Journal Name

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Identification of Enzyme Inhibitors Using Therapeutic Target Protein - Magnetic Nanoparticle Conjugates

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Supplymental materials

1. ***Preparation for GP extract***

20 mL water was added to 0.25 g dried powder of GP. The mixture was sonicated for 15 min. After being centrifuged, the supernatant was analyzed without further treatment. ESI-MS spectum obtained from the GP aqueous extract is shown in Fig. S1.

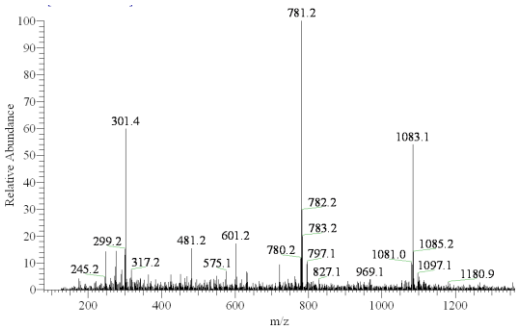

Figure S1. ESI-MS spectrums of GP aqueous extract.

2. ***Preparation of target protein-MNPs***

The protein-MNP conjugates were were prepared following the procedures we reported previously.[1-2] Briefly, Fe_3O_4 nanoparticles (MNPs) were prepared by coprecipitation with a molar ratio of Fe^{2+} : Fe^{3+} = 1 : 2. MNPs were firstly coated with SiO_2 using tetraethyl orthosilicate (TEOS). Secondly, the particles were dispersed in 3-aminopropyltrimethoxysilane (APTMS) to add -NH_2 to the SiO_2; the resultant particles were then dispensed in the 25% glutaraldehyde solution solution to provide -CHO to the silica coating. Finally, the silica coated MNPs with -CHO functionality was incubated for 24 hrs with target protein (α-glucosidase or protein tyrosine phosphatase 1B, purchased from Sigma–Aldrich) to obtain target protein-MNPs. They were suspended in ammonium acetate buffer solution (10 mM, pH 7.4) and kept at 4 °C before use.

3. ***Affinity extraction using α-glucosidase-MNPs:***

To carry out the extraction experiment, a portion of GP extract (1.0 mL) was mixed with 100 μL α-glucosidase-MNPs suspension prepared above in a 4-mL eppendorf tube. The mixture was shaken for 5 min on an oscillator shaker. The α-glucosidase-MNPs were separated from the liquid by means of an external magnet. The supernatant was completely removed. The α-glucosidase-MNPs were washed three times with 1 mL of ammonium acetate buffer solution (10 mM, pH 7.4). Elution of the α-glucosidase-MNPs was done with 1 mL buffer containing 50% ACN and incubated for 1 min. After the magnetic separation, the supernatant was collected for ESI-tandem MS analysis. Extraction with PTP1B-MNPs was carried out similarly.

4. ***Assay of α-glucosidase inhibitory activity:***

The inhibitory experiment was carried out by following a procedure in literature.[3] Briefly, A 100 μL reaction system containing 0.02 U of α-glucosidase, the test compound, and 67 mM potassium phosphate buffer (pH 6.8) was pre-incubated at 37 °C for 10 min. The reaction was initiated by adding 0.1 M maltose. The reaction mixture was incubated at room temperature for 10 min. Then, the glucose-detecting agent was added, and the absorbance (A) at 490 nm was recorded. (A negative control in the absence of test compound, and a blank control in the absence of either α-glucosidase or test compound were run simultaneously.) The test compound was initially assayed for its inhibition on α-glucosidase at a concentration of 10 μg/mL. The inhibition rate was calculated by the following equation:

$$Inhibition\,\% = \frac{A_{negative} - A_{test}}{A_{negative} - A_{blank}} \times 100\%$$

5. ***Assay of PTP1B inhibitory activity:***

A 200-μL reaction system containing PTP1B (recombinant, expressed in Escherichia coli), the test compound, and the buffer (25 mM HEPES, 50 mM sodium chloride, 2.5 mM EDTA, 0.1% BSA, pH 7.2) was pre-incubated at 37 °C for 10 min. The reaction was initiated by adding PNPP (4-nitrophenyl phosphate disodium salt hexahydrate, the substrate of PTP1B), and the reaction mixture was incubated at 37 °C for 30 min. Then, sodium carbonate was added to stop the reaction, and the absorbance (A) at 405 nm was recorded. (A negative control in the absence of test compound, and a blank control in the absence of either PTP1B or test compound were run simultaneously.) The test compound was initially assayed for its inhibition of PTP1B at a concentration of 10 μg /mL. The inhibition rate was also calculated by the equation shown above.

6. ***Structural elucidation of the ligands isolated from GP extract by MS/MS :***

The MS/MS spectra for the four ligands isolated are shown in Figure S2. Ligand I (*m/z* 301) shows fragments at *m/z* 283, 272, 257, and 229, matching the structural characteristics of ellagic acid; ligand Ⅱ(*m/z* 601) shows fragments at *m/z* 299 and 271 matching the structural characteristics of gallagic acid; ligand ⅢⅢ(*m/z* 781) shows fragments at *m/z* 721 and 601 matching the structural characteristics of punicalin; and ligand Ⅳ(*m/z* 1083)) shows fragments at *m/z* 781 and 601 matching the structural characteristics of punicalagin, respectively. The assignments are in consistance with those reported in literature.[4-5]

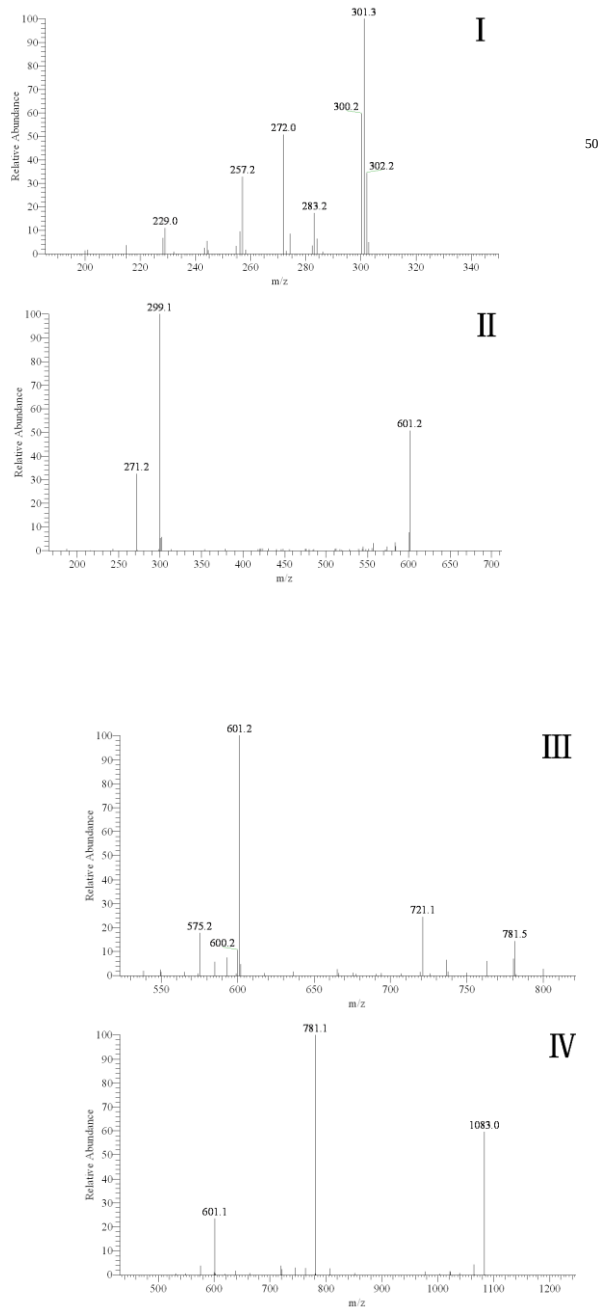

Figure S2. MS/MS spectra of ligands isolated from GP aqueous extract identified as ellagic acid (I), gallagic acid (II), punicalin (III), and punicalagin (IV), respectively.

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