Original Article

Compounds from *Astragalus membranaceus* (Huangqi) interact with PPAR- γ , DPP-IV, and GP to treat Type 2 Diabetes MellitusChe Chen^{1#}, Sheng Zhou^{2#}, Jianguo Niu³, Feng Wang⁴, Tao Sun⁴, Peng Wang^{*5}

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Type 2 diabetes mellitus (T2DM) is a metabolic disorder disease. Traditional Chinese Medicine (TCM) has a long history for treatment of T2DM. The TCM herb treatments effectively elevate the visceral functions of human body, adjust the inner body environment, balance the level of blood glucose, and cause less side effects. We aim to find core TCM herbs in the treatments of T2DM and discover the underlying mechanism of core herbs in treating T2DM. The prescriptions and herbs upon TCM for treating T2DM was searched in literatures and the complex network was constructed to find the core herbs. After selecting the core herbs, molecular docking was performed to find interactions between compounds of the core herb and related receptors by using LigandFit and CDocker. The results of the literature study shown that Milkvetch Root [*Astragalus membranaceus* (Fisch) Bunge, HQ, Huangqi in Chinese pinyin] was the most commonly used herb in treating T2DM. Molecular docking results shown that the compounds of HQ have comparatively interactions with the diabetes related receptors. According to the results, four compounds have interactions with peroxisome proliferator activated receptor (PPAR- γ), six compounds have interactions with dipeptidyl peptidase-IV (DPP-IV), and two compounds have interactions with Glycogen phosphorylase (GP). These results suggest that HQ can play a key role in the metabolism of human glucose and has a complex mechanism with the characteristic of multi-components and multi-targets in the treatment of T2DM.

Key Words: Traditional Chinese Medicine; Complex network; Huangqi; Molecular docking; Type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a worldwide long-term metabolic disorder disease. T2DM is featured by high blood sugar, insulin resistance, and relatively lack of insulin.¹ The medicines for treating T2DM commonly use chemical medicines. However, chemical medicines cause severer side effects, such as hypoglycemia, nausea, vomiting, or even inducing liver, and kidney dysfunction.² Therefore, the medicines that effectively control pathological process, and cause less side effects are populated for treating T2DM.

Recently, traditional Chinese Medicine (TCM) is highlighted upon treating T2DM by its bio-function on preventing the pathological process, and side effects. TCM has a long history of adjusting metabolism disorders that lead to many diseases including T2DM. In TCM theories, T2DM is caused by poor body quality, unhealthy life style, deficiency of *qi* and *yin*, and dryness-

heat in internal organs.³ According to TCM theories, the treatments for T2DM are considered by generating *qi*, clearing heat, nourishing *yin*, and tonifying *yang* based on the syndrome. For T2DM, the TCM treatments effectively elevate the visceral functions of human body, adjust the inner body environment,

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balance the level of blood glucose, and cause less side effects.⁴⁻⁵ However, the biggest challenge for developing TCM to cure T2DM is that several TCM herb treatments generate complex compounds, which result in hardly revealing the curative mechanism. To uncover the curative mechanism, the methodology termed 'Molecular docking' is employed to study ingredients, and predict their receptors.⁶ Molecular docking is based on the "Lock-Key Model Theory" of Fisher,⁷ which allows to analyze thousands of potential ingredients and their binding targets.

Given the role of TCM herbs for treating T2DM in TCM clinical practice, we unveil the potential mechanism. In this study, literatures are screened by NoteExpress and manually confirmed to select herbs used in prescriptions for treating T2DM. The complex network is created by the Gephi software package to analyze core TCM herb (Milkvetch Root, Huangqi, HQ) in treating T2DM. Following, the database of TCMSP is employed to predict the functional compounds in Huangqi and their binding targets. Finally, eight compounds are considered to interact with PPAR- γ receptor, DPP-IV receptor, and GP receptors by LigandFit and CDOCKER for treating T2DM.

MATERIALS AND METHODS

1 Search strategy

In order to simplify the methods, the diagram is shown in supplemental fig.1. The databases used in this study were China National Knowledge Infrastructure Database (CNKI), the Chinese Scientific Journal Database (VIP), Wan Fang Data, and PubMed Database from their inception to January 2018. The searching strategy included the topic retrieval, subject word retrieval, subject word combined with free word retrieval, and full-text search to ensure the comprehensiveness of the literature retrieval. The terms were searched individually or combined as follows: "type 2 diabetes", "type 2 diabetes mellitus", "type II diabetes", "type II diabetes mellitus", "Xiaoke", "T2DM", "TIIDM", "DMII", "TCM", "TCM treatment", "TCM herb", "traditional Chinese medicine", "Chinese medicine". The excluded terms were "Diabetic Nephropathy", "DN", "Diabetic Retinopathy", "Diabetes Foot", "Diabetic Peripheral Neuropathy", "Vasculopathy", "Nursing", "Acupuncture", and "Complication". The detailed criteria were as follows: A. Inclusion criteria, 1) Clinical literature on treating T2DM by TCM or Integrative Medicine treatments with detailed TCM prescriptions; 2) Literature of famous TCM doctors' cases reports and experience summaries with detailed TCM prescriptions to treat T2DM; 3) Literature of TCM prescriptions introductions of treating T2DM with detailed prescriptions information. B. Exclusion criteria, 1) Literature with incomplete prescription information; 2) Repeated articles (just keep the one with the most detailed records and exclude the rest); 3) Literature on animal experiments, acupuncture and epidemiology studying T2DM; 4) Literature on treating diabetes complications.

2 Literature Screening

The literature screening was performed by NoteExpress (V3.0) and manually confirmation (see supplemental fig 1). Articles are firstly screened by NoteExpress (V3.0). Then screening results were double checked by researchers. Finally, 675 articles were met requirements for this study. The frequency of herbs used in prescriptions were statistically analyzed and the most commonly

used herbs were studied.

3 Network visualization and analysis

The complex network was created according to prescriptions for treating T2DM. The Gephi software package (Version 0.9.1; The Gephi Consortium, Paris, France) was used to visualize the network.⁸ Each node of the network represented the herb, and the edge between nodes indicated the compatibility of herbs in the prescription. Topological analysis was used to analyze the connectivity of herbs.

4 Preparation of small molecules (Ligands)

According to the complex network analysis, Milkvetch Root (Huangqi, HQ) was the core herb for molecular docking. Compounds of HQ were searched in Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (<http://ibts.hkbu.edu.hk/LSP/tcmsp.php>, updated on 2014-05-31).^{9,10} The oral bioavailability (OB) and drug-likeness (DL) were main parameters to consider the substance in TCM herbs as drugs.¹¹ The structures of compounds with OB $\geq$ 30% and DL $\geq$ 0.18 were studied.

The structures of selected compounds were downloaded from PubChem database and TCMSP, and were saved as mol2 format files for the Discovery Studio software (Accelrys, San Diego, CA) to perform molecular docking. The "Prepare Ligands" in this software was selected to transfer the small molecules into 3D structures and the protonation state was pretreated. The "Minimize Ligands" was chosen to optimize the energy field.

5 Preparation of receptors for docking

The receptors related to curing T2DM were searched in TCMSP (<http://ibts.hkbu.edu.hk/LSP/tcmsp.php>, updated on 2014-05-31), Therapeutic Target Database (<http://bidd.nus.edu.sg/group/ttd/ttd.asp>, updated on 2017-09-15), and DrugBank Database (<http://www.drugbank.ca/>, version, 4.3).^{12,13} The searching terms were "type 2 diabetes" and "type 2 diabetes mellitus", "type II diabetes", "type II diabetes mellitus", "T2DM", "TIIDM" or "DMII". After searching, the peroxisome proliferator-activated receptor- γ (PPAR- γ), glycogen phosphorylase (GP), and dipeptidyl peptidase-IV (DPP-IV) were target receptors. As reported in diabetes pharmacological research, PPAR- γ , GP and DPP-IV were mostly related to glycometabolism.¹⁴⁻¹⁶ PPAR- γ regulated fatty acid storage and glucose metabolism.^{17,18} GP was a molecular therapeutic target for treating hyperglycemia associated with T2DM.^{19,20} DPP-IV played a major role in glucose metabolism, which was responsible for the degradation of incretins, such as GLP-1.¹⁷ The target proteins were retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank²¹ (<http://www.rcsb.org/pdb/home/home.do>) and were saved as PDF format files. For targets that contain multiple crystal structures, the one with complex substance and high resolution were selected (See in Table 1).²¹

Table1 Protein targets information

Name	PBD code	Resolution(Å)
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ)	4CI5	1.77
Glycogen phosphorylase (GP)	1A8I	1.78
Dipeptidyl Peptidase-IV (DPP-IV)	2OQV	2.8

The proteins were introduced into the Discovery Studio software as target receptors and were prepared by the procedure of “Clean Protein” and “Prepare Protein”: 1) removed the ligand molecules and water molecules in the structure; 2) deleted the conformation of proteins; 3) supplement incomplete amino acid residues; 4) added hydrogenation atoms for proteins; 5) added loop structures; 6) performed protonation state pretreatment. After the process described above, the proteins were saved in the dsf format.

6 Molecular docking and analysis

Molecular docking experiments were carried out by using Discovery Studio package that was developed by Accelrys Company. Before docking, the binding sites were determined, which was according to the locations of original ligands in crystal structures. The original ligands were able to bind with DPP-IV, PPAR- γ , or GP.

To ensure the accuracy of the docking results, two molecular docking methods, LigandFit and CDocker, were adopted in this study. The LigandFit was a molecular docking technique based on geometric matching to search for molecular binding conformations. In LigandFit, the Monte Carlo method was used to search the flexible conformation space of ligand molecules, which was scoring functions for docking. The CDocker was a semi-flexible docking program based on CHARMM. The high-temperature kinetics was used to search for the flexible conformation space of the ligand molecules. The simulated annealing method was used to optimize each conformation at the active site of the receptor. All calculations for protein-fixed ligand-flexible docking were done by the LigandFit and CDocker in Discovery Studio.²²

In the LigandFit, the Maximum Poses Retained was set to 10.

For each compound molecule, a maximum of 10 conformations were generated, and other parameters were defined as default values. The parameters of CDocker were defined as default values. In the docking process of both methods, the protein structures remained rigid and the ligand molecules were flexible. After docking, the scoring functions were used to evaluate the score. The Dock_Score was a geometry parameter that was derived after referring to other parameter values in LigandFit. Therefore, Dock_Score of LigandFit was used as the evaluation index in this study. In CDocker, the *CDocker Interaction Energy* was chosen as the scoring function for the binding capacity of the receptors to ligands.

RESULTS

1 HQ is the core herb in TCM prescriptions for treating T2DM by literature screening

In literature screening, 4336 articles in CNKI, 2483 articles in VIP, 4034 articles in Wan Fang, and 2286 articles in Pubmed are studied for treating T2DM. After selection, 675 articles finally meet the search strategy for this study. From analyzing these articles, we find 767 prescriptions, and 266 herbs are relative for treating T2DM (not show). After calculating the frequency of herbs used in prescriptions, the top 20 herbs are listed in table 2. The commonly used herbs for treating T2DM are Astragali Radix (Huangqi, HQ), Dioscoreae Rhizoma (Shanyao, SY), Rehmanniae Radix (Shengdi SD), Radix Salviae Miltiorrhiae (Danshen, DS), and Puerariae Lobatae Radix (Gegen, GG), whose frequency of be used is over 300 and the relevance is higher than 40%. Briefly, the HQ is mostly used herbs in prescriptions.

Table 2 The statistical analysis results of TCM herbs

No.	Herb	Chinese Pinyin	Latin Name	Relevance (%)	Frequency
1	HQ	Huangqi	Astragali Radix	61.41	471
2	SY	Shanyao	Dioscoreae Rhizoma	47.59	365
3	SD	Shengdi	Rehmanniae Radix	42.5	326
4	DS	Danshen	Radix Salviae Miltiorrhiae	41.2	316
5	GG	Gegen	Puerariae Lobatae Radix	40.81	313
6	HL	Huanglian	Coptidis Rhizoma	35.33	271
7	FL	Fuling	Poria	33.12	254
8	THF	Tianhuafen	Trichosanthis Radix	32.59	250
9	MD	Maidong	Ophiopogonis Radix	28.94	222
10	SZY	Shanzhuyu	Corni Fructus	24.9	191
11	ZM	Zhimu	Anemarrhenae Rhizoma	23.86	183
12	CZ	Cangzhu	Atractylodis Rhizoma	23.34	179
13	XS	Xuanshen	Scrophulariae Radix	23.21	178
14	GC	Gancao	Glycyrrhizae Radix	21.9	168
15	BZ	Baizhu	Atractylodis Macrocephalae Rhizoma	21.51	165
16	WWZ	Wuweizi	Schisandrae Chinensis Fructus	19.95	153
17	SDH	Shudihuang	Rehmanniae Radix	15.51	119
18	GQZ	Gouqizi	Lycii Fructus	15.51	119
19	ZX	Zexie	Alismatis Rhizoma	14.99	115
20	DSH	Dangshen	Codonopsis Radix	14.39	110

In order to confirm the most commonly used herbs in prescriptions, the Gephi software is used to create complex network (Fig. 1). In the complex network, the node represents the

herb, and the size shows the frequency of the herb used in treating T2DM. The edge indicates the herbal relationship, and the thickness shows the frequency of herbal compatibility. Still, the HQ

possesses the biggest node size, which indicates that the HQ is the mostly used and important herb for treating T2DM. Also, the HQ is commonly compatible with the SY, HL, SD, DS and GG in prescriptions. Furthermore, topological analysis is performed to show core herbs and their closeness (Table 3). The node degree of herb higher than 100, which can be considered as core herbs, which are listed in table 3. The node degree is quantified by edge numbers. The value of node degree presents the importance to the network. Here, the herb with more edge numbers, which get the high value, is more important for treating T2DM. Still, the HQ has highest score that is over 200 and make the HQ as the most important herb in the network for treating T2DM.

Except nodes, the weight of edges is quantified to show the chance of herbal pairs (table 4). The value of weight is calculated by the thickness of edge. The high value of weight indicates that herbs are more likely to be paired. The herbal pairs with value higher than 160 are listed in table 4, and still HQ takes up four top pairs whose values are higher than 200. Here, results show that the HQ normally pairs with SY, DS, GG and SD in prescription to treat T2DM. Clearly, HQ is highlighted in prescriptions with other herbs. However, the main functional compounds are still covered, which will be answered in next session.

Figure 1. The complex network of TCM herbs for treating T2DM. The node represents the TCM herb. The node size represents the using frequency and importance of the herb in prescriptions for treating T2DM. The edges represent the herbal relationship. The edge thickness represents the herbal compatibility frequency.

Table 3 The topology information of the core herbs in the network

Herb	Chinese Pinyin	Degree	Herb	Chinese Pinyin	Degree
HQ	Huangqi	227	WWZ	Wuweizi	139
SY	Shanyao	194	SDH	Shudihuang	132
HL	Huanglian	194	HJ	Hangjing	131
SD	Shengdi	192	HQIN	Huangqin	131
FL	Fuling	189	CH	Chaihu	125
DS	Danshen	185	MDP	Mudanpi	125
GG	Gegen	180	CP	Chenpi	125
BZ	Baizhu	174	DH	Dahuang	121
CZ	Cangzhu	158	SZ	Shanzha	120
GC	Gancao	158	RS	Renshen	120
DG	Danggui	153	CX	Chuanxiong	119
THF	Tianhuafen	152	TZS	Taizishen	115
MD	Maidong	150	YYR	Yiyiren	113
GQZ	Gouqizi	150	HH	Honghua	110
SZY	Shanzhuyu	150	CS	Chishao	109
BS	Baishao	147	TR	Taoren	106
XS	Xuanshen	147	FZ	Fuzi	106
DSH	Dangshen	144	PL	Peilan	104
ZX	Zexie	142	SG	Shigao	101
ZM	Zhimu	140			

2 Analysis of HQ Compounds and their Interactions

To clarify the functional compounds for treating T2DM, the database of TCMSP is used to predict the HQ compounds and their binding targets. The TCMSP contains data for herbs ingredients, and their binding targets. After searching, 87 compounds of HQ are found (not show). The compounds of TCM usually reach the targets in human body by absorption,

distribution, metabolism, and excretion (ADME). The oral bioavailability (OB) and drug-likeness (DL) are main parameters to consider the substance in TCM herbs as drugs[11]. The Oral bioavailability (OB) is the extent to which a drug or other substance is taken up by a specific tissue or organ after administration. The high value of OB shows that the drug or substance is more absorbed and OB≥30% indicates that the drug or substance is absorbed well.²³ Drug-likeness (DL) shows the

similarity of compounds with drugs, which assess whether the compound can be the drug. The standard of DL was usually set as 0.18,²⁴ and the standard of selected compound higher than 0.18 is potentially considered as the drug. According to OB% and DL values, 20 compounds are finally shown the ability to be easily absorbed and the possibility to be the drug for treating T2DM (Table 5). Here, the isoflavanone has highest OB value (109.99), the 7-O-methylisomucronulatol and the formononetin are following, whose OB values are 74.69 and 69.67. The methylnissoin (9,10-dimethoxypterocarpan-3-O- β -D-glucoside) has the highest DL value (0.92), and the 10,13-dimethyl-17-[(2R,5S)-5-propan-2-yl-octan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol and the mairin are behind, whose DL values are 0.78. These results indicates that these molecules are easily absorbed by body and potentially considered as drugs.

3 Molecular docking searches target Receptors of HQ compounds

According to literature screening, we find three receptors, DPP-IV, GP and PPAR- γ , which are targeted by these 20 compounds and related with glucose metabolism. In results of LigandFit, there are 20 compounds of HQ is docked successfully with DPP-IV; 12 compounds are docked with GP; 18 compounds are docked with

PPAR- γ (Table 6). To validate these results, the CDocker is also used to predict the receptors for compounds of HQ. Still, there are 12 compounds of HQ are targeted with DPP-IV, 12 compounds are assumed to bind with GP, and 12 compounds are probably coupled with PPAR- γ (Table 6). Here, by two different docking methodology, 20 compounds of HQ bind with DPP-IV, GP and PPAR- γ receptors, which regulate glucose metabolism.

Table 4 The weight of the edges of core herbs in the network

Source	Target	Type	Weight
HQ	SY	Undirected	279
HQ	DS	Undirected	248
HQ	GG	Undirected	242
HQ	SD	Undirected	229
SY	GG	Undirected	183
SY	SD	Undirected	183
SY	DS	Undirected	180
HQ	THF	Undirected	176
DS	GG	Undirected	164
DS	SD	Undirected	160

Table 5 Small Molecular Compounds Information of HQ

ID	Name	OB (%)	DL
MOL000379	9,10-dimethoxypterocarpan-3-O- β -D-glucoside	36.74	0.92
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yl-octan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78
MOL000211	Mairin	55.38	0.78
MOL000296	Hederagenin	36.91	0.75
MOL000433	Folic Acid	68.96	0.71
MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69
MOL000387	Bifendate	31.10	0.67
MOL000439	isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62
MOL000371	3,9-di-O-methylnissoin	53.74	0.48
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42
MOL000354	Isorhamnetin	49.60	0.31
MOL000378	7-O-methylisomucronulatol	74.69	0.30
MOL000398	Isoflavanone	109.99	0.30
MOL000239	Jaranol	50.83	0.29
MOL000098	Quercetin	46.43	0.28
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26
MOL000417	Calycosin	47.75	0.24
MOL000422	Kaempferol	41.88	0.24
MOL000392	Formononetin	69.67	0.21

By docking, the receptors have original ligands. The candidate ligands for receptors normally compare with original ligands to select the ideal ligands based on the docking score.²⁵ In docking score, the score of candidate ligands, which is higher than, or close with score of original ligands, is considered as ideal ligands. In this study, the original ligands in receptors are first removed from structures then candidate ligands are docked into binding sites.

The original ligand dock scores are recorded (Table 7). After the docking, the successful conformations are sorted from high to low according to their dock scores towards each target. It is generally believed that the compound with the dock score higher than the original ligand score or more than 80% of original ligand score can be regarded as the active compounds.²⁵ Therefore, the value of 80% of the original score is set as the threshold for active compounds

Table 6 Interactions between Compounds of HQ and Target Receptors

ID	Name	LigandFit			CDocker		
		DPP-IV	GP	PPAR- γ	DPP-IV	GP	PPAR- γ
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5- <i>propan-2-yl</i> octan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	+	-	+	-	-	-
MOL000098	Quercetin	+	+	+	+	+	+
MOL000211	Mairin	+	-	-	-	-	-
MOL000239	Jaranol	+	+	+	+	+	+
MOL000296	Hederagenin	+	-	+	-	-	-
MOL000354	Isorhamnetin	+	+	+	+	+	+
MOL000371	3,9-di-O-methylnissolin	+	+	+	+	+	+
MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	+	-	+	-	-	-
MOL000378	7-O-methylisomucronulatol	+	+	+	+	+	+
MOL000379	9,10-dimethoxypterocarpan-3-O- β -D-glucoside	+	-	+	-	-	-
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofuran o[3,2-c]chromen-3-ol	+	+	+	+	+	+
MOL000387	Bifendate	+	-	+	-	-	-
MOL000392	Formononetin	+	+	+	+	+	+
MOL000398	Isoflavanone	+	+	+	+	+	+
MOL000417	Calycosin	+	+	+	+	+	+
MOL000422	Kaempferol	+	+	+	+	+	+
MOL000433	Folic Acid	+	-	+	-	-	-
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	+	+	+	+	+	+
MOL000439	isomucronulatol-7,2'-di-O-glucosiole	+	-	-	-	-	-
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	+	+	+	+	+	+

selection. Finally, eight compounds (quercetin, isorhamnetin, formononetin, isoflavanone, Calycosin, kaempferol, Folic Acid, 1,7-Dihydroxy-3,9-dimethoxy pterocarpene) are considered as active compounds (Table 8). Next, we uncover the active compounds for receptors by LigandFit and CDocker.²⁵ Only those compounds that can both be scored by LigandFit and CDocker to interact with their receptors are considered. Still, four compounds (quercetin, isorhamnetin, Calycosin, and kaempferol) interact with PPAR- γ receptor, six compounds (quercetin, isorhamnetin, formononetin, Calycosin, kaempferol, and Folic Acid) target for DPP-IV receptor, and two compounds (isoflavanone, and 1,7-Dihydroxy-3,9-dimethoxy pterocarpene) bind with GP receptors (Table 8).

Table 7 The dock scores of original ligands

PDB code	Target	LigandFit Dock Score	CDocker Dock Score
2OQV	DPP-IV	73.175	37.802
1A8I	GP	79.597	40.219
4CI5	PPAR- γ	124.686	47.810

Still, the LigandFit and CDocker are used to show the interaction between ligands with receptors. According to the docking scores, we choose the top 3 compounds of each receptor from table 8 for further analysis, which are quercetin, isorhamnetin, kaempferol, isoflavanone and 1,7-Dihydroxy-3,9-dimethoxy pterocarpene. In Table 9, the interactions are shown in two-dimensional action diagrams. The imaginary lines in the pictures represent the interactions between compounds and receptors; pink bubbles represent hydrophobic bonds; grass green bubbles represent conventional hydrogen bonds; light green bubbles represent carbon hydrogen bonds; green bubbles represent van der Waals bonds; orange bubbles represent charge bonds; red bubbles represent unfavorable bonds. The detailed information of the interactions can be seen in supplementary Table 1.

DISCUSSION

T2DM is caused by a variety of genetic and environmental factors. It may lead to a group of clinical manifestations with glucose metabolism disorders as the main syndrome. T2DM pathogenesis involves insulin metabolism disorders. The basic characteristics of

Table 8 Dock scores of HQ compounds with targets receptors

ID	Name	Targets	LigandFit Dock Score	CDocker Dock Score
MOL000098	Quercetin	PPAR- γ	153.975	75.655
MOL000239	Jaranol	PPAR- γ	137.701	/
MOL000354	Isorhamnetin	PPAR- γ	156.755	80.940
MOL000392	Formononetin	PPAR- γ	/	56.059
MOL000398	Isoflavanone	PPAR- γ	/	61.550
MOL000417	Calycosin	PPAR- γ	117.769	59.258
MOL000422	Kaempferol	PPAR- γ	147.752	75.825
MOL000433	Folic Acid	PPAR- γ	178.881	/
MOL000098	Quercetin	DPP-IV	135.52	54.206
MOL000239	Jaranol	DPP-IV	88.208	/
MOL000354	Isorhamnetin	DPP-IV	107.732	58.091
MOL000378	7-O-methylisomucronulatol	DPP-IV	/	41.337
MOL000392	Formononetin	DPP-IV	73.337	41.691
MOL000398	Isoflavanone	DPP-IV	/	44.085
MOL000417	Calycosin	DPP-IV	77.849	42.485
MOL000422	Kaempferol	DPP-IV	110.871	56.842
MOL000433	Folic Acid	DPP-IV	102.114	47.085
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	DPP-IV	/	37.928
MOL000098	Quercetin	GP	/	53.444
MOL000354	Isorhamnetin	GP	/	46.132
MOL000392	Formononetin	GP	/	45.987
MOL000398	Isoflavanone	GP	76.622	43.974
MOL000417	Calycosin	GP	/	81.465
MOL000422	Kaempferol	GP	/	52.781
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	GP	/	60.657
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	GP	73.079	79.557

Table 9 Interactions between compounds and corresponding binding sites

Name	Targets	Docking Method	
		LigandFit	CDocker
Quercetin	PPAR- γ
Isorhamnetin	PPAR- γ

Name	Targets	Docking Method	
		LigandFit	CDOcker
Kaempferol	PPAR-γ	<p>Interactions</p> <ul style="list-style-type: none"> Salt Bridge Attractive Charge Conventional Hydrogen Bond Pi-Sulfur Pi-Pi Stacked Pi-Alkyl	<p>Interactions</p> <ul style="list-style-type: none"> Salt Bridge Attractive Charge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Cation Pi-Pi Stacked Pi-Pi T-shaped Pi-Alkyl
Quercetin	DPP-IV	<p>Interactions</p> <ul style="list-style-type: none"> Salt Bridge Attractive Charge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Alkyl	<p>Interactions</p> <ul style="list-style-type: none"> van der Waals Attractive Charge Conventional Hydrogen Bond Pi-Anion Pi-Pi Stacked Pi-Pi T-shaped
Isorhamnetin	DPP-IV	<p>Interactions</p> <ul style="list-style-type: none"> Salt Bridge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Pi Stacked Pi-Alkyl	<p>Interactions</p> <ul style="list-style-type: none"> Salt Bridge Attractive Charge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Anion Pi-Pi T-shaped
Kaempferol	DPP-IV	<p>Interactions</p> <ul style="list-style-type: none"> Salt Bridge Attractive Charge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Alkyl	<p>Interactions</p> <ul style="list-style-type: none"> Salt Bridge Attractive Charge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Alkyl
Isoflavonone	GP	<p>Interactions</p> <ul style="list-style-type: none"> Attractive Charge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Alkyl Pi-Alkyl	<p>Interactions</p> <ul style="list-style-type: none"> Attractive Charge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Alkyl
1,7-Dihydroxy-3,9-di methoxy pterocarpene	GP	<p>Interactions</p> <ul style="list-style-type: none"> Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Alkyl	<p>Interactions</p> <ul style="list-style-type: none"> Attractive Charge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Alkyl Pi-Alkyl

T2DM are defects of insulin secretion and insulin activity disorders with varying degrees of insulin resistance as well as pancreatic β -cell dysfunction.²⁶⁻²⁸ Chinese medicine has a long history in the treatment of T2DM with unique advantages. Over the years, thousands of articles have reported on the curative effects of Chinese herbal medicine treatments of T2DM.²⁹⁻³¹ A literature study was performed to collect and systematically analyze articles of T2DM treatments with TCM herbal prescriptions. However, we exclude the oral liquid and injections because they do not have all compounds of the herb or herb pairs in prescriptions.

According to the literature screening, in total 675 articles with 767 Chinese herbal prescriptions are included in this study. From the statistical results of the literature study, it can be seen that the usage frequency of HQ in those prescriptions is the highest among all herbs. The HQ is one of the most commonly used herbs in the TCM herbal prescriptions for treating T2DM. After that, a complex network was constructed to further analyze the results and select the core TCM herbs used in treating T2DM. The complex network is an analytical method to study complex phenomena in the form of network modeling that describes the relationships among the elements in a complex system.^{32,33} The node of HQ is the largest among all, which indicates that HQ is the core herb of this network. According to the analysis of the characteristic parameters of the network topology, we find that the top 10 herbs with the highest node degree are HQ, SY, SD, DS, GG, HL, FL, THE, MD and SZY, which suggests that these herbs are most commonly used in the TCM treatments of T2DM. The Degree of HQ is the highest of all. According to the weight of the edges, it can also be seen that HQ-SY, HQ-GG, HQ-DS and HQ-SD are most commonly used herbal pairs. The results indicate that the HQ is the most important herb in TCM prescriptions upon treating T2DM. Therefore, the HQ is selected as the core TCM herb for further study.

The HQ is a commonly used herb in TCM clinical practice. It is mainly used to tonify qi in body, and therefore to elevate the human body functions. The history of using HQ in China can be traced back to ancient times. The usage of HQ is first recorded in *Sheng Nong's Herbal Classic* (one of the Four Classical Traditional Chinese Medicine Books). In Han dynasty, the HQ is used to enrich qi and activate blood, which is recorded in *Jin Gui Yao Lue* (one of the Four Classical Traditional Chinese Medicine Books). From Tang dynasty, the HQ is used to treat "Xiaoke" (an ancient TCM disease caused by metabolic disorders which was similar to diabetes). Nowadays, it is widely used in TCM clinical practice to adjust metabolic disorders, such as T2DM. According to the literature study, we find that the HQ is mostly used in treating T2DM patients with qi deficiency, and usually is used with SY, GG, DS and SD to adjust complicated syndromes, such as spleen qi deficiency, deficiency of both qi and yin, or blood stasis with qi deficiency. In addition, according to many previous experimental and clinical reports related to the HQ,^{34,35} the HQ has a really good hypoglycemic effect and can effectively control the development of T2DM. However, as a natural medicine, the hypoglycemic mechanism of the HQ is still unclear. Therefore, a molecular docking is carried out in this study to find the potential mechanism of HQ treating T2DM.

Molecular docking is a virtual screen method which can help to simulate the interaction between compounds and target receptors. In this study, three T2DM related receptors are found to dock with compounds of HQ. The receptors are downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data

Bank, and the original ligands have been proven to have good interactions with those receptors.³⁶⁻³⁸ Comparing with the docking score of the original ligands in those proteins, the results of compounds of HQ turned out to be satisfactory. According to the results of molecular docking using LigandFit, the dock score of original ligand with PPAR- γ is 124.686; among 20 compounds, 5 compounds dock scores are higher than the original ligand, and one compound dock score is above threshold; the dock score of original ligand with DPP-IV is 73.175, and seven compounds of HQ have even higher dock scores; the dock score of original ligand with GP is 79.597, and two compounds of HQ are above the threshold. According to the results of molecular docking using CDocker, the dock score of original ligand with PPAR- γ is 47.81; among 20 compounds, 6 compounds dock scores are higher than the original ligand; the dock score of original ligand with DPP-IV is 37.802, and 8 HQ compounds have higher dock scores than the original ligand; the dock score of original ligand with Glycogen phosphorylase is 40.219, and 8 compounds of HQ have higher dock scores than the original ligand.

From the results of LigandFit with CDocker, we find that quercetin, isorhamnetin, formononetin, calycosin, kaempferol and folic acid in HQ are able to bind with DPP-IV. Therefore, the activity of GLP-1 increases to stimulate islet β cells secreting insulin, and inhibit islet α cells to produce glucagon, and thereby reduce blood glucose.^{36,39} In addition, quercetin, isorhamnetin, calycosin, and kaempferol are able to bind with PPAR- γ , which may therefore increase insulin sensitivity, and promote the expression of phosphoinositide 3-kinase (PI3K), and glucose transporter (GLUT4). Furthermore, the glucose uptake are increased and the blood glucose is regulated.^{40,37,41} Through the molecular docking, isoflavanone and 1,7-Dihydroxy-3,9-dimethoxy pterocarpene of HQ are able to bind with GP. GP can catalyze the phosphorylation of glycogen, which degrades glucose molecules from the surface of glycogen molecules and increase blood glucose, while inhibit the activity of GP can control the fasting blood glucose.^{38,42,43} The results indicate that these compounds from HQ possess capability to interact with target receptors, therefore, to adjust blood glucose.

The results of molecular docking also show that several compounds of HQ could interact with more than one receptor and each receptor has interacted with more than one ligand in HQ. For example, quercetin, isorhamnetin, and kaempferol are successfully docked with PPAR- γ as well as DPP-IV. In the results of LigandFit, the residues HIS323, HIS449 of PPAR- γ bonds with quercetin, isorhamnetin, and kaempferol. These results suggest that the interactions between HQ compounds and receptors are complicated with characteristic of multi-compounds with multi- targets.

The docking results also confirmed the macroscopic medical theory of TCM from the microscopic level. The TCM theory of medicine application is mainly based on the macroscopic state of human from overall perspective. In TCM medical theory, HQ is considered to tonify qi, and therefore, is usually used in treating patients of qi deficiency with the clinical manifestation of fatigue and tiredness. After taking HQ, the symptoms can often be relieved, and related functions of the body may also be elevated correspondingly [3]. The results of this molecular docking show that HQ can interact with many kinds of proteins in human body and achieve a series of complex functions. In addition to regulating blood glucose, HQ may also increase the gene expression of PI3K and GLUT4, slow down the decomposition of glycogen and muscle glycogen, inhibit cell apoptosis, and thus improve the body

function. From the molecular level, those improvements may eventually achieve the overall manifestation and relieve the symptoms of fatigue and tiredness corresponding to qi restoring. The results confirm the theory of TCM and reveal the functional mechanism of TCM from a molecular level.

CONCLUSION

In summary, according to the results of literature research and complex network analysis, HQ is found to be one of the TCM core herbs to treat T2DM. Molecular docking results indicate that HQ may act on several key points in the glucose metabolism process in treating T2DM, and its complex mechanism possesses multi-compounds and multi-targets mutual interactions. This study elucidates the molecular mechanisms of traditional Chinese medicine in the regulation of human blood glucose in treating

T2DM and provides a new inspiration for the follow-up study of systematical complexity of Chinese medicine.

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SUPPLEMENTARY MATERIALS

Supplementary table 1: The detailed interaction information of table 9.

Supplementary material 2: 20 compounds chemical structures of HQ downloaded from TCMSP . Search in <http://lsp.nwu.edu.cn/tcmsp.php?from=singlemessage>.

Supplementary Fig 1: The diagram of method

Supplementary Table 1 The detailed interaction information

Interactions	Bond Type	Resides	
		LigandFit	CDocker
MOL000098 with PPAR-γ	hydrogen bond	TYR473, CYS285, SER289	SER289
	hydrophobic bond	ARG288, HIS449, CYS285	CYS285, MET364
	charge interactions	HIS323, LYS367, HIS449, MET364	HIS323, HIS449, LYS367
MOL000354 with PPAR-γ	hydrogen bond	CYS285, TYR473, ILE326, LEU330	SER289
	hydrophobic bond	ARG288	CYS285, MET364
	charge interactions	HIS323, HIS449	HIS323, HIS449, LYS367
MOL000422 with PPAR-γ	hydrogen bond	TYR473	SER289
	hydrophobic bond	ARG288, CYS285	CYS285, PHE363, MET364
	charge interactions	HIS323, HIS449, MET364	HIS323, HIS449, LYS367
MOL000098 with DPP-IV	hydrogen bond	ARG356	SER209
	hydrophobic bond	ILE405, ARG356	TYR666, TYR662
	charge interactions	GLU361, ARG429, ARG356	ARG669, GLU205, ARG125, HIS740, TYR662
	van der Waals bond	/	TYR547
	unfavorable bond	ARG358	/
MOL000354 with DPP-IV	hydrogen bond	GLU361, SER360, GLU205, ARG358	SER209, SER630
	hydrophobic bond	PHE357, ARG358	PHE357
	charge interactions	ARG356, ARG358	ARG669, GLU205, ARG125, HIS740
MOL000422 with DPP-IV	hydrogen bond	SER630	SER552, GLN553
	hydrophobic bond	/	TYR547, LYS554
	charge interactions	HIS740, GLU205, ARG669, ARG125	HIS740, ARG125, LYS554
MOL000398 with GP	hydrogen bond	ASN284, ASN484, SER674, GLU672, GLY135, GLU88, ASN282	LEU136, LLP680, GLU672, GLY135, ASN284, ALA673, TYR573, SER674
	hydrophobic bond	VAL455, ALA673, LEU136	VAL455, LEU139
	charge interactions	HIS377, ASP283	HIS377
MOL000442 with GP	hydrogen bond	ASP339, GLU672, GLY135, ASP283, PHE285, ALA383	THR671, ASN284, LEU136, ASN338, ASN484, GLU672, SER674
	hydrophobic bond	LEU136	ALA673, TYR573
	unfavorable bond	/	ASN284, LEU139

Supplementary Fig.1 The diagram of method

REFERENCES

- Zhang N, Yang X, Zhu X, Zhao B, Huang T, Ji Q. Type 2 diabetes mellitus unawareness, prevalence, trends and risk factors: National Health and Nutrition Examination Survey (NHANES) 1999-2010. *J Int Med Res* 2017; 45 (2): 594-609.
- Xi M, Hai C, Tang H, Chen M, Fang K, Liang X. Antioxidant and antiglycation properties of total saponins extracted from traditional Chinese medicine used to treat diabetes mellitus. *Phytother Res* 2008; 22 (2): 228-237.
- Zhou ZY. *Chinese Internal Medicine*, 2nd. China: Chinese Medicine Press, 2007.
- Xia R, Huang P, Shao GM. Nourishing Yin and promoting blood circulation of TCM to treat hemorheologic disorder induced by diabetes mellitus in rats. *Evid Based Complement Alternat Med* 2007; 4 (2): 203-207.
- Chen D, Li C, Michalsen A, Kessler C, Huang Y, Meng J, Ke B, Wang Y, Zhang J, Qin J. Modified Ling-Gui-Zhu-Gan decoction combined with short-term fasting improves therapeutic response in type 2 diabetic patients. *Eur J Integr Med* 2012; 4 (3): e309-e314.
- Gschwend DA, Good AC, Kuntz ID. Molecular docking towards drug discovery. *J Mol Recognit* 1996; 9 (2): 175-186.
- Jorgensen W. Rusting of the lock and key model for protein-ligand binding. *Science* 1991; 254 (5034): 954.
- Deng J, Sitou K, Zhang Y, Yan R, Hu Y. Analyzing the Chinese landscape in anti-diabetic drug research: leading knowledge production institutions and thematic communities. *Chin Med* 2016; 11 (1): 13.
- Ru J, Li P, Wang J, Zhou W, Li B, Huang C, Li P, Guo Z, Tao W, Yang Y, Xu X, Li Y, Wang Y, Yang L. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform* 2014; 6 (1): 13.
- Zheng C, Pei T, Huang C, Chen X, Bai Y, Xue J, Wu Z, Mu J, Li Y, Wang Y. A novel systems pharmacology platform to dissect action mechanisms of traditional Chinese medicines for bovine viral diarrhea disease. *Eur J Pharm Sci* 2016; 94: 33-45.
- Zhu F, Shi Z, Qin C, Tao L, Liu X, Xu F, Zhang L, Song Y, Liu X, Zhang J, Han B, Zhang P, Chen Y. Therapeutic target database update 2012: a resource for facilitating target-oriented drug discovery. *Nucleic Acids Res* 2012; 40: D1128-1136.
- Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M. DrugBank: a knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Res* 2008; 36: D901-906.
- Daisy P, Jaculine L, Manikkam R. Identification of dual agonistic novel ligands for insulin receptor and PPAR γ through molecular docking. *Int J Pharm Sci* 2012; 4 (4): 142-147.
- Pereira ALE, Dos Santos GB, Franco MSF, Federico LB, da Silva CHTP, Santos CBR. Molecular modeling and statistical analysis in the design of derivatives of human dipeptidyl peptidase IV. *J Biomol Struct Dyn* 2018; 36 (2): 318-334.
- Schweiker SS, Loughlin WA, Lohning AS, Petersson MJ, Jenkins ID. Synthesis, screening and docking of small heterocycles as glycogen phosphorylase inhibitors. *Eur J Med Chem* 2014; 84 (18): 584-594.
- Lowell BB. PPAR- γ : an essential regulator of adipogenesis and modulator of fat cell function. *Cell* 1999; 99 (3): 239-242.
- Wang Q, Imam MU, Yida Z, Wang F. Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ) as a Target for Concurrent Management of Diabetes and Obesity-Related Cancer. *Curr Pharm Des* 2017; 23 (25): 3677-3688.
- Oikonomakos NG. Glycogen phosphorylase as a molecular target for type 2 diabetes therapy. *Curr Protein Pept Sci* 2002; 3 (6): 561.
- Treadway JL, Mendys P, Hoover DJ. Glycogen phosphorylase inhibitors for treatment of type 2 diabetes mellitus. *Expert Opin Investig Drugs* 2001; 10 (3): 439-454.
- Berman H, Westbrook J, Feng Z, Gilliland GL. The protein data bank/ nucleic acids research. *Nucleic Acids Res* 2000; 17 (20): 3588.
- Lu F, Jiang L, Chen Y, Luo G, Liu F, Li G, Zhang Y. Multi-target neuroprotective effect of Buyang Huanwu Decoction against cerebral ischemia injury. *Bei Jing Zhong Yi Yao Da Xue Xue Bao* 2016; 39 (9): 744-749.
- Li J, Zhao P, Li Y, Tian Y, Wang Y. Systems pharmacology-based dissection of mechanisms of Chinese medicinal formula Bufe Yishen as an effective treatment for chronic obstructive pulmonary disease. *Sci Rep* 2015; 5: 15290.
- Xu X, Zhang W, Huang C, Li Y, Yu H, Wang Y, Duan J, Ling Y. A novel chemometric method for the prediction of human oral bioavailability. *Int J Mol Sci* 2012; 13 (6): 6964-6982.
- Tao W, Xu X, Wang X, Li B, Wang Y, Li Y, Yang L. Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal Radix Curcumae formula for application to cardiovascular disease. *J Ethnopharmacol* 2013; 145 (1): 1-10.
- Jiang LD, He YS, Chen X, Tao O, Li GY, Zhang YL. Study on anti-hyperlipidemia mechanism of high frequency herb pairs by molecular docking method. *Zhong Guo Zhong Yao Za Zhi* 2015; 40 (12): 2413-2419.
- Kahn SE, Lachin JM, Zinman B, Haffner SM, Aftiring RP, Paul G, Kravitz BG, Herman WH, Viberti G, Holman RR. the ADOPT Study Group. Effects of rosiglitazone, glyburide, and metformin on beta-cell function and insulin sensitivity in ADOPT. *Diabetes* 2011; 60 (5): 1552-1560.
- Meier JJ, Bonadonna RC. Role of reduced beta-cell mass versus impaired beta-cell function in the pathogenesis of type 2 diabetes. *Diabetes Care* 2013; 36 (Suppl 2): S113-119.
- Halban PA, Polonsky KS, Bowden DW, Hawkins MA, Ling C, Mather KJ, Powers AC, Rhodes CJ, Sussel L, Weir GC. Beta-cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. *Diabetes Care* 2014; 37 (6): 1751-1758.
- Liu JP, Li JL, Zhang M, Wang WY. Chinese herbal medicines for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2004; 3: Cd003642.

30. Zhao HL, Sui Y, Qiao CF, Yip KY, Leung RK, Tsui SK, Lee HM, Wong HK, Zhu X, Siu JJ, He L, Guan J, Liu LZ, Xu HX, Tong PC, Chan JC. Sustained Antidiabetic Effects of a Berberine Containing Chinese Herbal Medicine Through Regulation of Hepatic Gene Expression. *Diabetes* 2012; 61 (4): 933.
31. Tong XL, Dong L, Chen L, Zhen Z. Treatment of diabetes using traditional Chinese medicine: past, present and future. *Am J Chin Med* 2012; 40 (5): 877-886.
32. Newman MEJ. Who Is the Best-Connected Scientist? A Study of Scientific Coauthor Ship Networks. *Working Papers* 2000; 650: 337-370.
33. Newman MEJ. The structure and function of complex networks. *SIAM Rev* 2003; 45 (2): 167-256.
34. Lv L, Wu SY, Wang GF, Zhang JJ, Pang JX, Liu ZQ, Xu W, Wu SG, Rao JJ. Effect of astragaloside IV on hepatic glucose-regulating enzymes in diabetic mice induced by a high-fat diet and streptozotocin. *Phytother Res* 2010; 24 (2): 219-224.
35. Tian H, Lu J, He H, Zhang L, Dong Y, Yao H, Feng W, Wang S. The effect of *Astragalus* as an adjuvant treatment in type 2 diabetes mellitus: A (preliminary) meta-analysis. *J Ethnopharmacol* 2016; 191: 206-215.
36. Pei Z, Li X, Geldern TWV, Longenecker K, Pireh D, Stewart KD, Backes BJ, Lai C, Lubben TH, Ballaron SJ, Beno DW, Kempf-Grote AJ, Sham HL, Trevillyan JM. Discovery and Structure–Activity Relationships of Piperidinone and Piperidine-Constrained Phenethylamines as Novel, Potent, and Selective Dipeptidyl Peptidase IV Inhibitors. *J Med Chem* 2007; 50 (8): 1983-1987.
37. dos Santos JC, Bernardes A, Giampietro L, Ammazalorso A, De Filippis B, Amoroso R, Polikarpov I. Different binding and recognition modes of GL479, a dual agonist of Peroxisome Proliferator-Activated Receptor alpha/gamma. *J Struct Biol* 2015; 191 (3): 332-340.
38. Gregoriou M, Noble ME, Watson KA, Garman EF, Krulle TM, de la Fuente C, Fleet GW, Oikonomakos NG, Johnson LN. The structure of a glycogen phosphorylase glucopyranose spirohydantoin complex at 1.8 Å resolution and 100 K: The role of the water structure and its contribution to binding. *Protein Sci* 1998; 7 (4): 915.
39. Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Digest Dis Sci* 1993; 38 (4): 665-673.
40. Oikonomakos NG. Glycogen phosphorylase as a molecular target for type 2 diabetes therapy. *Curr Protein Pept Sci* 2002; 3 (6): 561.
41. Anandharajan R, Pathmanathan K, Shankernarayanan NP, Vishwakarma RA, Balakrishnan A. Upregulation of Glut-4 and PPAR gamma by an isoflavone from *Pterocarpus marsupium* on L6 myotubes: a possible mechanism of action. *J Ethnopharmacol* 2005; 97: 253-260.
42. Somsák L, Czifrák K, Tóth M, Bokor E, Chrysina ED, Alexacou KM, Hayes JM, Tiraidis C, Lazoura E, Leonidas DD, Zographos SE, Oikonomakos NG. New inhibitors of glycogen phosphorylase as potential antidiabetic agents. *Curr Med Chem* 2008; 15 (28): 2933-2983.
43. Bernard H, Reinhard S. Glycogen Phosphorylase Inhibitors. *Mini Rev Med Chem* 2006; 6 (8): 845.