Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Materials

A high-throughput screening assay for dipeptidyl peptidase-IV inhibitors using human plasma

Jing Zhang [a], Xing-Kai Qian [a], Pei-Fang Song [a], Xiao-Dong Li [a], An-Qi Wang [a], Hong Huo [c], Jing-Chun Yao [b], Gui-Min Zhang [b,*], Li-Wei Zou [a,*]

[a]Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, Shanghai, 201203, China

[b] State Key Laboratory of Generic Manufacture Technology of Traditional Chinese Medicine, Lunan Pharmaceutical Group Co.LTD, Linyi, 276006, China

[c] Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

*Corresponding author

E-mail addresses: gmzhanglunan@163.com (G.-M. Zhang) and chemzlw@163.com (L.-W. Zou)

* Corresponding author. Tel.: +86-215-132-3182; e-mail: gmzhanglunan@163.com

* Corresponding author. Tel.: +86-215-132-3182; e-mail: chemzlw@163.com

2.1 Materials

There were 69 kinds of natural alkaloids in total, among which Reserpine, Rutecarpine, Monocrotaline, Lapatinib, Quinidine and Nonivamide were purchased from Dalian Meilun Biotechnology Co, Ltd., Evodiamine, Brucine and Toddaline were purchased from Sichuan Vicky Biotechnology Co., Ltd.; Oxymatrine and Gelsemine were purchased from Chengdu pfeider Biotechnology Co., Ltd.; Trigonelline was purchased from China Institute of pharmaceutical and biological products; Loperamide hydrochloride was purchased from tichia (Shanghai) chemical industry Development limited and Quinine.

The following 54 alkaloid samples obtained from the Dalian Institute of Chemical Technology of the Chinese Academy of Sciences: Uncarine E, Senecionine, Catharanthine, Dehydroevodiamine, Jervine, Veratramine, Vinblastine sμLfate ,Vinpocetine, Colchicine, Piperine, Arecoline hydrobromide, Cyclopamine, Hordenine, Theophylline, Harmine, 7-Ethyl-10-Hydroxycamptothecin, Camptothecin, Berbamine, Berbamine dihydrochloride, Boldine, Cephalotaxine, Corynoxeine, Cyclovirobuxine D, Fangchinoline, Harmine hydrochloride, Huperzine B, Lappaconitine, Hypaconitine, Mesaconitine, Ranaconitine, Imperialine, Isorhynchophylline, Lycorine hydrochloride, Magnoflorine, Peiminine, Scopoletin, Sinomenine hydrochloride, Solargine, Khasianine, and Solanine Sophocarpine, Sophoridine, Sanguinarine, Berberine hydrochloride, Coptisine Chloride, Dehydrocorydalin, Jatrorrhizine, Palmatine Chloride, Chelidonine, D-Tetrahydrop-almatine, Phellodendrine chloride, Tetrahydroberberine, Rotundinum and Tetrahydrocop-tisine.

3.1 Methodological optimization

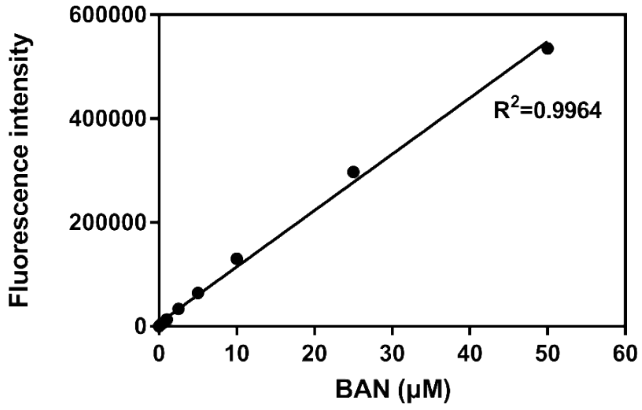

Fig. S1. Standard curves for BAN in PBS and acetonitrile (v/v 1:1), The product BAN standard curve is

expressed as: Y = 10845*X + 6190 (P < 0.0001). Data were shown as mean ± SD (n=3).

3.2. Enzymatic kinetics of DPP-IV-mediated hydrolysis of fluorescent substrates (GP-BAN and GP-AMC)

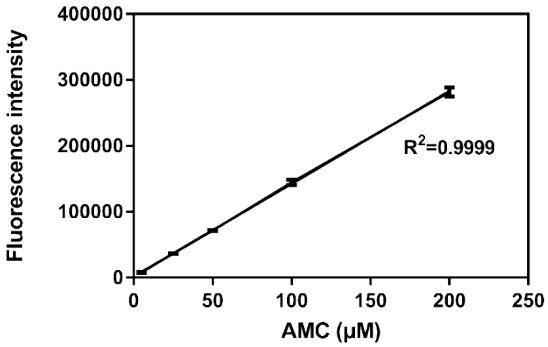

Fig. S2. Standard curves for AMC in PBS and acetonitrile (v/v 1:1), The product AMC standard curve was expressed as: Y = 1407*X + 1598 (P < 0.0001). Data were shown as mean ± SD (n=3).

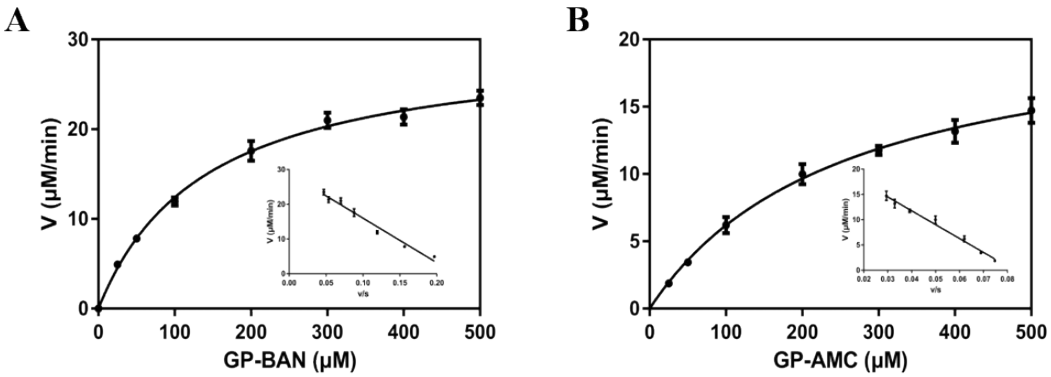

Fig. S3 Enzyme kinetics study of DPP-IV-mediated hydrolysis of substrates in human plasma. **A)** Enzyme kinetics study of GP-BAN hydrolysis in human plasma, the K_m and V_{max} values of GP-BAN were 140.70 μM, and 29.84 μM/min, Eadie-Hofstee linear R^2=0.974; **B)** Enzyme kinetics study of GP-AMC hydrolysis in human plasma, the K_m and V_{max} values of GP-AMC were 255.70 μM and 22.01 μM/min. Eadie-Hofstee linear R^2=0.979. Data were shown as mean ± SD (n=3).

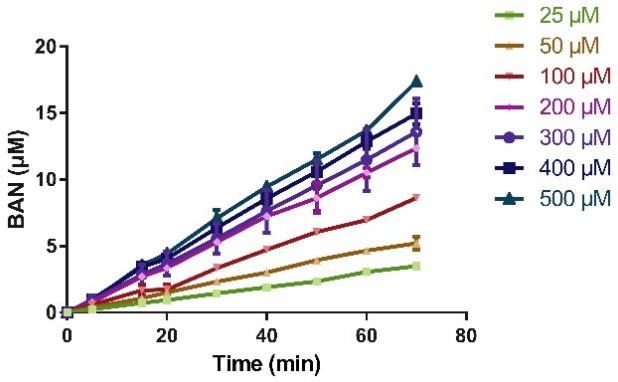

Fig. S4 Dependence curve of product generation over time. Data were shown as mean ± SD (n=3).

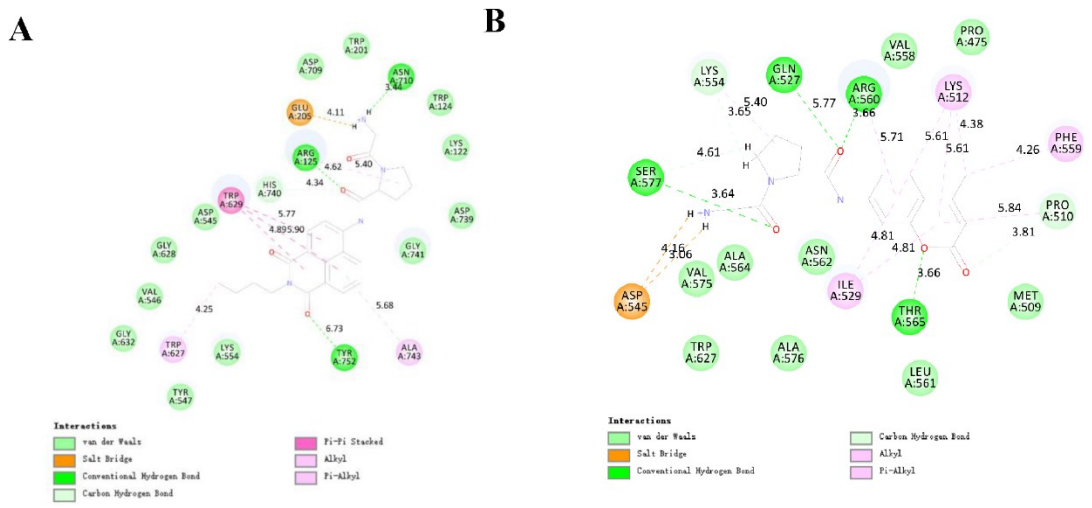

Fig. S5. 2D represents the key interaction between GP-BAN (**A**), GP-AMC (**B**) and the amino acids surrounding the DPP-IV pocket.