The perceptions of natural compounds against dipeptidyl peptidase 4 in diabetes: from in silico to in vivo

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Abstract: Dipeptidyl peptidase IV (DPP-4), an incretin glucagon-like peptide-1 (GLP-1) degrading enzyme, contains two forms and it can exert various physiological functions particular in controlling blood glucose through the action of GLP-1. In diabetic use, the DPP-4 inhibitor can block the DDP-4 to attenuate GLP-1 degradation and prolong GLP-1 its action and sensitize insulin activity for the purpose of lowering blood glucose. Nonetheless the adverse effects of DPP-4 inhibitors severely hinder their clinical applications, and notably there is a clinical demand for novel DPP-4 inhibitors from various sources including chemical synthesis, herbs, and plants with fewer side effects. In this review, we highlight various strategies, namely computational biology (in silico), in vitro enzymatic and cell assays, and in vivo animal tests, for seeking natural DPP-4 inhibitors from botanic sources including herbs and plants. The pros and cons of all approaches for new inhibitor candidates or hits will be under discussion.

Keywords: diabetes, dipeptidyl peptidase 4, herbal, in silico, natural compounds

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Introduction
According to the statistics from International Diabetes Federation (IDF), there were 425 million diabetes mellitus (DM) patients in 2017 worldwide, and that number is expected to increase to 629 million by 2045. Abnormally high blood glucose caused by insulin insufficiency or insensitivity can lead to severe complications such as chronic renal failure, microvascular complication, cerebrovascular accident, and infarction induced by high glycated serum and blood vessel proteins. Moreover, insufficient insulin signal leads to the decreased glucose uptake from the blood that, in turn, can result in ulcers, gangrene, diabetic retinopathy, and neuropathy. Recent DM treatment is inclined to maintain the blood glucose level within normal limits by (e.g. nutritional therapy and physical management) and medication due to the incurable nature of DM. Diabetic medications can be characterized into five strategies based on their acting mechanisms: raising insulin secretion (e.g. sulfonylurea and meglitinide analogs), reducing intestinal glucose absorption (e.g. acarbose), triggering insulin-independent glucose uptake signaling (e.g. thiazolidinedione and biguanide), reducing urinal glucose reabsorption (e.g. gliflozins), and prolonging insulin sensitive [e.g. dipeptidyl peptidase 4 (DPP-4) inhibitor and glucagon-like peptide 1 (GLP-1) receptor agonists] [American Diabetes Association, 2019b]. These antidiabetic drugs help DM patients to maintain their blood glucose levels with various adverse effects (Table 1), such as urine-tract infection, lactoacidosis, hypoglycemia, and obesity. These drug-related adverse effects can deteriorate the quality of life of DM patients and create unsurmountable difficulties for proper dosing regimens in a clinical setting. It has been observed that DPP-4 inhibitors can exert a similar efficacy in reducing blood glucose levels without severe adverse effects such as hypoglycemia as compared with sulfonylurea. Nevertheless,
various adverse side effects associated with the current DPP-4 inhibitor can still be observed that, in turn, can severely limit their practical application. As such, there is a clinical demand for novel DPP-4 inhibitors from various sources including chemical synthesis and botanic sources containing herbs and plants with fewer side effects. In this article, we have reviewed various approaches including in silico, in vitro enzymatic and cell assays, and in vivo animal tests in the search for natural DPP-4 inhibitors for the treatment of type 2 diabetes.

### Overview of DPP-4 and its biological function

**Two forms of DPP-4**

DPP-4, which is a 88 kDa serine protease, contains one region of cytoplasmic region (amino acids 1–6) coupled with transmembrane domain (amino acids 7–28) and extracellular region (amino acids 29–766) with the main catalytic domain. There are two DPP-4 isoforms in the body: membrane-bound DPP-4 (mDPP-4) composed of full-length DPP-4 peptide; and soluble DPP-4 (sDPP-4), whose cytoplasmic and transmembrane regions are absent. Both forms can exert various biological activities in regulation of physiology and pathology.

**Biological function of soluble form DPP-4**

sDPP-4 is secreted by lymphocytes, circulates in the blood, and shows high concentration in kidney. It has been observed that sDPP-4 plays various roles in improving skeleton muscle activity, immunocyte activation, chemotaxis, and homeostasis. sDPP-4 can secrete into serum via the response of skeletal muscle cells upon acute physical activities or feeding protein hydrolysate. Secreted sDPP-4 can reduce vasoconstriction that is caused by neuropeptide Y (NPY) and subsequently increase the arteriolar diameter of skeletal muscle that provides a physiological explanation for raising training efficiency caused by sDPP-4. In addition to arteriolar diameter of skeletal muscle, secreted sDPP-4 acts as myokine, which stimulates inflammation in smooth muscles from blood vessel through activating protease-activated receptor 2 (PAR2)/ERK/NF-κB signaling pathway, increasing proinflammatory cytokine release and finally stimulating smooth muscle cell proliferation. However, sDPP-4-induced smooth muscle inflammation is not always good to the body. For instance, Romacho et al. reported sDPP-4 might cause microvascular endothelial dysfunction, which is the cause of chronic kidney disease in elders, through the same signaling with smooth muscle inflammation. Thus, Dubé et al. illustrated that cardiovascular inflammation can be attenuated by DPP-4 inhibitor in the process of HIV treatment.

### Table 1. Current hypoglycemic agents and their side effect.

| Class | Name | Mechanism | Side effect | Reference |
|-------|------|-----------|-------------|-----------|
| Sulfonylurea | Glimepiride, Glyburide, Gliclazide | Insulin secretion | Hypoglycemia, obesity | Deacon and Lebovitz |
| Meglitinides agonist | Repaglinide, Nateglinide | Insulin secretion | Hypoglycemia, obesity | Grant et al. |
| Thiazolidinedione | Pioglitazone, Rosiglitazone, Lobeclitazone | Increase insulin sensitivity | Obesity, edema | Kung and Henry |
| DPP-4 inhibitors | Sitagliptin, Vildagliptin, Saxagliptin | Increase insulin sensitivity | Indigestion, rash | Chin et al. |
| Biguanide | Metformin | Reduce gluconeogenesis | Lactoacidosis, indigestion | Defronzo et al. |
| Amylase/glucosidase inhibitors | Acarbose, Miglitol, Voglibose | Inhibit starch digestion | Diarrhea, flatulence | Zhang et al. |
| Sodium/glucose transporter inhibitors | Dapagliflozin, Canagliflozin, Empagliflozin | Reduce urine glucose re-absorption | Urinary and genital tract infection | Lupsa et al. |
and that leads to diminish cardiovascular morbidity of HIV treatment.\textsuperscript{24} In T-cell activation, sDPP-4 can activate T-cell proliferation \textit{via} co-stimulation with T-cell receptor (TCR) signaling and Toll-like receptor, whose activation is neither associated with its enzymatic activity nor with adenosine deaminase binding.\textsuperscript{25–28} On the other hand, sDPP-4 with its enzymatic activity nor with adenosine receptor, whose activation is neither associated with T-cell receptor (TCR) signaling and Toll-like can activate T-cell proliferation \textit{via} co-stimulation.\textsuperscript{15} GLP-1 is involved in insulin sensitivity and secretion, food reward, and appetite through ghrelin and leptin, and cellular metabolism with adiponectin.\textsuperscript{15,33–35} NPY blocks melanocortin-4 receptor signaling and leads to the reduction of energy consumption and possibility of obesity.\textsuperscript{36,37} After secretion from intestinal L cell, GLP-1 is rapidly degraded by sDPP-4 into inactive GLP-1 amide, in which half-life is shorter than 2 min.\textsuperscript{38} In dysglycemic patients, active GLP-1 content can be further decreased and cause more severe hyperglycemia.\textsuperscript{39} Accordingly, inhibiting sDPP-4 activity can keep more active GLP-1 and NPY in serum and thus improve insulin efficiency.\textsuperscript{40} Other biological activities including non-immunodeficient virus infection are also explored. It has been demonstrated by the case study of Middle East respiratory syndrome coronavirus (MERS-CoV) infection that sDPP-4 can potentially function to block viral infection.\textsuperscript{41,42} Truncated C-X-C chemokine 10 (CXCL10) secretion in persistent infection of hepatitis C is essential,\textsuperscript{43} implying that DPP-4 may play a role in HCV persistent infection as manifested by a case report, in which a DM patient complicated with HCV infection showed HCV replication reduction after sitagliptin treatment.\textsuperscript{44} sDPP-4 is essential for maintaining immune activity and chemotaxis, especially in inflammatory regulation, based on all available information of its biological activities. Thus, the serum activity of sDPP-4 can be an indicator of physiological or immunological stages.

The effect of serum sDPP-4 activity can be classified into several categories: infection related damage, transplantation or autoimmune disease, respiratory disease, and response to diabetic medications and complications, as discussed in detail in the following. Serum sDPP-4 activity in HIV infection is referred to the HIV-induced intestinal damage that is caused by Th17 cell depletion.\textsuperscript{45,46} It has been observed that rheumatoid arthritis and multiple sclerosis patients have lower serum sDPP-4 activity than healthy people,\textsuperscript{47,48} whereas HIV patients have higher serum sDPP-4 activity.\textsuperscript{49} Moreover, Leicht \textit{et al.} analyzed sDPP-4 activity in end-stage renal disease patients before and after kidney transplantation, and sDPP-4 activities were found to decrease after kidney transplantation.\textsuperscript{50} These results suggest that serum sDPP-4 activity can be a potential biomarker for monitoring the progress of autoimmune disease and the prognosis of organ transplantation. Interestingly, serum sDPP-4 activity can be associated with the progress of chronic obstructive pulmonary disease (COPD),\textsuperscript{51} which is highly correlated with respiratory inflammation, obviously indicating the relationship between serum sDPP-4 activity and COPD progression.\textsuperscript{52} However, the prognosis of malignant pleural mesothelioma (MPM), which is a rare pulmonary malignancy, can be predicted by sDPP-4 activity in pleural fluid.\textsuperscript{53} The pathologic correlation between sDPP-4 activity and MPM prognosis is still veiled. These reports provide interesting suggestions about sDPP-4 levels as the biomarkers of various diseases, which are not easy to monitor in serum or other body fluid.

\textbf{Biological function of membrane-bound DPP-4}

Remarkably, mDPP-4 can be found mainly in the kidney, gastrointestinal tract, T lymphocytes, and reproductive organs.\textsuperscript{54,55} Biological activities of mDPP-4 include the regulation of immune response and blood vessel function.\textsuperscript{15} mDPP4, also named CD26, is a T-cell co-stimulator of T-cell receptor responding to antigen-presenting cells.\textsuperscript{56} Thus, mDPP-4 recently has been
considered as a potent target in treatment of transplantation and autoimmune disease. Dolanbay et al. reported an interesting study about the impact of mDPP-4 inhibition in early pregnancy that can be important in treating recurrent implantation failure. In hematopoietic stem cell transplantation, graft-versus-host disease (GVHD) is a common complication, which is critical in survival rate after transplantation. Zhang et al. proved the association between Th17 cells and GVHD and which Th17 cell can be regulated by mDPP-4 inhibition that indicates current DPP-4 inhibitors can help ameliorating the onset of GVHD. The impact of mDPP-4 in immune regulation is also implicated in autoimmune disease and hypersensitivity. mDPP-4 expression levels in CD8+ T cells of Hashimoto’s thyroiditis patients are significantly lower than healthy subjects that is plausibly attributed to disease progression. In contrast, mDPP-4 levels are almost 11-fold higher in psoriatic skin than in normal skin, asserting the involvement of mDPP-4 in psoriatic development. mDPP-4 plays a positive role in asthma progress through promoting T-cell activation. These reports have unequivocally demonstrated the positive role played by mDPP-4 in immune regulation. mDPP-4 acts on both endothelial and epithelial cells in regulation of blood vessel function. In addition to endothelial inflammation caused by sDPP-4, the role of mDPP-4 in endothelial generally involves the endothelial migration, angiogenesis, and proliferation under hypoxia status, which can be found in the development of endometriosis. Xu et al. have pointed out that DPP-4 inhibitors that can alleviate pulmonary artery remodeling and, finally, delay the development of pulmonary hypertension. In the regulation of cardiovascular function, mDPP-4 inhibition can reverse diastolic left ventricular dysfunction via inhibiting mDPP-4/SDF-1α related angiogenesis. mDPP-4 involves in epithelial-mesenchymal transition (EMT) for epithelial cell, suggesting the potential implication of mDPP-4 in promoting cancer development. In fact, breast cancer metastasis can be triggered by DPP4 inhibition through CXCL12/CXCR4/mTOR pathway. However, DPP-4 inhibitor shows the opposite activity in non-small cell lung cancer, which suppresses cancer cell growth via macrophage-mediated natural killer (NK) cell activation. Collectively, these studies have furnished comprehensive descriptions of mDPP-4 biological functions in the whole body and further manifest that DPP-4 inhibition (sDPP-4 or mDPP-4) can produce unexpected side effects.

**Role of DPP-4 in diabetes treatment**

The endocrinological impact of DPP-4 is more prominent in the mediation of blood glucose. DPP-4 inhibition is a predominant approach for treating diabetes because of prolonged incretin half-lives within serum, especially in type 2 DM. In addition, it has been suggested that sitagliptin can preserve pancreatic β-cell function and subsequently stabilize insulin secretion as shown by two 4-year clinical trials, in which sitagliptin was adopted to treat slowly progressive type 1 DM (SPTIDDM) and latent autoimmune diabetes adult (LADA). In addition to clinical treatment, DPP-4 levels can be used as a biomarker. For instance, high serum sDPP-4 levels can be referred to the elevated glycation end products, which subsequently evoke endothelial cell damage and diabetic nephropathy incidence. In addition, high serum sDPP-4 levels also indicate worse drug response to DPP-4 inhibitor and hyperglycemia, which are the indicators of poor glycemic control and advanced disease progress. The above information emphasizes the effect of DPP-4 inhibition and monitoring in DM treatment. The method of screening DPP-4 inhibitor and the recent known natural DPP-4 inhibitors are presented in the following.

**Methods for screening novel DPP-4 inhibitors**

**In silico screening of DPP-4 inhibitors**

Virtual screening has been seamlessly integrated into drug discovery and development and its success significantly relies on compound library, especially the structural diversity of compound library. For instance, microalgal metabolites were screened for DDP-4 inhibitors. Compared with the synthesized chemicals, natural compound libraries generally consist of more structurally diverse compounds than their synthetic counterparts, providing a better screening resource. As such, numerous studies have adopted various natural compound libraries to find novel DDP-4 inhibitors as listed in Table 2.

It is not uncommon to observe that docking studies were carried out based on a single DDP-4 crystal structure, despite the fact that a great...
number of DDP-4-inhibitor co-complex structures have been deposited in the Protein Data Bank (PDB). Deng et al.88 for instance, docked a series of synthesized triazole-based uracil derivatives into the linagliptin-DDP4 co-complex structure (PDB code: 2RGU) using the standard precision (SP) Glide (Schrödinger, Inc.), which places internally generated ligand conformations with various positions and orientations into the binding pocket. Deng et al.89 employed Gold (Cambridge Crystallographic Data Center), which is a genetic algorithm (GA)-based scheme to explore the conformational flexibility of ligand and the rotational flexibility of receptor, to dock synthesized pyrazolo inhibitors into the quinazolinone-DDP4 co-complex structure (PDB code: 2ONC).90 It should be noted that both Glide and Gold are flexible docking algorithms.91 Nevertheless, DDP-4 is unrestrained per se as manifested by the fact that DDP-4 consists of various binding subsites, namely S₁, S’₁, S₂, S’₂, and extensive S₂, etc., to which the corresponding amino acids of the DDP4 substrate peptide designated by P₁, P’₁, P₂, P’₂, etc. from the nearest to the farthest cleavage point can bind as shown in Figure 1.92 and S₂, which is composed of various hydrophobic residues, namely GLU205 and GLU206 dyad and ARG125, is highly plastic.93 Furthermore, Nabeno et al. categorized inhibitors into three different classes (Figure 1) based on the interactions between inhibitor and DDP-4 subsites as listed in Table 3, from which it can be observed that inhibitors of different classes bind to different DDP-4 subsites, and S₁ and S₂ are the

| Natural compound library | Reference |
|-------------------------|-----------|
| Traditional Chinese Medicine Database (TCM Database@Taiwan) | Chen80 |
| Naturally Occurring Plant-based Anti-cancer Compound-Activity-Target database (NPACT) | Mangal et al.81 |
| Natural Products subset of the ZINC database | Irwin and Shoichet et al.82 |
| The Binding Database (BindingDB) | Liu et al.83 |
| Antidiabetic natural compounds database (ADNCD) | Khatoon et al.84 |
| Phenol-explorer | Rothwell et al.85 |
| In-house natural products database (NPD) | Zhang et al.86 |
| The NuBBE Database (NuBBEDB) | Nguyen et al.87 |

**Table 2.** Natural compound libraries adopted by various studies.
common binding subsites. In fact, the root means square deviation (RMSD) between alogliptin-bound protein conformation (PDB: 3G0B) and teneligliptin-bound one (PDB: 3VJK) is 1.07 Å, denoting the promiscuous nature of DDP-4 that can be further demonstrated by Figure 2, in which the six DDP4 co-complex structures (PDB codes are 3W2T, 1X70, 3BJM, 2RGU, 3VJK, and 3G0B) are superimposed. Moreover, the diverse structures of inhibitors can also manifest the plasticity of DPP4. Lee et al. for instance, analyzed the chemical structures of those launched DDP-4 inhibitors and divided them into different classes that, in turn, can be further noticeable the promiscuity of target protein. As such, the promiscuity of DDP-4 cannot be fully taken into account unless more sophisticated structure-based ensemble docking schemes such as SVM-Pose/SVM-Score combinatorial ensemble docking or analog-based pharmacophore ensemble schemes such as pharmacophore ensemble/support vector machine can be adopted. It can be argued that molecular dynamics (MD) can be used to address the flexibility of DDP4 as illustrated by the study of Liu et al. Nevertheless, the lengthy MD calculation will substantially increase computational time and expense, making it impractical to be carried out in a high-throughput fashion, let alone the more resource-demanded quantum mechanical (QM)/molecular mechanics (MM) algorithm.

Table 3. Three classes of inhibitors based on their subsites, their corresponding Protein Data Bank (PDB) entry, and references.

| Inhibitor     | PDB code | Class | Reference   |
|---------------|----------|-------|-------------|
| Vildagliptin  | 3W2T     | 1     | Nabeno et al.92 |
| Saxagliptin   | 3BJM     | 1     | Metzler et al.100 |
| Alogliptin    | 3G0B     | 2     | Zhang et al.101 |
| Linagliptin   | 2RGU     | 2     | Eckhardt et al.102 |
| Sitagliptin   | 1X70     | 3     | Kim et al.103 |
| Teneligliptin | 3VJK     | 3     | Yoshida et al.104 |

Direct test of compounds against DPP-4

There are four types of assay methods for screening DPP-4 inhibitors for direct testing: direct enzymatic assay, in vitro cell assay, ex vivo assay, and in vivo animal tests. DPP-4 and tested compounds are mixed in the direct enzymatic assay, followed by adding specific substrate peptides such as glypro-p-nitroanilide. The chemical p-nitroanilide will be released from peptides and the amount is determined by optical absorption at 405 nm at noninhibition state. This method is fast for analysis and can be used to evaluate the inhibition pattern from calculated $K_i$ values. However, the minimum changes within direct enzymatic assay cannot be directly translated into the actual bioactivity in cells and animals. Ex vivo assay can simulate the biological interaction within body, whereas it needs fresh serum or tissue sample as the source of DPP-4. Moreover, previous studies reported that mucosal DPP-4 inhibition can be possibly related to the onset of coeliac disease, which is an autoimmune disorder due to the immune response to gluten. However, a modern version of DPP4 activity assay needs to homogenize the whole intestinal biopsy that, in turn, can lead to mucosal DPP-4 inhibition. Yazbeck et al. have derived a new DPP4 substrate with $^{13}$C isotope that can be released upon reaction.
with DPP-4.\textsuperscript{111} As such, the requirement to homogenize the biopsy is completely exonerated, leading to higher correlation as compared with its conventional counterparts.\textsuperscript{111}

Myocytes and pancreatic cells are often used in the cell-based assays to discover DPP-4 inhibitors. Because β-cells in pancreatic islet is an important GLP-1 target, the downstream signaling of GLP-1 in pancreatic cells can be an indicator or biomarker of DPP-4 activity.\textsuperscript{112} In addition, GLP-1 attenuates lipopolysaccharide (LPS)-induced cardiomyocyte inflammation. The variations of inflammatory signaling including NF-κB, ERK, and TNF-α within LPS-induced cardiomyocyte can indirectly gauge DPP-4 activity.\textsuperscript{113} Nevertheless, the results of cell-based assay can be an authentic representative of the realistic situation mainly due to the fact that they do not consider \textit{in vivo} pharmacodynamic and pharmacokinetic factors. Nevertheless, direct action upon target cells can be very helpful in detailing intracellular dynamics prior to clinical or animal tests.

DPP-4 inhibitors have been highlighted as potential regimen for autoimmune-disease based on the characteristics in T-cell activation and inflammation.\textsuperscript{55} Notably, autoimmune animal model becomes a platform for testing \textit{in vivo} efficacy of DPP-4 inhibitors in long-term administration.\textsuperscript{114} Alternatively, \textit{in vivo} assay of DPP-4 inhibitory efficacy can be verified by diabetic animal model despite the fact that DPP-4 can degrade GLP-1, leading to insulin desensitization and secretion decrease.\textsuperscript{112} The most unvanquished limitation of \textit{in vivo} test is that only end-point observation can be observed in pre-testing drug candidate despite the fact that it is more related to clinical situations. The data retrieved from \textit{in vitro} and direct enzymatic assay can be synergistically essential for understanding the conceivable hypoglycemic mechanism.

**Natural compounds as novel DPP-4 inhibitors**

The effects of DPP-4 studies were mainly focused on immune, endocrine, and neuron system from the end of 1990s to early of 2000s.\textsuperscript{115-117} A study reported in 2006 that incretin was the molecular target of DPP-4, suggesting the implication of DPP-4 inhibitor in diabetes treatment. Consequently, the diabetic research has turned into a new paradigm for searching for antidiabetic DPP-4 inhibitors.\textsuperscript{118} To date, only very limited natural DPP-4 inhibitors from various sources/origins have been reported (Table 4). Natural DPP-4 inhibitors from different origins using different approaches for screening the compound to reach the target are summarized as follows. In addition to plant source, DPP-4 inhibitors from animals and microbes are single subclasses, in which DPP-4 inhibitors from animals and microbes are peptides and macrolides, respectively. Interestingly, the most predominant subclasses of DPP-4 inhibitors are terpenoids, peptides, phenolics, and flavonoids. These findings have implicated that alkaloids are not suitable as DPP-4 inhibitors or their applications in DPP4 inhibition have not been well explored. In addition to pure compounds, some crude extracts of natural materials or protein hydrolysates can exert DPP-4 inhibition as well. For instance, the DPP-4 inhibition activities of methanol extracts of \textit{Ficus benghalensis}, \textit{Syzygium cumini}, \textit{Ocimum sanctum}, and \textit{Eucalyptus sp.} have been demonstrated.\textsuperscript{119,120} The hypoglycemic efficacy of traditional Chinese antidiabetic medicines decoction of \textit{Schizandra chinensis} Baill., \textit{Coptis chinensis}, \textit{Psidium guajava} L., and \textit{Morus alba} L. has been verified by DPP-4 inhibition by \textit{in vivo} test.\textsuperscript{121} The protein hydrolysates from whey, barbel, and yam can reduce the DPP-4 activity in enzymatic measurements.\textsuperscript{122-125} Through partition by molecular sieve, the highest inhibition peptide sequences of DPP-4 such as Ala-Pro, Leu-Pro-Val-Pro-Gln, Trp-Ser-Gly, and Phe-Ser-Asp have been found.\textsuperscript{126-128} Nevertheless, these results from \textit{in vitro} enzymatic assays cannot guarantee a promising future since physiological regulation of DPP-4 is far more complicated than bench-top experiments. Therefore, the results from direct enzymatic assay for new candidates or hits require further validation such as \textit{in vivo} investigations to confirm their actual therapeutic values when compared with clinical medicines.

**Caution of using DPP-4 inhibitors**

Previous sections have described the biological functions, assay methods, and known natural DPP-4 inhibitors, which are purported to treat DM. However, DPP-4 inhibition can possibly cause unexpected sequelae owing to its entanglement with immune response and endothelial functions. In fact, the roles of DPP-4 in tumorigenesis and progression, respectively, have been reviewed recently.\textsuperscript{144,145} In lung cancer and
Table 4. Natural DPP-4 inhibitors from different origins.

| Structure subclass | Compound name | Source | Testing method | Reference |
|--------------------|---------------|--------|----------------|-----------|
| Plant origin       | Ephedrine     | Ephedra spp. | Enzymatic      | Ojeda-Montes et al.129 |
|                    | Berberine     | Coptis chinensis | Enzymatic      | Al-Masri et al.130 |
| Diarylheptanoid    | Calebin A     | Curcuma longa | Enzymatic      | Oliveira et al.131 |
| Flavonoids         | Christsin      | Passiflora caerulea | In vitro   | Kalhotra et al.132 |
|                    | Kaempferol, Kaempferol 7-O-A-L-Rhamnoside, Vitexin, Lepidoside, Rutin | Smilax china L. | Enzymatic      | Zhao et al.133 |
|                    | Aspalathin     | Aspalathus linearis | In vivo      | Muller et al.134 |
| Glycoside          | Linustatins A, Linustatins B, Linustatins C, Linustatins D, Linustatins E | Linum usitatissimum L. | Enzymatic      | Yang et al.135 |
| Peptide            | Soybean hydrolysate | Glycine max | Ex vivo      | Lammi et al.108 |
|                    | Lupin hydrolysate | Lupinus spp. | Ex vivo      | Lammi et al.108 |
|                    | AP peptide, IPA Peptide | Euphausia superba | Enzymatic      | Ji et al.128 |
| Phenolics          | Emolin        | Rheum palmatum Linn | In vivo   | Wang et al.136 |
|                    | Salvianolic Acid C | Xiaokean formula | Enzymatic      | Wu et al.137 |
|                    | (±)-Vitisin A | Vitis thunbergii var. taiwani ana | Enzymatic      | Lin et al.138 |
|                    | (–)-Vitisin B | Magnolia officinalis | Enzymatic      | Yan et al.139 |
| Sterol             | Stigmasterol  | Fagonia cretica | Enzymatic | Saleem et al.140 |
| Terpenoids         | 16-hydroxycleroda-3,13-dien-15,16-olide | Polyalthia longifolia | In vivo       | Huang et al.141 |
|                    | Quinovic Acid, Quinovic acid-3B-O-B-D-glycopyranoside | Fagonia cretica | Enzymatic      | Saleem et al.140 |
|                    | Quinovic acid-3B-O-B-D-glucopyranosyl-(28→1)-B-D-glucopyranosyl ester | Magnolia officinalis | Enzymatic      | Yan et al.139 |
|                    | Ginsenoside Rg, Timosaponin Al | Xiaokean formula | Enzymatic      | Wu et al.137 |
|                    | Two norsesquiterpenoids | Magnolia officinalis | Enzymatic      | Yan et al.139 |
|                    | Lupeol        | Hedera nepalensis | Enzymatic      | Saleem et al.140 |
|                   | Xanthonoid    | Magnifera indica | In vivo      | Suman et al.142 |
| Animal origin      | Peptide       | LPVPQ peptide, IPM peptide | milk | Nongonierma et al.126 |

(Continued)
pancreatic cancer, DPP-4 inhibitor can assuredly reduce cancer progression and promote the overall survival.\textsuperscript{146} However, in breast cancer, prostate cancer, and endometrial carcinoma, DPP-4 inhibition would cause the opposite consequence which promotes cancer progression.\textsuperscript{67,147,148} The role of DPP-4 inhibition in cancer treatment is inconclusive, but it is certain that DPP-4 inhibition in tumorigenesis and tumor development in site-specific tumor should be considered.\textsuperscript{144,145} In addition to cancer development, opportunistic infection is another issue of DPP-4 inhibition. Anno \textit{et al.} reported a 69-year-old DM patient, who developed fever after taking vildagliptin for 1 week owing to hypercytokinemia.\textsuperscript{149} It is obvious that numerous chemokines such as CXCL3, CXCL4, CXCL 5, and CXCL10 can also function as DPP-4 substrates.\textsuperscript{56} Chen \textit{et al.} have found that DM patients with short-term DPP-4 inhibitor treatment are at higher risk of herpes zoster infection as compared with non-DPP-4 treatment patients after surveying the Longitudinal Health Insurance Database 2000.\textsuperscript{150} The development of Hashimoto’s thyroiditis and celiac disease are inversely correlated with DPP4 levels (\textit{vide supra}), suggesting that DPP-4 inhibition can promote disease progression. Inflammatory bowel disease (IBD), which is a general term for Crohn’s disease and ulcerative colitis, is caused by opportunistic infection or immune cell infiltration.\textsuperscript{151,152} A meta-analysis published by Radel \textit{et al.} has indicated that DPP-4 inhibition can increase the risk of Crohn’s disease.\textsuperscript{153} In conclusion, DPP-4 inhibitors can be used to treat DM, whereas their complications with other immune disease or cancer should be seriously considered.

**Perspectives and future research**

The effect of virtual screening \textit{via} computational biology or informatics further combined with \textit{in vitro} enzymatic and cell assay, and \textit{in vivo} animal tests offer a promising approach to discover candidates or hits for expediting the preclinical development process (Figure 3). Nevertheless, poor or ill drug absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) properties make substantial contributions to drug attritions,\textsuperscript{154} and little effort has been dedicated to profiling ADME/Tox properties of DDP-4 inhibitors. As such, it is necessary to predict ADME/Tox parameters in the process of virtual screening, which should be carried by adopting schemes that can consider the unstructured nature of DDP-4, to minimize the late-stage failures.

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### Table 4. (Continued)

| Structure subclass | Compound name | Source | Testing method | Reference |
|--------------------|---------------|--------|----------------|-----------|
| WSG peptide, FSD peptide | Barbus sp. | Enzymatic | Sila \textit{et al.}\textsuperscript{127} |
| Microbial origin | Grassypeptolide A | marine | \textit{in vitro} | Kwan \textit{et al.}\textsuperscript{143} |

FSD, Phe-Ser-Asp; IPM, Ile-Pro-Met; LPVPQ, Lys-Pro-Val-Pro-Gln; WSG, Trp-Ser-Gly.

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**Figure 3.** Effect of natural products in DPP-4 inhibition and the screening methods.
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References
1. Cho NH, Shaw JE, Karuranga S, et al. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018; 138: 271–281.
2. Dodds S. The how-to for type 2: an overview of diagnosis and management of type 2 diabetes mellitus. Nurs Clin North Am 2017; 52: 513–522.
3. Park S, Kang HJ, Jeon JH, et al. Recent advances in the pathogenesis of microvascular complications in diabetes. Arch Pharm Res 2019; 42: 252–262.
4. American Diabetes Association a. 5. Lifestyle management: standards of medical care in diabetes-2019. Diabetes Care 2019; 42: S46–S60.
5. American Diabetes Association b. 9. Pharmacologic approaches to glycemic treatment: standards of medical care in diabetes-2019. Diabetes Care 2019; 42: S90–S102.
6. Zhou JB, Bai L, Wang Y, et al. The benefits and risks of DPP4-inhibitors vs. sulfonylureas for patients with type 2 diabetes: accumulated evidence from randomised controlled trial. Int J Clin Pract 2016; 70: 132–141.
7. Grant JS and Graven LJ. Progressing from metformin to sulfonylureas or meglitinides. Workplace Health Saf 2016; 64: 433–439.
8. Deacon CF and Lebovitz HE. Comparative review of dipeptidyl peptidase-4 inhibitors and sulphonylureas. Diabetes Obes Metab 2016; 18: 333–347.
9. Kung J and Henry RR. Thiazolidinedione safety. Expert Opin Drug Saf 2012; 11: 565–579.
10. Chin HJ, Nam JH, Lee EK, et al. Comparative safety for cardiovascular outcomes of DPP-4 inhibitors versus glimepiride in patients with type 2 diabetes: a retrospective cohort study. Medicine (Baltimore) 2017; 96: e7213.
11. Defronzo R, Fleming GA, Chen K, et al. Metformin-associated lactic acidosis: current perspectives on causes and risk. Metabolism 2016; 65: 20–29.
12. Zhang L, Chen Q, Li L, et al. Alpha-glucosidase inhibitors and hepatotoxicity in type 2 diabetes: a systematic review and meta-analysis. Sci Rep 2016; 6: 32649.
13. Lupsa BC and Inzucchi SE. Use of SGLT2 inhibitors in type 2 diabetes: weighing the risks and benefits. Diabetologia 2018; 61: 2118–2125.
14. Lambeir AM, Durinx C, Scharpe S, et al. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. Crit Rev Clin Lab Sci 2003; 40: 209–294.
15. Mulvihill EE and Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. Endocr Rev 2014; 35: 992–1019.
16. Casrouge A, Sauer AV, Barreira Da Silva R, et al. Lymphocytes are a major source of circulating soluble dipeptidyl peptidase 4. Clin Exp Immunol 2018; 194: 166–179.
17. Hasan AA and Hocher B. Role of soluble and membrane-bound dipeptidyl peptidase-4 in diabetic nephropathy. J Mol Endocrinol 2017; 59: R1–R10.
18. Neidert LE, Mobley CB, Kephart WC, et al. The serine protease, dipeptidyl peptidase IV as a myokine: dietary protein and exercise mimetics as a stimulus for transcription and release. Physiol Rep 2016; 4: e12827.
19. Just TP, Cooper IR and Delorey DS. Sympathetic vasoconstriction in skeletal muscle: adaptations to exercise training. Exerc Sport Sci Rev 2016; 44: 137–143.
20. Neidert LE, Al-Tarhuni M, Goldman D, et al. Endogenous dipeptidyl peptidase IV modulates skeletal muscle arteriolar diameter in rats. Physiol Rep 2018; 6: e13564.
21. Wronkowitz N, Gorgens SW, Romacho T, et al. Soluble DPP4 induces inflammation and proliferation of human smooth muscle cells via protease-activated receptor 2. Biochim Biophys Acta 2014; 1842: 1613–1621.
22. Romacho T, Vallejo S, Villalobos LA, et al. Soluble dipeptidyl peptidase-4 induces microvascular endothelial dysfunction through proteinase-activated receptor-2 and
thromboxane A2 release. *J Hypertens* 2016; 34: 869–876.

23. Seliger SL, Salimi S, Pierre V, et al. Microvascular endothelial dysfunction is associated with albuminuria and CKD in older adults. *BMC Nephrol* 2016; 17: 82.

24. Dube MP, Chan ES, Lake JE, et al. A randomized, double-blinded, placebo-controlled trial of sitagliptin for reducing inflammation and immune activation in treated and suppressed HIV infection. *Clin Infect Dis*. Epub ahead of print 10 December 2018. DOI: 10.1093/cid/ciy1051.

25. Kim SJ, Nian C and McIntosh CH. Sitagliptin (MK0431) inhibition of dipeptidyl peptidase IV decreases nonobese diabetic mouse CD4+ T-Cell migration through incretin-dependent and -independent pathways. *Diabetes* 2010; 59: 1739–1750.

26. Yu DM, Slaitini L, Gysbers V, et al. Soluble CD26/dipeptidyl peptidase IV enhances human lymphocyte proliferation in vitro independent of dipeptidyl peptidase enzyme activity and adenosine deaminase binding. *Scand J Immunol* 2011; 73: 102–111.

27. Lee DS, Lee ES, Alam MM, et al. Soluble DPP-4 up-regulates toll-like receptors and augments inflammatory reactions, which are ameliorated by vildagliptin or mannose-6-phosphate. *Metabolism* 2016; 65: 89–101.

28. Kitagawa N, Hamaguchi M, Majima S, et al. Dipeptidyl peptidase-4 inhibitors have adverse effects for the proliferation of human T Cells. *J Clin Biochem Nutr* 2018; 63: 106–112.

29. Lee DS, Lee ES, Alam MM, et al. Soluble DPP-4 up-regulates toll-like receptors and augments inflammatory reactions, which are ameliorated by vildagliptin or mannose-6-phosphate. *Metabolism* 2016; 65: 89–101.

30. Ikeda T, Kumagai E, Iwata S, et al. Soluble CD26/dipeptidyl peptidase IV enhances the transcription of IL-6 and TNF-α in THP-1 cells and monocytes. *PLoS One* 2013; 8: e66520.

31. Tansi FL, Blanchard V, Berger M, et al. Interaction of human dipeptidyl peptidase IV and human immunodeficiency virus type-1 transcription transactivator in Sf9 cells. *Virol* 2010; 7: 267.

32. Mortier A, Gouwy M, Van Damme J, et al. CD26/dipeptidylpeptidase IV-chemokine interactions: double-edged regulation of inflammation and tumor biology. *J Leukoc Biol* 2016; 99: 955–969.

33. Broxmeyer HE. Counteracting the enzymatic activity of dipeptidylpeptidase 4 for potential therapeutic advantage, with an emphasis on cord blood transplantation. *Korean J Intern Med* 2013; 28: 639–645.

34. Ronveaux CC, Tome D and Raybould HE. Glucagon-like peptide 1 interacts with ghrelin and leptin to regulate glucose metabolism and food intake through vagal afferent neuron signaling. *J Nutr* 2015; 145: 672–680.

35. Achari AE and Jain SK. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. *Int J Mol Sci* 2017; 18: e1321.

36. Kuhnhen P, Krude H and Bieberrmann H. Melanocortin-4 receptor signalling: importance for weight regulation and obesity treatment. *Trends Mol Med* 2019; 25: 136–148.

37. Bennett LA, Mokrosinski J, Mendes De Oliveira E, et al. Human gain-of-function MC4R variants show signaling bias and protect against obesity. *Cell* 2019; 177: 597–607.e599.

38. Sharma D, Verma S, Vaidya S, et al. Recent updates on GLP-1 agonists: current advancements & challenges. *Biomed Pharmacother* 2018; 108: 952–962.

39. Singh AK. Glucagon-like peptide 1 and dysglycemia: conflict in incretin science. *Indian J Endocrinol Metab* 2015; 19: 182–187.

40. Nadkarni P, Chepurny OG and Holz GG. Regulation of glucose homeostasis by GLP-1. *Prog Mol Biol Transl Sci* 2014; 121: 23–65.

41. Inn KS, Kim Y, Aigerim A, et al. Reduction of soluble dipeptidyl peptidase 4 levels in plasma of patients infected with Middle East respiratory syndrome coronavirus. *Virology* 2018; 518: 324–327.

42. Letko M, Miazgowicz K, Mcminn R, et al. Adaptive evolution of MERS-CoV to species variation in DPP4. *Cell Rep* 2018; 24: 1730–1737.

43. Riva A, Laird M, Casrouge A, et al. Truncated CXCL10 is associated with failure to achieve spontaneous clearance of acute hepatitis C infection. *Hepatology* 2014; 60: 487–496.

44. Yanai H. Dipeptidyl peptidase-4 inhibitor sitagliptin significantly reduced hepatitis C virus replication in a diabetic patient with chronic hepatitis C virus infection. *Hepatobiliary Pancreat Dis Int* 2014; 13: 556.

45. Paiardini M and Muller-Trutwin M. HIV-associated chronic immune activation. *Immunol Rev* 2013; 254: 78–101.
46. Ploquin MJ, Casrouge A, Maden Y, et al. Systemic DPP4 activity is reduced during primary HIV-1 infection and is associated with intestinal RORC+ CD4+ cell levels: a surrogate marker candidate of HIV-induced intestinal damage. *J Int AIDS Soc* 2018; 21: e25144.

47. Ulusoy H, Kamanli A, Ilhan N, et al. Serum levels of soluble CD26 and CD30 and their clinical significance in patients with rheumatoid arthritis. *Rheumatol Int* 2012; 32: 3857–3862.

48. Tejera-Alhambra M, Casrouge A, De Andres C, et al. Low DPP4 expression and activity in multiple sclerosis. *Clin Immunol* 2014; 150: 170–183.

49. Mahmoudi M, Hedayat M, Aghamohammadi A, et al. Soluble CD26 and CD30 levels in patients with common variable immunodeficiency. *J Investig Allergol Clin Immunol* 2013; 23: 120–124.

50. Leicht S, Shipkova M, Klett C, et al. CD26/dipeptidyl peptidase IV: a comparative study of healthy persons and kidney transplant recipients before and early after transplantation. *Clin Biochem* 2013; 46: 1383–1388.

51. Somborac-Bacura A, Buljescic S, Rumora L, et al. Decreased soluble dipeptidyl peptidase IV activity as a potential serum biomarker for COPD. *Clin Biochem* 2012; 45: 1245–1250.

52. Cho WK, Lee CG and Kim LK. COPD as a disease of immunosenescence. *Yonsei Med J* 2019; 60: 407–413.

53. Fujimoto N, Ohnuma K, Aoe K, et al. Clinical significance of soluble CD26 in malignant pleural mesothelioma. *PLoS One* 2014; 9: e115647.

54. Uhlen M, Fagerberg L, Hallstrom B, et al. Proteomics. Tissue-based map of the human proteome. *Science* 2015; 347: 1260419.

55. Wang X, Zheng P, Huang G, et al. Dipeptidyl peptidase-4(DPP-4) inhibitors: promising new agents for autoimmune diabetes. *Clin Exp Med* 2018; 18: 473–480.

56. Ohnuma K, Dang NH and Morimoto C. Revisiting an old acquaintance: CD26 and its molecular mechanisms in T cell function. *Trends Immunol* 2008; 29: 293–301.

57. Dolanbay EG, Yardimoglu M, Yalcinkaya E, et al. Expression of trophinin and dipeptidyl peptidase IV in endometrial co-culture in the presence of an embryo: a comparative immunocytochemical study. *Mol Med Rep* 2016; 13: 3961–3968.

58. Shokouhi S, Bray S, Bakhtiyari S, et al. Effects of AGVHD and CGVHD on survival rate in patients with acute myeloid leukemia after allogeneic stem cell transplantation. *Int J Hematol Oncol Stem Cell Res* 2015; 9: 112–121.

59. Zhang L, Chu J, Yu J, et al. Cellular and molecular mechanisms in graft-versus-host disease. *J Leukoc Biol* 2016; 99: 279–287.

60. Liu Y, Li Y, Gong Y, et al. CD26 expression is down-regulated on CD8+ T cells in patients with hashimoto’s thyroiditis. *Int Immunopharmacol* 2018; 54: 280–285.

61. Van Lingen RG, Van De Kerkhof PC, Seyger MM, et al. CD26/dipeptidyl-peptidase IV in psoriatic skin: upregulation and topographical changes. *Br J Dermatol* 2008; 158: 1264–1272.

62. Nieto-Fontarigo JJ, Gonzalez-Barcala FJ, San Jose E, et al. CD26 and asthma: a comprehensive review. *Clin Rev Allergy Immunol* 2019; 56: 139–160.

63. Tan CW, Lee YH, Tan HH, et al. CD26/DPPIV down-regulation in endometrial stromal cell migration in endometriosis. *Fertil Steril* 2014; 102: 167–177.e169.

64. Xu J, Wang J, He M, et al. Dipeptidyl peptidase IV (DPP-4) inhibition alleviates pulmonary arterial remodeling in experimental pulmonary hypertension. *Lab Invest* 2018; 98: 1333–1346.

65. Shigeta T, Aoyama M, Bando YK, et al. Dipeptidyl peptidase-4 modulates left ventricular dysfunction in chronic heart failure via angiogenesis-dependent and -independent actions. *Circulation* 2012; 126: 1838–1851.

66. Peng CH, Yang YS, Chan KC, et al. Hibiscus sabdariffa polyphenols alleviate insulin resistance and renal epithelial to mesenchymal transition: a novel action mechanism mediated by type 4 dipeptidyl peptidase. *J Agric Food Chem* 2014; 62: 9736–9743.

67. Yang F, Takagaki Y, Yoshitomi Y, et al. Inhibition of dipeptidyl peptidase-4 accelerates epithelial-mesenchymal transition and breast cancer metastasis via the CXCL12/CXCR4/mTOR axis. *Cancer Res* 2019; 79: 735–746.

68. Jang JH, Junker F, De Meester I, et al. The CD26/DPP4-inhibitor vildagliptin suppresses lung cancer growth via macrophage-mediated NK cell activity. *Carcinogenesis* 2019; 40: 324–334.
69. Luo Y, Lu K, Liu G, et al. The effects of novel antidiabetic drugs on albuminuria in type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. *Clin Drug Investig* 2018; 38: 1089–1108.

70. Awata T, Shimada A, Maruyama T, et al. Possible long-term efficacy of sitagliptin, a dipeptidyl peptidase-4 inhibitor, for slowly progressive type 1 diabetes (SPIDDM) in the stage of non-insulin-dependency: an open-label randomized controlled pilot trial (SPAN-S). *Diabetes Ther* 2017; 8: 1123–1134.

71. Ishibashi Y, Matsui T, Maeda S, et al. Advanced glycation end products evoke endothelial cell damage by stimulating soluble dipeptidyl peptidase-4 production and its interaction with mannose 6-phosphate/insulin-like growth factor II receptor. *Cardiovasc Diabetol* 2013; 12: 125.

72. Zheng T, Liu Y, Qin S, et al. Increased plasma dipeptidyl peptidase-4 activities are associated with high prevalence of diabetic nephropathy in Chinese patients with newly diagnosed type 2 diabetes: a cross-sectional study. *Diab Vasc Dis Res* 2016; 13: 127–136.

73. Aso Y, Ozeki N, Terasawa T, et al. Serum level of soluble CD26/dipeptidyl peptidase-4 (DPP-4) predicts the response to sitagliptin, a DPP-4 inhibitor, in patients with type 2 diabetes controlled inadequately by metformin and/or sulfonylurea. *Transl Res* 2012; 159: 25–31.

74. Aso Y, Terasawa T, Kato K, et al. The serum level of soluble CD26/dipeptidyl peptidase 4 increases in response to acute hyperglycemia after an oral glucose load in healthy subjects: association with high-molecular weight adiponectin and hepatic enzymes. *Transl Res* 2013; 162: 309–316.

75. Fara DC, Oprea TI, Prossnitz ER, et al. Integration of virtual and physical screening. *Drug Discov Today Technol* 2006; 3: 377–385.

76. Hajduk PJ, Galloway WR and Spring DR. Drug discovery: a question of library design. *Nature* 2011; 470: 42–43.

77. Muegge I and Oloff S. Advances in virtual screening. *Drug Discov Today Technol* 2006; 3: 377–385.

78. Selvaraj G, Kaliamurthi S, Cakmak ZE, et al. Computational screening of dipeptidyl peptidase IV inhibitors from microalgal metabolites by pharmacophore modeling and molecular docking. *Phycol Res* 2016; 64: 291–299.

79. Harvey AL. Natural products in drug discovery. *Drug Discov Today* 2008; 13: 894–901.

80. Chen CY. TCM Database@Taiwan: the world’s largest traditional Chinese medicine database for drug screening in silico. *PLoS One* 2011; 6: e15939.

81. Mangal M, Sagar P, Singh H, et al. NPACT: naturally occurring plant-based anti-cancer compound-activity-target database. *Nucleic Acids Res* 2013; 41: D1124–D1129.

82. Irwin JJ and Shoichet BK. Zinc—a free database of commercially available compounds for virtual screening. *J Chem Inf Model* 2005; 45: 177–182.

83. Liu T, Lin Y, Wen X, et al. BindingDB: a web-accessible database of experimentally determined protein-ligand binding affinities. *Nucleic Acids Res* 2007; 35: D198–D201.

84. Khatoon A, Rashid I, Shaikh S, et al. ADNCD: a compendious database on anti-diabetic natural compounds focusing on mechanism of action. *3 Biotech* 2018; 8: 361.

85. Rothwell JA, Perez-Jimenez J, Neveu V, et al. Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. *Database (Oxford)* 2013; 2013: bat070.

86. Zhang S, Lu W, Liu X, et al. Fast and effective identification of the bioactive compounds and their targets from medicinal plants via computational chemical biology approach. *Med Chem Commun* 2011; 2: 471–477.

87. Nguyen HT, Munnier E, Souce M, et al. Novel alginate-based nanocarriers as a strategy to include high concentrations of hydrophobic compounds in hydrogels for topical application. *Nanotechnology* 2015; 26: 255101.

88. Deng X, Han L, Zhou J, et al. Discovery of triazole-based uracil derivatives bearing amidoxime moieties as novel dipeptidyl peptidase-IV inhibitors. *Bioorg Chem* 2017; 75: 357–367.

89. Peng F, Shen J, Zhu H, et al. Surrogating and redirection of pyrazolo[1,5-a]pyrimidin-7(4H)-one core, a novel class of potent and selective DPP-4 inhibitors. *Bioorg Med Chem* 2018; 26: 903–912.

90. Feng J, Zhang Z, Wallace MB, et al. Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV. *J Med Chem* 2007; 50: 2297–2300.
91. Pagadala NS, Syed K and Tuszyński J. Software for molecular docking: a review. *Biophys Rev* 2017; 9: 91–102.

92. Nabeno M, Akahoshi F, Kishida H, et al. A comparative study of the binding modes of recently launched dipeptidyl peptidase IV inhibitors in the active site. *Biochem Biophys Res Commun* 2013; 434: 191–196.

93. Lee HK, Kim MK, Kim HD, et al. Unique binding mode of evogliptin with human dipeptidyl peptidase IV. *Biochem Biophys Res Commun* 2017; 494: 452–459.

94. Gao M and Skolnick J. A comprehensive survey of small-molecule binding pockets in proteins. *PLoS Comput Biol* 2013; 9: e1003302.

95. Leong MK, Syu RG, Ding YL, et al. Prediction of N-methyl-D-aspartate receptor GluN1-ligand binding affinity by a novel SVM-pose/SVM-score combinatorial ensemble docking scheme. *Sci Rep* 2017; 7: 40053.

96. Leong MK. A novel approach using pharmacophore ensemble/support vector machine (PHE/SVM) for prediction of hERG liability. *Chem Res Toxicol* 2007; 20: 217–226.

97. Liu J, Huan Y, Li C, et al. Establishment of a selective evaluation method for DPP4 inhibitors based on recombinant human DPP8 and DPP9 proteins. *Acta Pharm Sin B* 2014; 4: 135–140.

98. Al-Awar A, Almasi N, Szabo R, et al. Novel potentials of the DPP-4 inhibitor sitagliptin against ischemia-reperfusion (I/R) injury in rat ex-vivo heart model. *Int J Mol Sci* 2018; 19: E3226.

99. Lammi C, Bollati C, Ferruzza S, et al. Soybean- and lupin-derived peptides inhibit DPP-IV activity on in situ human intestinal caco-2 cells and ex vivo human serum. *Nutrients* 2018; 10: E1082.

100. Metzler WJ, Yanchunas J, Weigelt C, et al. Involvement of DPP-IV catalytic residues in enzyme-saxagliptin complex formation. *Protein Sci* 2008; 17: 240–250.

101. Eckhardt M, Langkopf E, Mark M, et al. 8-(3-(R)-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropropurine-2,6-dione (Bi 1356), a highly potent, selective, long-acting, and orally bioavailable DPP-4 inhibitor for the treatment of type 2 diabetes. *J Med Chem* 2007; 50: 6450–6453.

102. Kim D, Wang L, Beconi M, et al. (2r)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydropyrrolidin-2-yl]butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 2005; 48: 141–151.

103. Yoshida T, Akahoshi F, Sakashita H, et al. Discovery and preclinical profile of teneligliptin (3-[(2s,4s)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)piperazin-1-yl][pyrrolidin-2-yl][carbonyl]thiazolidine): a highly potent, selective, long-lasting and orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *Bioorg Med Chem* 2012; 20: 5705–5719.

104. Liu M, Sun X and Zhao X. Investigating the contributions of residues to dipeptidyl peptidase-IV inhibitor binding by molecular dynamics simulation. *Lett Drug Des Discov* 2014; 11: 886–893.

105. Walters WP and Namchuk M. Designing screens: how to make your hits a hit. *Nat Rev Drug Discov* 2003; 2: 259–266.

106. Al-Awar A, Almasi N, Szabo R, et al. Novel potentials of the DPP-4 inhibitor sitagliptin against ischemia-reperfusion (I/R) injury in rat ex-vivo heart model. *Int J Mol Sci* 2018; 19: E3226.

107. Lebwohl B, Sanders DS and Green PHR. Coeliac disease. *Lancet* 2018; 391: 70–81.

108. Nazbeck R, Jaenisch S, Squire M, et al. Development of a (13)C stable isotope assay for dipeptidyl peptidase-4 enzyme activity a new breath test for dipeptidyl peptidase activity. *Sci Rep* 2019; 9: 4906.

109. Rohrborn D, Wronkowitz N and Eckel J. DPP4 in diabetes. *Front Immunol* 2015; 6: 386.

110. Lin CH and Lin CC. Sitagliptin attenuates inflammatory responses in lipopolysaccharide-stimulated cardiomyocytes via nuclear factor-kB pathway.
pathway inhibition. *Exp Ther Med* 2016; 11: 2609–2615.

114. Yu X and Petersen F. A methodological review of induced animal models of autoimmune diseases. *Autoimmun Rev* 2018; 17: 473–479.

115. Clark RA and Kupper TS. IL-15 and dermal fibroblasts induce proliferation of natural regulatory T cells isolated from human skin. *Blood* 2007; 109: 194–202.

116. Frerker N, Raber K, Bode F, et al. Phenotyping of congenic dipeptidyl peptidase 4 (DP4) deficient dark agouti (DA) rats suggests involvement of DP4 in neuro-, endocrine, and immune functions. *Clin Chem Lab Med* 2009; 47: 275–287.

117. Kleemann C, Schade J, Pabst R, et al. Phenotyping of congenic dipeptidyl peptidase 4 (DP4) deficient dark agouti (DA) rats suggests involvement of DP4 in neuro-, endocrine, and immune functions. *Clin Chem Lab Med* 2009; 47: 275–287.

118. Drucker DJ and Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; 368: 1696–1705.

119. Dey B, Mitra A, Katakam P, et al. Exploration of natural enzyme inhibitors with hypoglycemic potentials amongst eucalyptus Spp. by in vitro assays. *World J Diabetes* 2014; 5: 209–218.

120. De B, Bhandari K, Singla RK, et al. Chemometrics optimized extraction procedures, phytosynergistic blending and in vitro screening of natural enzyme inhibitors amongst leaves of Tulsi, Banyan and Jamun. *Pharmacogn Mag* 2015; 11: S522–S532.

121. Wang HJ and Chiang BH. Anti-diabetic effect of a traditional Chinese medicine formula. *Food Funct* 2012; 3: 1161–1169.

122. Lacroix IM and Li-Chan EC. Inhibition of dipeptidyl peptidase (DPP)-IV and α-glucosidase activities by pepsin-treated whey proteins. *J Agric Food Chem* 2013; 61: 7500–7506.

123. Konrad B, Anna D, Marek S, et al. The evaluation of dipeptidyl peptidase (DPP)-IV, α-glucosidase and angiotensin converting enzyme (ACE) inhibitory activities of whey proteins hydrolyzed with serine protease isolated from Asian pumpkin (Cucurbita ficifolia). *Int J Pept Res Ther* 2014; 20: 483–491.

124. Shih SL, Lin YS, Lin SY, et al. Effects of yam dioscorin interventions on improvements of the metabolic syndrome in high-fat diet-induced obese rats. *Bot Stud* 2015; 56: 4.

125. Sila A, Martinez-Alvarez O, Haddar A, et al. Recovery, viscoelastic and functional properties of Barbel skin gelatine: investigation of anti-DPP-IV and anti-prolyl endopeptidase activities of generated gelatine polypeptides. *Food Chem* 2015; 168: 478–486.

126. Nongonierma AB and Fitzgerald RJ. Structure activity relationship modelling of milk protein-derived peptides with dipeptidyl peptidase IV (DPP-IV) inhibitory activity. *Peptides* 2016; 79: 1–7.

127. Sila A, Alvarez OM, Haddar A, et al. Purification, identification and structural modelling of DPP-IV inhibiting peptides from barbel protein hydrolysate. *J Chromatogr B Analyt Technol Biomed Life Sci* 2016; 1008: 260–269.

128. Ji W, Zhang C and Ji H. Purification, identification and molecular mechanism of two dipeptidyl peptidase IV (DPP-IV) inhibitory peptides from Antarctic krill (Euphausia superba) protein hydrolysate. *J Chromatogr B Analyt Technol Biomed Life Sci* 2017; 1064: 56–61.

129. Ojeda-Montes MJ, Ardid-Ruiz A, Tomasz-Hernandez S, et al. Ephedrine as a lead compound for the development of new DPP-IV inhibitors. *Future Med Chem* 2017; 9: 2129–2146.

130. Al-Masri IM, Mohammad MK and Tahaa MO. Inhibition of dipeptidyl peptidase IV (DPP IV) is one of the mechanisms explaining the hypoglycemic effect of berberine. *J Enzyme Inhib Med Chem* 2009; 24: 1061–1066.

131. Oliveira AL, Martinez SE, Nagabushnam K, et al. Calebin A: analytical development for pharmacokinetics study, elucidation of pharmacological activities and content analysis of natural health products. *J Pharm Pharm Sci* 2015; 18: 494–514.

132. Kalhotra P, Chitteputi V, Osorio-Revilla G, et al. Structure(-) activity relationship and molecular docking of natural product library reveal chrysin as a novel dipeptidyl peptidase-4 (DPP-4) inhibitor: an integrated in silico and in vitro study. *Molecules* 2018; 23: E1368.

133. Zhao BT, Le DD, Nguyen PH, et al. PTP1B, α-glucosidase, and DPP-IV inhibitory effects for chromene derivatives from the leaves of Smilax china L. *Chem Biol Interact* 2016; 253: 27–37.
134. Muller CJ, Joubert E, De Beer D, et al. Acute assessment of an aspalathin-enriched green rooibos (Aspalathus linearis) extract with hypoglycemic potential. *Phytomedicine* 2012; 20: 32–39.

135. Yang QY, Song L, Zhang JF, et al. Cyanogenetic glycosides and simple glycosides from the linseed meal. *Fitoterapia* 2015; 106: 78–83.

136. Wang Z, Yang L, Fan H, et al. Screening of a natural compound library identifies emodin, a natural compound from *Rheum palmatum* Linn that inhibits DPP4. *PeerJ* 2017; 5: e3283.

137. Wu XL, Wang Y and Zhao XP. Screening and identification of DPP-4 inhibitors from Xiaokean formula by a fluorescent probe. *Zhongguo Zhong Yao Za Zhi* 2016; 41: 1241–1245.

138. Lin YS, Chen CR, Wu WH, et al. Anti-α-glucosidase and anti-dipeptidyl peptidase-IV activities of extracts and purified compounds from vitis thunbergii var. Taiwianiana. *J Agric Food Chem* 2015; 63: 6393–6401.

139. Yan RY, Liu HL, Zhang JY, et al. Phenolic glycosides and other constituents from the bark of Magnolia officinalis. *J Asian Nat Prod Res* 2014; 16: 400–405.

140. Saleem S, Jafri L, Ul Haq I, et al. Plants Fagonia cretica L. and Hedera nepalensis K. Koch contain natural compounds with potent dipeptidyl peptidase-4 (DPP-4) inhibitory activity. *J Ethnopharmacol* 2014; 156: 26–32.

141. Huang PK, Lin SR, Riyaphan J, et al. Polyalthia clerodane diterpene potentiates hypoglycemia via inhibition of dipeptidyl peptidase 4. *Int J Mol Sci* 2019; 20: pii: E530.

142. Suman RK, Mohanty IR, Maheshwari U, et al. Natural dipeptidyl peptidase-IV inhibitor mangiferin mitigates diabetes- and metabolic syndrome-induced changes in experimental rats. *Diabetes Metab Syndr Obes* 2016; 9: 261–272.

143. Kwan JC, Liu Y, Ratmayake R, et al. Grasspeptolides as natural inhibitors of dipeptidyl peptidase 8 and T-cell activation. *ChemBioChem* 2014; 15: 799–804.

144. Overbeek JA, Bakker M, Van Der Heijden A, et al. Risk of dipeptidyl peptidase-4 (DPP-4) inhibitors on site-specific cancer: a systematic review and meta-analysis. *Diabetes Metab Res Rev* 2018; 34: e3004.

145. De S, Banerjee S, Kumar SKA, et al. Critical role of dipeptidyl peptidase IV: a therapeutic target for diabetes and cancer. *Mini Rev Med Chem* 2019; 19: 88–97.

146. Bishnoi R, Hong YR, Shah C, et al. Dipeptidyl peptidase 4 inhibitors as novel agents in improving survival in diabetic patients with colorectal cancer and lung cancer: a Surveillance Epidemiology and Endpoint Research Medicare study. *Cancer Med* 2019; 8: 3918–3927.

147. Yang X, Zhang X, Wu R, et al. DPPIV promotes endometrial carcinoma cell proliferation, invasion and tumorigenesis. *Oncotarget* 2017; 8: 8679–8692.

148. Russo JW, Gao C, Bhasin SS, et al. Downregulation of dipeptidyl peptidase 4 accelerates progression to castration-resistant prostate cancer. *Cancer Res* 2018; 78: 6354–6362.

149. Anno T, Kaneto H, Kawasaki F, et al. Drug fever and acute inflammation from hypercytokinemia triggered by dipeptidyl peptidase-4 inhibitor vildagliptin. *J Diabetes Investig* 2019; 10: 182–185.

150. Chen HH, Lin CL, Yeh SY, et al. Short-term dipeptidyl peptidase-4 inhibitor use increases the risk of herpes zoster infection in Asian patients with diabetes. *QJM* 2016; 109: 91–95.

151. Inflammatory Bowel Disease Group, Chinese Society of Gastroenterology and Chinese Medical Association. Evidence-based consensus on opportunistic infections in inflammatory bowel disease (republication). *Intest Res* 2018; 16: 178–193.

152. Lee SH, Kwon JE and Cho ML. Immunological pathogenesis of inflammatory bowel disease. *Intest Res* 2018; 16: 26–42.

153. Radel JA, Pender DN and Shah SA. Dipeptidyl peptidase-4 inhibitors and inflammatory bowel disease risk: a meta-analysis. *Ann Pharmacother* 2019; 53: 697–704.

154. Van De Waterbeemd H and Gifford E. ADMET in silico modelling: towards prediction paradise? *Nat Rev Drug Discov* 2003; 2: 192–204.