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Identification of Antidiabetic Hit Molecule from the Phytoconstituents of *Tinospora cordifolia* by *In silico* Studies

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ABSTRACT

Current antidiabetic drugs are not adequate to manage progression of diabetes. Since natural products have been a good source of drugs, it is necessary to find out new molecules with natural origin for antidiabetic drug development. Tinospora cordifolia has proven efficacy in diabetes. In silico analysis in this work revealed that components of Tinospora cordifolia have good affinity for multiple diabetic targets which may account for its antidiabetic efficacy. Further in silico analysis revealed that aglycone of syringin can be the most appropriate hit molecule which can be optimized for antidiabetic drug development.

Keywords: *Tinospora cordifolia*, Diabetes, Hit, Aglycone of syringin

INTRODUCTION

The incidences of diabetes are ever increasing. Type-2 Diabetes (T2D) accounts for more than 90% of all diabetes [1]. Chronic diabetes has been a risk factor for severe conditions including nephropathy, retinopathy, neuropathy and atherosclerotic cardiovascular diseases. The increases in complications of diabetes have suggested the inadequacies of current antidiabetic drugs to manage diabetes [2]. In this scenario, it is necessary to continuously make effort to develop new drugs which can potentially provide a better solution to the issue. Natural products have long been used as medication. Accordingly, many new drugs have been developed by capitalizing on the natural compounds. Thus natural products used against diabetes can also be a source for development of new molecules against diabetes.

Tinospora cordifolia has been used as a complimentary/alternative medicine against diabetes [3]. Its antidiabetic properties are highly appreciated in Ayurveda. Studies on its extracts and isolated phytoconstituents (Like tinosporin, berberine, jatrorrhizine, etc.) have reported it as a preventive and curative antidiabetic herb, which has been substantiated by clinical trials. Effect of natural products is ascribed to a holistic combination of phytoconstituents on multiple targets. Although exact antidiabetic mechanism of *T. cordifolia* is not clear, efforts have been made to propose mode of action by analyzing affinity of its components for specific diabetic targets. Affinity of its phytoconstituents for glycogen phosphorylase (PDB ID: 1LWO) has been reported as one of the modes of its antidiabetic effect [4]. However multiple ways are expected to be involved in antidiabetic action of *T. cordifolia*. So it is necessary to analyze multiple targets of diabetes to justify the effects.

Hit is generally understood as a molecule with potential affinity for a specific target. Identification of hit is the primary step in the sequence drug development which is followed by its optimization to lead molecule and further optimization to drug candidate. Hit selection primarily involves identification of hit in High-Throughput Screening (HTS) *in vitro* [5]. Over the years, virtual screening has been accepted as an efficient complement to experimental HTS to identify hits. Keeping these factors in view, this study was undertaken to understand multiple mode of action of *T. cordifolia* and identify a hit with affinity for multiple targets of diabetes.

MATERIALS AND METHODS

Selection of target and optimization of target structure

The targets were selected based on their role in diabetes. Excessive cortisol production is considered as a factor for the pathogenesis of type II diabetes and obesity. Human 11β-hydroxy steroid dehydrogenase type I (11β-HSD1) is an endoplasmic reticulum(ER)-localized membrane protein that catalyzes the inter conversion of cortisone and cortisol. Thus, its structure (PDB ID: 1XU7) was taken as target [6]. The fructose-6-phosphate amido transferase (PDB ID: 2ZJ3) was taken as another target due to its role in regulation of type 2 diabetes [7]. The glycogen phosphorylase (PDB ID: 1LWO) was also taken as another target. Since inhibition of Sirt6 is shown to improve glucose tolerance in a type 2 diabetes mouse model [8,9], it was taken as another target (PDB ID: 3K35). Considering the role of α-glucosidase and pancreatic alpha-amylase

in diabetes, their structures (PDB ID: 5KZW; 5E0F) were taken as other targets. The X-ray crystallographic structures of these targets were recovered in PDB format from protein data bank and optimized following methods reported by us earlier for further use [10].

Molecular docking studies

The AutoDock-Vina program [11,12] was used for molecular docking study. PDB structures of targets were converted to target molecules. Structures of ligands were dawn in the ChemSketch and optimized using the Argus Lab. Package. Co-crystallized ligand of glycogen phosphorylase (PDB ID: 1LWO) was used as control to validate molecular docking. The molecular binding affinity (Kcal/Mole) of the ligands obtained from the docking study were tabulated and analyzed. Ligand efficiency was calculated by dividing binding affinity with number of heavy atoms in the ligands. Three ligands with better ligand efficiency across all targets were selected for further studies.

Prediction of Adsorption, Distribution, Metabolism, Excretion (ADME) and off targets

The smiley notations of ligands were generated from their structure using the ChemSketch. This was submitted to the online servers (Swiss ADME and Swiss Target Prediction) for prediction and analysis of different drug properties and off-targets in human system.

RESULTS AND DISCUSSION

Antidiabetic action of *T. cordifolia* is widely reported. Although mechanism of this effect is yet to be clearly elucidated, *in silico* studies have shown glycogen phosphorylase as one of the target [4]. Since multiple etiologies are believed to be behind the progression of diabetes and its complication, other targets which can potentially contribute to diabetes were selected for evaluation. Obesity is one of the risk factor for diabetes. So 11β-HSD1 was selected as one of the target. *In vitro* and *in vivo* studies have strongly suggested role of fructose-6-phosphate amidotransferase in insulin resistance [13]. Accordingly, effect of components of *T. cordifolia* was evaluated against this. Sirtuin 6 (SIRT6) is a sirtuin family member with ability to repress the expression of glucose transporters and glycolytic enzymes [8].

Table 1: Affinity of ligands for diabetic targets

Ligands	1XU7		2ZJ3		3K35		5E0F		5KZW		1LWO	
	Aff.	LE.	Aff.	LE.	Aff.	LE.	Aff.	LE.	Aff.	LE.	Aff.	LE.
Cardifolioside A	-8.9	0.33	-6.2	0.23	-3.7	0.14	-11	0.43	-13	0.481	-6.6	0.24
Cardifolioside B	-8.9	0.33	-7.6	0.28	-3.7	0.14	-3.8	0.14	34.5	1.28	-6.6	0.24
Cardifolioside C	-8.9	0.33	-8	0.3	-3.8	0.14	-4.6	0.17	-33	1.22	-6.8	0.25
Cardifolioside D	-8.8	0.32	-7.7	0.29	-3	0.11	-2.2	0.08	-35	1.29	-5.5	0.2
Cardifolioside E	-8.8	0.32	-7.7	0.28	-3.8	0.14	-4.7	0.17	-10	0.37	-5.5	0.23
Tinocordiside	-7.5	0.44	-7.7	0.45	-4.4	0.26	-5.7	0.33	-1.2	0.07	-7.4	0.43
Cordioside	-8.7	0.33	-7	0.24	-4	0.15	-3.8	0.15	-2.5	0.1	-6.3	0.24
Tinocordifolioside	-7.1	0.39	-8.6	0.48	-4	0.22	-5.7	0.32	-1.3	0.1	-7.6	0.42
Tinospraside	-8.1	0.34	-8.1	0.34	-0.2	0.01	-6.9	0.29	-36	1.5	-4.9	0.2
Syringin	-6.4	0.43	-6.1	0.41	-5.5	0.37	-5.1	0.34	-2.8	0.19	-6.1	0.4
Aporphin	-7.7	0.43	-7.3	0.4	-7.1	0.39	-5.4	0.3	-5	0.28	-9.6	0.53
Choline	-4.5	0.64	-4.5	0.64	-4.4	0.63	-3.9	0.56	-3.3	0.47	-4.7	0.67
Columbin	-9.6	0.37	-7.4	0.28	-1.8	0.07	-2.4	0.09	-45	1.7	-4.6	0.18
Isocolumbin	-9.1	0.35	-8.8	0.34	-1.2	0.05	-4.2	0.16	-47	1.8	-6.6	0.25
Jatrorrhizine	-8.3	0.32	-6.6	0.26	-5.2	0.21	-4.3	0.17	-1	0.04	-6.2	0.25
Magnoflorine	-7.7	0.31	6.4	0.26	3.6	0.14	-2.2	0.09	-28	1.14	-7.7	0.31
Palmitine	-8.4	0.32	6.4	0.25	6.4	0.25	-3.6	0.14	-2.3	0.1	-4.7	0.18
Tembetarine	-8.3	0.33	6.9	0.28	2.6	0.1	-3.4	0.14	-27	1.1	-8.1	0.32
Tetrahydropalmitine	-8.4	0.32	6.1	0.23	6.2	0.24	-1.9	0.07	-1.7	0.1	-4.4	0.17
Berberin	-9.7	0.39	7.1	0.28	7.2	0.29	-6.4	0.26	-3.3	0.13	-5.7	0.23
Tinosporine	-9.6	0.37	8.2	0.31	4.6	0.18	-4.4	0.17	-13	0.51	-8.6	0.33

Aff: Affinity of ligand (Kcal/mol) for the target; LE: Ligand efficiency (Binding affinity/no of heavy atom); Ligands include aglycones of glycosides and alkaloids

Table 2: Predicted properties of ligands

Parameter	Aglycone of tinocordiside	Aporphine	Aglycone of syringin
Consensus Log P	2.47	3.23	1.47
GI absorption	High	High	High
BBB permeant	Yes	Yes	Yes
Pgp substrate	No	Yes	No
CYP 1A2/2C19/2C9/3A4	No	No	No
CYP2D6 inhibitor	No	Yes	No
Lipinski #violations	0	0	0
Bioavailability Score	0.55	0.55	0.55
PAINS #alerts	0	0	0
Brenk #alerts	0	0	0
Leadlikeness #violations	1	1	1
Synthetic Accessibility	4.3	2.94	2.12

Although berberin as a component of *T. cordifolia* has been validated against diabetes, contribution from other phytocomponents of *T. cordifolia* to its antidiabetic effect cannot be ruled out [14]. Thus major alkaloids and glycosides were included in the analysis. Since the aglycone functionality is primarily responsible for bioactivity [15], respective aglycones were used as ligands along with alkaloids. Molecular docking was validated using the co-crystallized ligand of PDB ID: 1LWO which showed strong affinity (-8.1 Kcal/mol) for this target. Further analysis revealed (Table 1) that many components of *T. cordifolia* have good affinity (> 6 Kcal/mol) against the targets. Accordingly the antidiabetic

effect can be considered to be mediated by inhibition of these multiple targets. However, affinity alone cannot reveal the utility of ligand. This is because big molecules tend to have higher affinity and may lead to false positives in the screening. Hence ligand efficiency was used as a parameter to compare the ligands. It revealed that four compounds including aglycone of tinocordiside, aglycone of syringin, aporphin and choline showed higher ligand efficiency against at least three targets (Table 1). Choline is a small but ionic compound. So it was considered as a false positive and excluded from further analysis. Thus aglycone of tinocordiside, aglycone of syringin and aporphin (Figure 1) were evaluated for their suitability as hit.

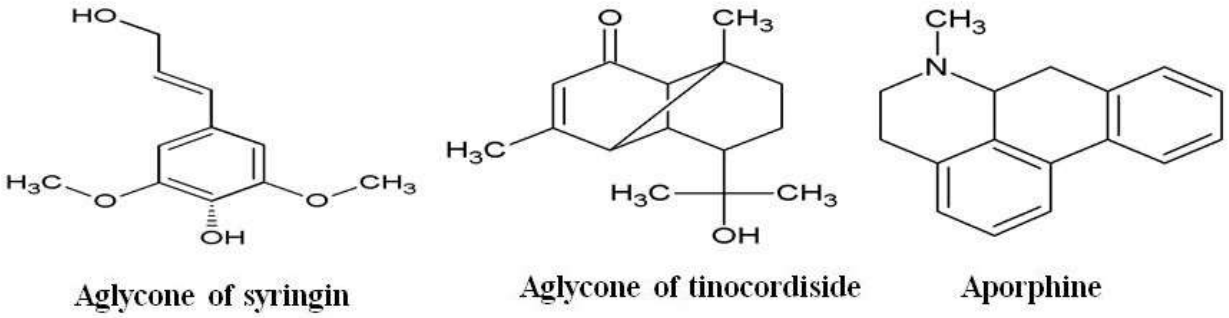

Figure 1: Hits from *Tinospora cordifolia* with higher (> 0.4) ligand efficiency against multiple targets (Minimum 3) of diabetes

The ADME and toxicity properties were analyzed following reported methods [16]. All three compounds were predicted to have good oral absorption and bioavailability (Table 2). They were also not found to be frequent hitters of drug targets which support their novelty as hit candidates [17]. Further, there was no violation of drug likeness which is again in support of their suitability for drug development (Table 2). They also exhibited only one violation of lead likeness as their molecular weight was less than 250. However, this is an advantage for a hit. This is because the hit will be further modified during lead optimization which will increase the molecular weight. So smaller compounds offer higher flexibility and hence can be accepted as better hits. Synthesis accessibility or ease of synthesis is a very critical parameter of hit selection as hits with higher score are difficult to synthesize, which limit the options for lead optimization process. Since aglycone of syringin was most accessible (lowest score) for synthesis, it can be considered as most suitable for optimization.

Table 3: Predicted off targets of ligands

Tinocordiside		Aporphine		Syringin	
Target	**p**	**Target**	**p**	**Target**	**p**
Cytochrome P450 19A1	0.84	5-hydroxytryptamine receptor 1A	0.95	Carbonic anhydrase 1	0.57
Glucocorticoid receptor	0.84	D(2) dopamine receptor	0.95	Carbonic anhydrase 2	0.57
Mineralocorticoid receptor	0.84	5-hydroxytryptamine receptor 1B	0.95	Carbonic anhydrase 3	0.57
Muscle blind-like protein 1	0.79	5-hydroxytryptamine receptor 7	0.95	Carbonic anhydrase 5A	No
Muscle blind-like protein 2	0.79	D(3) dopamine receptor	0.95	Carbonic anhydrase 7	0.57
Muscle blind-like protein 3	0.79	Sodium-dependent serotonin transporter	0.84	Carbonic anhydrase 9	0.57
Corticosteroid 11β-dehydrogenase isozyme	0.78	D(1A) dopamine receptor	0.78	Carbonic anhydrase 13	0.57
Androgen receptor	0.75	D(4) dopamine receptor	0.78	Carbonic anhydrase 5B	Yes
Sigma non-opioid intracellular receptor 1	0.73	D(1B) dopamine receptor	0.78	Carbonic anhydrase 12	0.54
Corticosteroid 11β-dehydrogenase isozyme 1	0.73	5-hydroxytryptamine receptor 2A	0.72	Carbonic anhydrase 6	0.54
Hydroxysteroid 11β-dehydrogenase 1-like protein	0.73	5-hydroxytryptamine receptor 2C	0.72	Carbonic anhydrase 14	0.54
Sodium-dependent noradrenaline transporter	0.72	5-hydroxytryptamine receptor 2B	0.72	Prostaglandin G/Hsynthase1	0.54
Mitogen-activated protein kinase 3	0.72	Sigma non-opioid intracellular receptor 1	0.68	Prostaglandin G/H synthase 2	0.54
Mitogen-activated protein kinase 1	0.72	Alpha-2A adrenergic receptor	0.63	Epidermal growth factor receptor	0.5
Sodium-dependent serotonin transporter	0.72	Alpha-2B adrenergic receptor	0.63	Receptor tyrosine protein kinase erb-2	0.5

p: Probability/likeliness of the off-target based on structure similarity with known substrates

Prediction of off-targets [18] showed relatively higher probability for binding to non-targets in case of aglycone of tinocordiside and aporphin (Table 3). Thus, these two ligands may have higher affinity for these non-targets. Besides, the numbers of off-targets were also more for these ligands. This suggests their potential for generating unwanted effects because of action on multiple targets in homosopiens. In contrast to this, aglycone of syringin showed affinity for relatively fewer types of off-targets and may likely to have fewer side effects. Accordingly aglycone of syringin can be considered as the most suitable hit for further optimization to develop lead/drug candidates against diabetes.

CONCLUSION

The molecular docking analysis reveals that the antidiabetic action of *T. cordifolia* may be attributed to inhibition of multiple targets of diabetes including 11β-HSD1, fructose-6-phosphate amidotransferase, glycogen phosphorylase, Sirt6, α-glucosidase and pancreatic alpha-amylase by its components. Aglycone of tinocordiside, aglycone of syringin and aporphin were found to be better ligands for these targets. Prediction of ADME and off-targets revealed that aglycone of syringin is the most appropriate hit which can be further optimized to develop new antidiabetic drug.

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