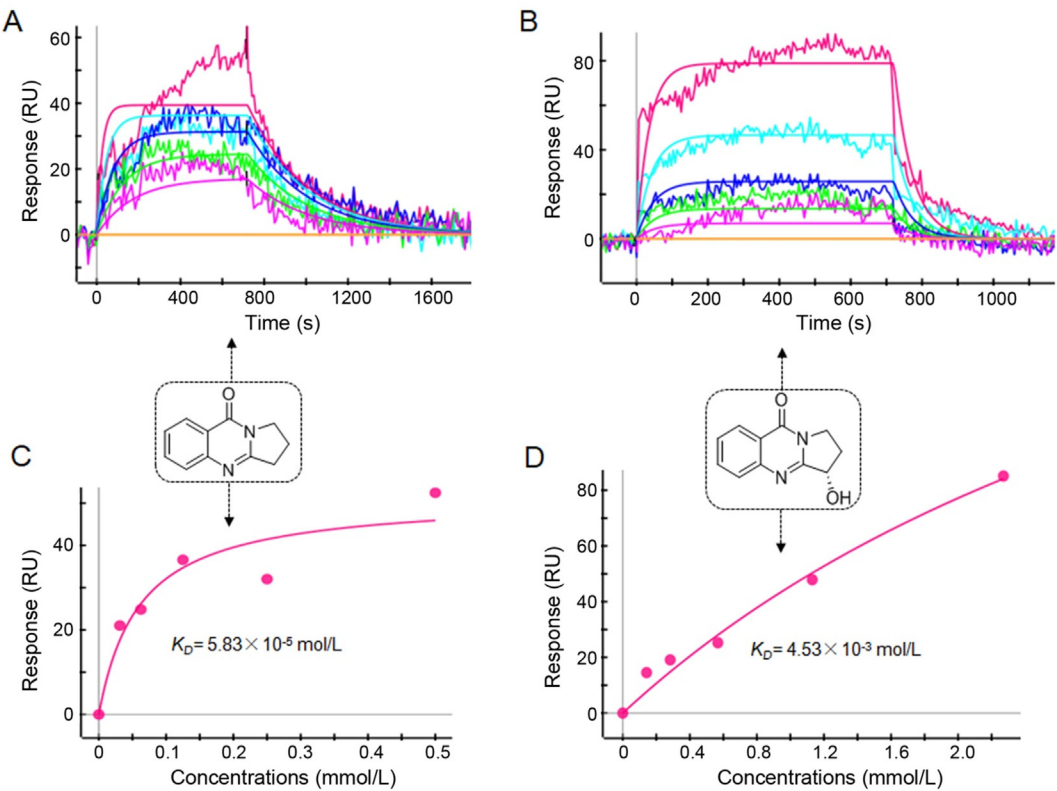


Fig. 5. Interactions between two compounds and α-Man measured by SPR at 25 °C. The sensorgrams represent a real-time dynamic interaction process. The smooth curves were obtained by performing a global fit of sensorgrams. Kinetic interaction between deoxyvasicinone (A), vasicinone (B) and α-Man. The fitted curves for different concentrations of deoxyvasicinone (C), vasicinone (D) binding to α-Man using equilibrium steady-state analysis.

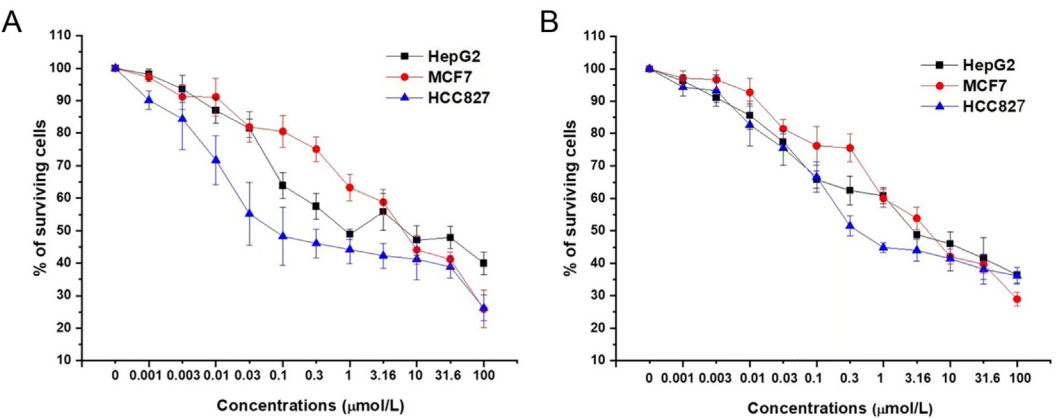


Fig. 6. Inhibitory effects of candidate components deoxyvasicinone (A), gefitinib (B) on three kinds of cancer cells ($n = 5$).

Table 2

Anti-proliferative activities of deoxyvasicinone against cancer cells.

Compounds	IC ₅₀ (μmol/L)		
	HepG2	MCF7	HCC827
Deoxyvasicinone	5.70	7.21	0.75
Gefitinib	4.69	3.95	1.65

3.5. Anti-proliferative activity of deoxyvasicinone

Although deoxyvasicinone is reported to be a potential target drug candidate for Alzheimer's disease as a kind of acetylcholinesterase inhibitor (Du, Liu, Xie, & Ma, 2019; Deng et al., 2019), there have been limited studies on its anti-cancer activity. Therefore, three kinds of cancer cells, including human hepatoma cells (HepG2), human breast cancer cells (MCF7) and human lung cancer cells (HCC827), respectively were used to evaluate the cytotoxic activity of deoxyvasicinone, and gefitinib was used as a positive control compound. The toxicity of deoxyvasicinone on normal cells was evaluated by the proliferation inhibition of mouse fibroblast cells (L929). The safe doses of deoxyvasicinone and gefitinib were 20 and 40 μmol/L under the condition of keeping 90% cell viability, respectively, showing that they were less toxic to the normal cell (Fig. S6, in Supplementary Data). The results of anti-proliferative activity were shown in Fig. 6. It can be found in Table 2 that deoxyvasicinone showed good effects on inhibited proliferation of HepG2 (IC₅₀ = 5.7 μmol/L), MCF7 (IC₅₀ = 7.21 μmol/L) and HCC827 (IC₅₀ = 0.75 μmol/L), respectively. In particular, the inhibitory effect of deoxyvasicinone on HCC827 was stronger than the positive drug gefitinib (IC₅₀ = 1.65 μmol/L). These results also verified the feasibility of the screening strategy.

4. Conclusion

In this paper, a comprehensive strategy of directional screening potential cytotoxic components from herb based on biomolecular interaction and chromatography was established. Deoxyvasicinone was initially isolated from *A. inebrians*, a plant with promising anti-cancer properties. To the best of our knowledge, this is the first time that deoxyvasicinone is reported as an effective anti-cancer component. Furthermore, the method significantly increased the speed, efficiency and accuracy of screening active components from natural plants with complex matrixes, and it is expected that the screening strategy will be extensively employed in medicinal herb-drug development.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chmed.2022.06.008>.

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