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Research Article

AN IN SILICO INSIGHT INTO MOLECULAR MECHANISM OF HYPOGLYCEMIC ACTIVITY OF SCOPARIC ACID D, A DITERPENOID FROM SCOPARIA DULCIS L.

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ABSTRACT

Despite availability of preclinical report about antidiabetic activity of the molecule Scoparic Acid D isolated from *Scoparia dulcis* L. no clinical trial has reported to be initiated possibly because of the fact that molecular mechanism of hypoglycemic effect of the molecule was not explained properly. Considering the reported hypoglycemic potential of the molecule and looking at the alarming increase of diabetic population worldwide and particularly in India we wanted to explain the mode of action of the molecule using virtual screening technique.

Target Fishing Technique was employed to find out suitable target for the molecule using PharmMapper server taking human targets as database. From the result, the topmost diabetes drug target was selected and Scoparic Acid D was docked with it using FlexX. Collecting from PubChem database, descriptors of 54 existing antidiabetic molecules were considered for developing QSAR model and activity of Scoparic Acid D was predicted with reference to the QSAR model developed. In a similar manner Docking score of two known commercially available human α-glucosidase enzyme inhibitor drugs, viz. Miglitol and Voglibose and their IC50 values were recorded and compared with that of Scoparic Acid D.

The target fishing of Scoparic acid D revealed that it has a high potentiality to inhibit the human α-glucosidase enzyme, which is a target for Diabetes mellitus. Docking studies showed greater affinity of Scoparic Acid D towards the active site of human α-glucosidase with a docking score of -8.1675, which is better than that of existing 22 inhibitors out of 54 used in the study and is also comparable to that of two commercial drugs, which target human α-glucosidase enzyme. Predicted IC50 value of Scoparic Acid D was found to be 3.98μM which is better than that of the known human α-glucosidase enzyme inhibitor drug i.e., Voglibose (IC50: 5.6 μM).

We, therefore, suggest that Scoparic Acid D may be a potent inhibitor of human α-glucosidase enzyme and thereby delaying digestion of starch and sucrose, flattening postprandial blood glucose excursions and mimicking the effects of dieting on hyperglycemia may be a potent drug for treating diabetes.

Keywords: Scoparic Acid D, hypoglycemic effect, Diabetes mellitus, human α-glucosidase, docking, QSAR

INTRODUCTION

Diabetes mellitus is one of the major life threatening diseases all over the world. Global estimates for the year 2010 predict a further growth of almost 50%, with the greatest increases in the developing countries of Africa, Asia, and South America (Stumvoll, 2005). India has become the Diabetes capital of the world. As per the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently is around 40.9 million. The number is expected to rise to 69.9 million by 2025 (Mohan *et al.,* 2007). So, there is always a high demand for effective drug molecules to combat this disease and plant derived drugs are always of top priority in treating any ailment.

Scoparic acid D (SAD), a diterpenoid isolated from the ethanol extract of *Scoparia dulcis* L. has shown promising hypoglycemic effect in streptozotocin (STZ)-induced diabetic male Wistar rats (Latha *et al.,* 2009). Although a number of other molecules have been isolated from this plant viz., Scoparic acid A (Hayashi *et al.,* 1999), Scoparic acid B (Hayashi *et al.,*1992), Scopadulcic acid A (Riel *et al.,* 2002) and B (Hayashi *et al.,* 1997; Hayashi *et al.,* 1999), Scopadulciol (Hayashi *et al.,* 1991; Hayashi *et al.,* 1997), and Scopadulin (Hayashi *et al.,*1990) and the plant is traditionally reported to be used in a number of remedies including stomach troubles (Satyanarayana, 1969), hypertension (Chow *et al.,* 1974), diabetes (Perry, 1980), bronchitis (Gonzalez-Torres, 1986) and as analgesic and antipyretic agents (De Farias Freire *et al.,* 1993). No other molecule of the plant except Scoparic acid D (SAD) is reported to have hypoglycemic effect. Nath (1943) though reported that a glycoside Amellin isolated from fresh plant can relieve a number of associated ailments of diabetes.

Despite publication of hypoglycemic activity report of Scoparic acid D (SAD) in a journal like Natural product Research in 2009, neither the compound has appeared in the market as standard drug nor any clinical trial with this compound is reported to be initiated probably because of the fact that mechanism of action of the compound in lowering blood glucose has not been explained yet. Interestingly, in spite of all the latest advances in drug discovery process there has been no "fully potent no side effect" drug appeared in the market for treating diabetes. During past few decades however, advent of genomics and proteomics has helped in understanding the molecular alteration characteristics of NIDDM and currently various approaches are available to curb the disease from molecular point of view. Accordingly some molecular inhibitors of human α-glucosidase enzyme like Miglitol and Voglibose are now available in the market (Saqib and Siddiqi, 2009).

In the present work we, therefore, have given an insight into molecular mechanism of action of SAD using computational tools as virtual screening offers immense potential in identification of novel drug candidates (Venkatesan *et al.*, 2010). Probable target for SAD was searched out using target fishing tool and its ability to block the target was determined by molecular docking technique. Probable potency of the compound in question was predicted by developing QSAR model.

METERIALS AND METHOD

Preparation of Scoparic Acid D structure for *in silico* analysis

The structure of Scoparic Acid D (Latha *et al.,* 2009) was drawn in ACD ChemSketch and was converted to 3D structure in Sybyl MOL2 format using OpenBabel (O'Boyle *et al.,* 2011). The MOL2 file was loaded in PharmMapper server (Liu *et al.,* 2010) taking the human targets as database. From the result, the topmost diabetes drug target was selected for docking studies using FlexX. Some known inhibitors of the target were also obtained from NCBI PubChem BioAssay database in SDF Format for developing QSAR model.

The ADME/Tox parameters of SAD and other known inhibitors were studied using online server Mobyle@RPBS maintained by the University of Paris. The compounds were input in the server in SMILES format using the following parameters:

Molecular weight : min 200.0 max 600.0

Hydrogen donors : min 0.0 max 6.0

Hydrogen acceptors : min 0.0 max 12.0

Flexible bonds : min 0.0 max 15.0

Rigid bonds : min 0.0 max 50.0

Ring number : min 0.0 max 7.0

Ring size : min 0.0 max 12.0

Atom number : min #carbons: 5.0 min #non carbons 2.0

Ratio carbon/hetero : min 0.1 max 1.0

Charge number : min 0.0 max 3.0

Total charge : min -2.0 max 2.0

logP : min -2.0 max 6.0

Polar Surface Area : min 0.0 max 150.0

Active site identification

The structure of the drug target was obtained from Protein Data Bank and SWISS Model Repository. The PDB file was loaded into Q-Site Finder (Burgoyne and Jackson, 2006; Laurie and Jackson, 2005) to identify the active site amino acids. The amino acids of the first site were selected as active site for docking study.

Molecular Docking using FlexX

The PDB file of the target was loaded in the BioSolveIT FlexX (Sato *et al.*, 2006). The active site amino acids were defined in the target molecule during the target preparation step of FlexX. A sphere of 10Å radius was defined as active site. The MOL2 file of Scoparic Acid D was loaded in FlexX as docking library. The Protein Ligand clash was set to 2.9 Å and Intra Ligand clash was set to 0.6 in the docking. The docking was performed to study the binding efficacy of Scoparic Acid D and the drug target. To compare binding efficacy, some known inhibitors obtained from NCBI PubChem were also docked in the same active site of the target protein. The docked ligand-target complexes were analyzed carefully to identify the interactions. The docking score was noted down and docking poses were saved for reference. In a similar manner docking score of two known commercially available human α-glucosidase enzyme inhibitor, viz. Miglitol and Voglibose were also recorded.

QSAR studies

For QSAR study, some known inhibitors of the target enzyme were obtained in SDF format. The QSAR descriptors viz. Volume, Hydration Energy, LogP, Refractivity, Polarizability, Mass, Molar Refractivity, Parachor, Index of Refraction, Surface Tension and Density were generated for each of the molecule using HyperChem and ACD ChemSketch softwares. The descriptors were tabulated in a MS Excel Sheet against their bioactivities (log IC_{50}^{-1}) obtained from PubChem BioAssay. The descriptors and activities were loaded in EasyQSAR software for multiple linear regression analysis. From the regression, the QSAR equation was generated and the activities for each of the molecules were predicted. The predicted activities were plotted against the actual activities of the molecules to validate the QSAR equation. The activity of Scoparic Acid D was predicted by putting its QSAR descriptors in the equation and the activity was compared with that of the commercially available drugs Miglitol and Voglibose.

RESULTS

The target fishing of Scoparic acid D revealed that it has a high potentiality to inhibit the human α-glucosidase enzyme, which is a target for Diabetes mellitus. The structure of human α-glucosidase was obtained in PDB format. The active site characterization of the enzyme using Q-site finder showed that PRO 365, TRP 367, ARG 437, GLU 500, VAL 503, ALA 504, HIS 507 and PHE 512 are the key amino acids forming active site.

In ADME/Tox screening, out of 54 standard inhibitors of α-glucosidase enzyme (PubChem Bioassay ID: 2111), 2 molecules, viz. Compound-11 and Compound-54 were found to be toxic. High formal charge, low molecular weight and low logP values are the key factors for the toxicity of these molecules. So, these 2 molecules were discarded from QSAR study. The QSAR analysis using 52 molecules each with 11 descriptors showed significant correlation with R square value of 57.79% and F statistics of 4.98. The equation generated out of QSAR analysis is as follows:

$\log (IC50)^{-1}$ = 9.63144E-001 - 4.37431E-001*(Volume) - 3.40575E-001*(HydrationEnergy) + 1.33617E-002*(LogP) + 2.11499E-001*(Refractivity) + 3.03255E-002*(Polarizability) - 6.94475E-002*(Mass) - 5.57817E-002*(MolarRefractivity) + 5.751573E-001*(Parachor) - 7.460862 E+ 000*(IndexofRefraction) - 7.50638E-001*(SurfaceTension) + 5.25595 E+ 001*(Density)

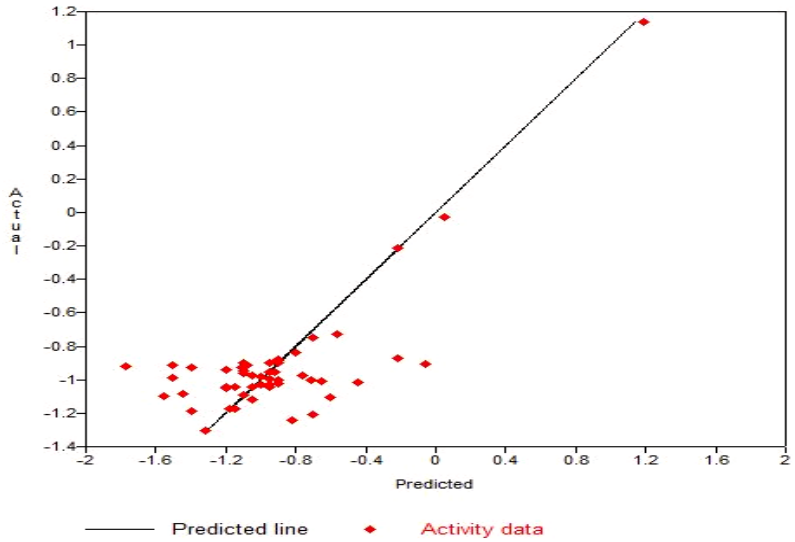

Fig 1: The QSAR Plot.

By putting QSAR descriptors in the above equation, the IC50 value of SAD was found to be 3.98µM. Molecular docking studies of SAD and all 54 standard inhibitors with human α-glucosidase revealed high potentiality (Score -8.1675) of SAD as inhibitor of human α glucosidase. Whereas the docking score for Miglitol and Voglibose is recorded to be -13.8328 and -9.0441 respectively. The IC50 value of Miglitol is 0.59 µM (Natori *et al.,* 2011) and of Voglibose is 5.6 µM (Kuriyama *et al.,* 2008). SAD forms four H-bonds with GLN 429, LYS 418 and ASP 423 and weak interactions with PHE 146, ALA 426, ASP 423, LYS 418 and PRO 425.

Table 1: Chemical descriptors and Activity (IC50) of 54 known inhibitors of human α-glucosidase

Compounds	Surface Area	Volume	Hydration Energy	Log P	Refractivity	Polarizability	Mass	Molar Refractivity	Parachor	Index of Refraction	Surface Tension	Density	IC50 (µM)
Compound-1	517.37	1029.41	-16.47	8.87	54.48	37.39	401.44	101.77	781.5	1.664	66	1.464	0.8913
Compound-2	509.44	1025.2	-14.9	6.78	57.54	37.31	387.45	101.66	776.3	1.659	63	1.406	7.9433
Compound-3	510.09	1007.01	-11.31	8.56	53.04	36.75	385.44	100.47	768.2	1.656	62.4	1.41	0.0651
Compound-4	605.33	1143.62	-9.18	10.61	67.58	42.45	468.35	114.54	878.7	1.693	75.1	1.56	10
Compound-5	559.83	1090.01	-11.37	8.8	59.52	39.23	415.46	106.61	824.9	1.63	57.5	1.386	7.9433
Compound-6	515.91	1029.19	-8.8	8.19	56.69	36.4	425.43	54.37	781.32	1.73	69.92	1.53	5.0119
Compound-7	478.63	973.08	-16.22	7.29	52.54	35.47	373.43	100.22	738.72	1.6	61.37	1.34	3.9811
Compound-8	524.5	1028.24	-10.56	7.22	57.58	37.31	387.45	65.25	780.6	1.76	63.68	1.4	5.0119
Compound-9	475.81	960.15	-9.07	7.71	51.06	34.74	375.42	56.04	728.9	1.56	61.7	1.35	8.9125
Compound-10	517.85	1007.07	-13.56	7.28	56.92	36.69	382.44	83.79	764.52	1.74	62.85	1.38	6.3096
Compound-11*	528.02	1015.98	-15.2	7.5	57.15	36.55	402.42	101.53	778.1	1.664	65.5	1.471	31.6228
Compound-12	476.01	985.44	-5.53	6.18	70.62	41.47	399.34	34.17	748.1	2.16	65.63	1.44	0.9431
Compound-13	470.23	1006.34	-5.19	6.18	70.62	41.47	399.34	32.07	763.97	2.16	65.63	1.44	8.9125
Compound-14	529.22	1046.57	-8.06	5.73	66.98	39.4	374.44	49.8	794.51	2.05	61.54	1.35	4.4668
Compound-15	556.33	1060.46	-8.23	4.19	69.72	40.72	376.47	50.85	805.06	2.13	61.87	1.36	7.9433
Compound-16	567.07	1063.83	-8.7	4.19	69.72	40.72	376.47	53.76	807.61	2.13	61.87	1.36	1.6343

Compounds	Surface Area	Volume	Hydration Energy	Log P	Refractivity	Polarizability	Mass	Molar Refractivity	Parachor	Index of Refraction	Surface Tension	Density	IC50 (µM)
Compound-17	560.66	1054.36	-4.61	5.55	70.84	41.29	358.5	28.48	800.43	2.16	58.92	1.29	7.9433
Compound-18	581.19	1136.79	-7.55	4.85	78.94	44.39	404.53	46.65	863	2.41	66.49	1.46	7.9433
Compound-19	595.68	1073.67	-4.69	5.55	70.84	41.29	358.5	28.98	815.08	2.16	58.92	1.29	11.2202
Compound-20	560.59	1037.72	-6.24	6.18	70.62	41.47	399.34	38.56	787.79	2.16	65.63	1.44	8.9125
Compound-21	580.34	1057.62	-4.61	5.55	70.84	41.29	358.5	28.48	802.9	2.16	58.92	1.29	11.2202
Compound-22	-180.9	1054.59	-2.43	5.55	70.84	41.29	358.5	15.01	800.6	2.16	58.92	1.29	8.9125
Compound-23	549.63	1078.89	-9.95	4.26	72.5	41.79	388.48	61.48	819.05	2.21	63.85	1.4	2.8184
Compound-24	562.51	1058.13	-4.84	5.55	70.84	41.29	358.5	29.91	803.29	2.16	58.92	1.29	12.5893
Compound-25	561.53	1162.59	-11.18	5.38	63.97	46.71	424.52	69.08	882.59	1.95	69.77	1.53	10
Compound-26	511.52	998.46	-8.51	3.63	65.2	38.89	362.45	52.58	757.99	1.99	59.57	1.31	5.7988
Compound-27	606.56	1113.88	-8.66	4.39	74.36	42.56	390.5	53.51	86.45	2.27	64.18	1.41	3.6588
Compound-28	501.14	1021.82	-5.02	5.55	70.84	41.29	358.5	31.02	775.72	2.16	58.92	1.29	1.6343
Compound-29	529.58	1014.49	-6.31	5.32	66.23	39.45	344.47	38.99	770.16	2.02	56.61	1.24	5.1682
Compound-30	575.41	1052.6	-6.24	6.18	70.62	41.47	399.34	38.56	799.09	2.16	65.63	1.44	1.157
Compound	578.4	1095.	-6.87	4.4	74.47	42.56	390.	42.45	831.91	2.27	64.18	1.41	15.84

-31	5	84		4			5						89
Compound -32	487.76	1079.42	-4.55	4.44	74.47	42.56	390.5	28.11	819.45	2.27	64.18	1.41	12.5893
Compound -33	508.04	946.79	-7.28	4.19	67.68	37.14	336.47	44.98	718.76	2.07	55.3	1.21	15.8489
Compounds	**Surface Area**	**Volume**	**Hydration Energy**	**Log P**	**Refractivity**	**Polarizability**	**Mass**	**Molar Refractivity**	**Parachor**	**Index of Refraction**	**Surface Tension**	**Density**	**IC50 (µM)**
Compound -34	643.04	1217.27	-7.32	5.13	83.97	46.23	418.55	45.23	924.1	2.56	68.79	1.51	15.8489
Compound -35	573.48	1045.38	-6.25	6.18	70.62	41.47	399.34	38.62	793.61	2.16	65.63	1.44	6.6769
Compound -36	578.95	1103.83	-8.71	4.44	74.47	42.56	390.5	53.82	837.98	2.27	64.18	1.41	14.1254
Compound -37	523.64	1071.98	-8.24	4.44	74.47	42.56	390.5	50.91	813.8	2.27	64.18	1.41	11.2202
Compound -38	531.51	1077.23	-8.78	4.44	74.47	42.56	390.5	54.25	817.79	2.27	64.18	1.41	12.5893
Compound -39	525.8	996.73	-5.52	5.24	66.23	39.45	344.47	34.11	756.67	2.02	56.61	1.24	8.4057
Compound -40	563.08	1044.94	-8.33	4.68	68.05	40.09	360.47	51.47	793.27	2.08	59.24	1.3	8.9125
Compound -41	522.64	1001.36	-5.94	5.55	66.12	39.54	364.89	36.7	760.19	2.02	59.97	1.31	25.1189
Compound -42	465.61	1001.73	-5.33	5.55	66.12	39.54	364.89	32.93	760.47	2.02	59.97	1.31	14.1254
Compound -43	545.65	1035.16	-6.92	4.68	68.05	40.09	360.47	42.76	785.85	2.08	59.24	1.3	14.9478
Compound -44	564.16	1022.9	-5.68	5.24	66.23	39.45	344.47	35.1	776.54	2.02	56.61	1.24	31.6228
Compound -45	560.03	1040.39	-6.12	6.18	70.62	41.47	399.34	37.82	789.82	2.16	65.63	1.44	11.8734
Compound -46	553.48	1015.86	-6.4	5.55	66.12	39.54	364.89	39.55	771.2	2.02	59.97	1.31	15.8489
Compound -47	517.1	1000.72	-5.9	5.24	66.23	39.45	344.47	36.46	759.7	2.02	56.61	1.24	25.1189
Compound -48	563.61	1042.92	-8.53	4.68	68.05	40.09	360.47	52.71	791.74	2.08	59.24	1.3	12.5893
Compound -49	564.77	1099.89	-8.96	9.77	62.43	40.52	433.91	55.36	834.99	1.91	71.31	1.56	12.982
Compound -50	538.62	1060.51	-9.51	9.14	57.94	38.59	399.46	58.76	805.09	1.77	65.65	1.44	20.575
Compounds	**Surface Area**	**Volume**	**Hydration Energy**	**Log P**	**Refractivity**	**Polarizability**	**Mass**	**Molar Refractivity**	**Parachor**	**Index of Refraction**	**Surface Tension**	**Density**	**IC50 (µM)**
Compound -51	471.83	1154.16	-7.78	5.21	68.68	48.55	438.54	48.07	876.19	2.1	72.07	1.58	59.5081
Compound -52	458.68	854.76	-8.58	7.51	54.53	28.93	323.37	53.02	648.9	1.67	53.15	1.16	35.4813
Compound -53	615.89	1125.52	-8.92	4.44	74.47	42.56	390.5	55.12	854.45	2.27	64.18	1.41	28.1838
Compound -54*	253.99	487.74	-20.88	-1.87	36.57	14.91	163.17	37.4	314.2	1.582	61.9	1.456	35.4831

*** Toxic compounds**

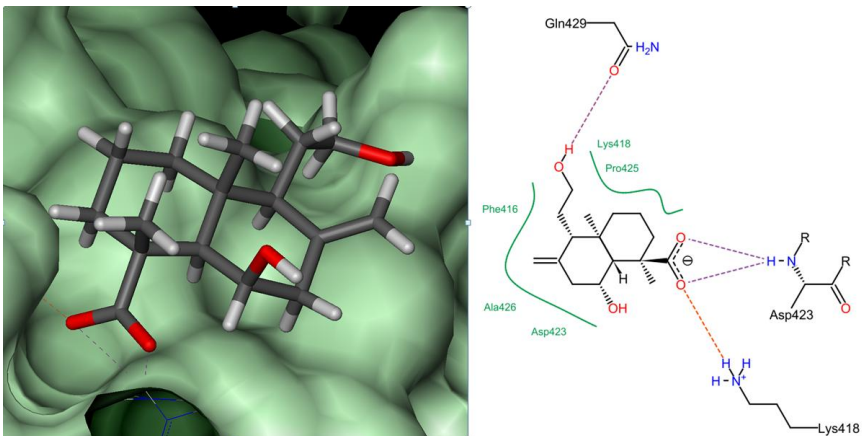

Fig-2: Docking pose of Scoparic acid D with human α-glucosidase

The docking studies of existing 54 inhibitors with human α-glucosidase revealed that the docking score of Scoparic Acid D is better than that of 22 existing inhibitors and also comparable to that of commercially available human α-glucosidase inhibitors like Miglitol and Voglibose. Among the existing inhibitors, Compound-1, i.e., 4-(4-(2-oxoindolin-5-ylsulfonyl)piperazin-1-yl)benzoic acid has the highest docking score with -13.2469 and Compound-16, i.e., 6-propyl-1-(2-(thiophen-3-yl)ethyl)-2-thioxo-2,3,5,6,7,8-hexahydropyrimido[4,5-d]pyrimidin-4(1H)-one being lowest with a docking score of -2.5931.

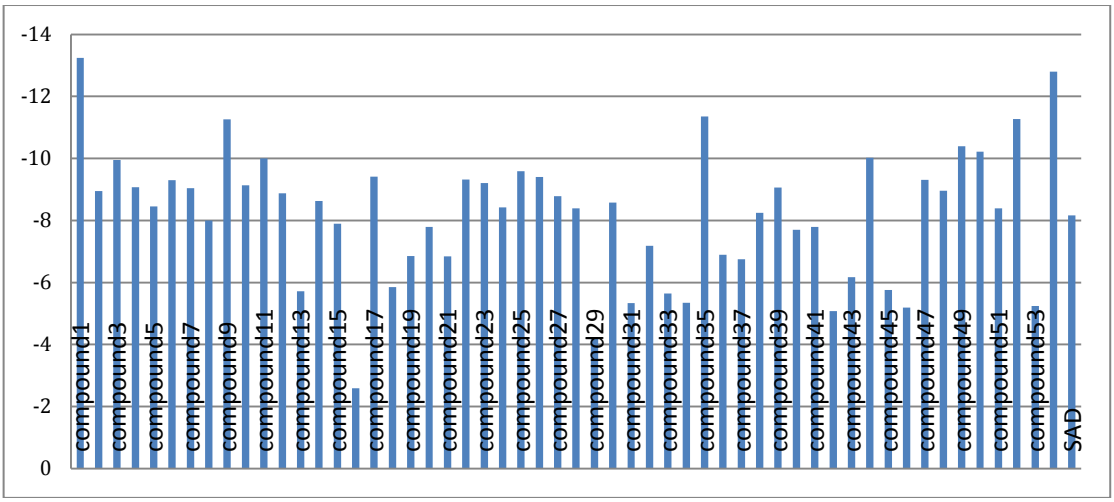

Fig 3: Plot showing docking scores of existing 54 inhibitors and Scoparic Acid D

Studying the Hydrogen bonding patterns of 54 inhibitors with α-glucosidase as shown in Figure-4, it has been observed that Hydrogen bonding with OD1-ASP-513-A is the most conserved one. This bond was found in 19 out of 54 existing inhibitors.

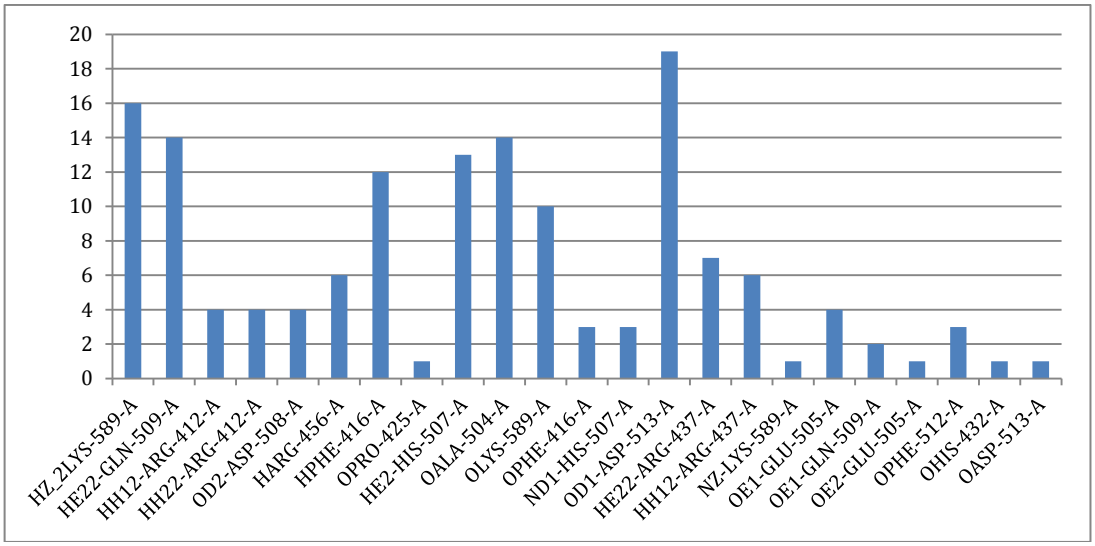

Fig 4: Plot showing Hydrogen bond forming amino acids of human α-glucosidase with existing 54 inhibitors

DISCUSSION

In the present study *in silico* target fishing of Scoparic Acid D revealed its inhibition potential of human α-glucosidase enzyme. Recently, α-glucosidase inhibitors have been put in a new light as they can prevent or delay the development of type II diabetes. The mechanism of α-glucosidase inhibition represents the pharmacological optimization of the dietary principle of delayed carbohydrate absorption. Inhibition of intestinal α-glucosidase delays the digestion of starch and sucrose, flattens the postprandial blood glucose excursions, and thus mimics the effects of dieting on hyperglycemia, hyperinsulinaemia and hypertriglyceridemia (Bischoff, 1994). Moreover, inhibition of the same enzyme reported to be shown a reduced risk of cardiovascular disease and hypertension the most commonly associated attributes of Diabetes. Currently only few α-glucosidase inhibitors are commercially available as drugs of which Miglitol and Voglibose (Van de Laar FA *et al*., 2005) are used in the study.

In the present study, we observed that Scoparic Acid D also has sufficient affinity towards active site of human α-glucosidase enzyme in comparison to existing 22 inhibitors out of a total of 54 inhibitors reported in PubChem BioAssay ID 2111. It is recorded in the present study that docking score of Scoparic Acid D is comparable to that of two commercially available human α-glucosidase inhibitors Miglitol and Voglibose. Predicted IC50 value of Scoparic Acid D (3.98μM) is better than that of one of the commercially available drugs, i.e. Voglibose (5.6 μM).

CONCLUSION

Our study, therefore, concludes as follows-

1. Human α-glucosidase is the target enzyme of SAD. By inhibiting this enzyme SAD reflects hypoglycemic activity probably with the technique of delaying digestion of starch and sucrose, flattening postprandial blood glucose excursions, and thus mimicking the effects of dieting on hyperglycemia.
2. Scoparic Acid D has comparable capacity to block Human α-glucosidase enzyme with reference to that of Miglitol and Voglibose.
3. Inhibitory concentration of Scoparic Acid D as predicted from the QSAR model is better than that of Voglibose.
4. Scoparic Acid D may be recommended for clinical trial making Human α-glucosidase enzyme as target as preclinical data are already available.
5. Suggestion put forwarded in the present work if considered, may contribute to marketing of a Human α-glucosidase targeted new antidiabetic drug.

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