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In Silico screening of phytochemicals to inhibit myo-inositol oxygenase (MIOX), an enzyme overactive in Type 2 diabetes

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Abstract

Selected phytochemicals are screened by bioinformatics tools to inhibit the myo-inositol oxygenase (MIOX) in an effort to find a novel drug against Type 2 diabetes. Hentria contane, a phytochemical from *Citrullus colocynthis* could be a potential drug that could block the enzyme activity.

Keywords: bioinformatics, diabetes, computation screening, phytochemicals, myo-inositol oxygenase, Hentriacontane

INTRODUCTION

Diabetes, is a serious metabolic disease currently with an estimated 124 million patients worldwide. Further more the prevalence of type 2 diabtetes is on the rise. (Perfetti and D'Amico, 2005). So, there is a great demand for finding new drugs to treat diabetes.

Myo-inositol oxygenase (MIOX) is an enzyme that is understood to be solely responsible for breaking down inositol, a sugar critical to the insulin signalling pathway in humans. MIOX is overactive in type 2 diabetes (Palmano *et al.*, 1997) leading to defected inositol levels, resulting in insulin resistance. The present paper deals with an attempt to find a novel diabetes drug to inhibit MIOX, boost inositol level and therefore restore insulin signalling in Type 2 diabetes.

METHODS

I. Retrieval of the Structure for Human MIOX

The tertiary structure for Human MIOX was retrieved from the Protein Data Bank (http://www.rscb.org/pdb/home/home.do) with the PDB ID being 2IBN.

II. Retrieval of Ligands structure

The structures of selected ligands were obtained from the "KEGG Data base" (http://www.genome.jp/kegg/compound) and their KEGE IDs are given below.

Name of Ligand	KEGG ID	Plant Source
i. Emodin	- C10343	*Cassia auriculata*
ii. Hentria Contane	- C08376	*Citrullus colocynthis*
iii. Quercetin	- C00389	*Eugenia jambolana*
iv. Kaempferol	- C05903	*Eugenia jambolana*
v. Myricetin	- C10107	*Eugenia jambolana*
vi. Beta Sito Sterol	- C01753	*Salvadora persica*

From the KEGG database, we have got the SDF file formats of all the ligands. Then the SDF files were converted to .pdb files by using "Open Babel" software (http://www.openbabel.org/).

III. Calculation of free energy for the Human MIOX receptor

The free energy computations for Human MIOX were done with "GROMOS96" implementation of "SPDB Viewer". While calculating and minimizing the free energy, the bonds, angles, torsion, improper, non bonded, electrostatic and constraint parameters were considered. As a result of this process, optimized structure having the minimum energy was obtained.

IV. Identification of the active sites of the receptor

For this analysis, we have used "Active site prediction tool" from SCFBio Server [http://www.scfbio-iitd.res.in/research/sanjeevini.htm]. It requires a proper .pdb file as an input and the results obtained from this tool explains the total number of active sites present in our query pdb file, along with information on their aminoacid sequence. In addition to that it also gives their cavity points and the average volume of the cavity.

V. Docking process

'HEX' a common and freely available software that can be downloaded and installed into any computer, was used to calculate the binding energy requirements of different ligands with the Human MIOX receptor.
In HEX, we have used full rotation search mode and

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shape only correlation type for docking. The docking parameters employed were as follows.

1.Search Mode	:	Full rotation
2.Correlation type	:	Shape only
3.Radial filter	:	None
4.Post processing	:	None
5.Grid dimension	:	0.6
6.Solution	:	500 samples : 642
7. Receptor range	:	180 samples : 642
8. Ligand range	:	180 samples : 128
9. Twist range	:	360
10. Distance range	:	40
11. Scan step	:	1.0
12. Substep	:	2

The dockings were performed for the receptor (before and after energy minimized structures were considered) and different ligands individually. After the completion of binding processes, we obtained the minimum and maximum energy requirements for bindings of the receptor with different ligand molecules from the HEX message box. Least energy represents the easy binding characters of ligand and receptor.

VI. Identification of Ligand Binding Sites

After the docking process was completed, the docked structure from the Hex, was saved as .pdb file.Then this complexed structure was opened in "Q-SiteFinder" tool and this tool can be accessed *via* http://www.modelling.leeds.ac.uk/qsitefinder/. It predicts different sites for ligand in an receptor along with their site volume, min & max co-ordinates and their aminoacid composition. In addition to that, the structure for our query could be viewed by Jmol viewer.

VII. Ligand property prediction

Lipinski rule of five helps in distinguishing drug-like and non drug-like properties and predicts high probability of success or failure due to drug likeliness for molecules. The Lipsinki filter helps in early preclinical assessment and thereby avoiding costly late stage preclinical and clinical failures. This tool which can be accessed from http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp. was used for ligand property prediction.

VIII. Analysis of "Physical property" and "Bio-activity"

"Mol inspiration tools" which can be accessed from http://www.molinspiration.com/cgi-bin/properties were used to analyze the following physical properties *viz.*,

(i) miLogP *i.e.*, sum of fragment based contributions and correction factors.

(ii) Molecular Polar Surface Area (TPSA), calculated as a sum of fragment contributions O- and N- centered polar fragments. PSA (Polar Surface Area) has been shown to be a very good descriptor characterizing drug absorption including intestinal absorption, bioavailabilty, $CaCO_2$ permeability and blood - brain barrier penetration.

(iii) Molecular volume which was obtained by fitting sum of fragment contributions to "real" 3D volume for a training set of about 12,000, mostly drug-like molecules.

(iv) No. of Rotatable bonds – nrotb:- This simple topological parameter is a measure of molecular flexibility. It has been shown to be a very good descriptor of oral bioavailability of drugs. It is defined as any single non ring bond, bounded to nonterminal heavy atom. Amide C-N bonds are not considered because of their high rotational energy barrier.

(v) Drug likeness, which may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs.

These properties, mainly hydrophobicity, electronic distribution hydrogen bonding characteristics, molecule size and flexibility and the presence of various pharmacophoric features influence the behaviour of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others.

Bioactivity analysis included features such as GPCR ligand, Ion channel modulators, Kinase inhibitor and Nuclear receptor ligand.

IX. Bioactivity Analyses

The Bioproperty analysis were carried out by "chem exper" accession (http://www.chemexper.com)

The analyse included evaluation of Mutagenic property, Tumerogenic property, Irritant property, Reproductive effect, cLogP (log of partition coefficient between n-octanol and water) *i.e.*, the measure of hydrophilicity), log S (the aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with a bad absorption and therefore the general aim is to avoid poorly soluble compounds. The estimated logS value in a unit of stripped logarithm (basel O) of the solubility measures in mol/l. Normal range greater than – 4), Molecular weight (Optimizing compounds for high activity on a biological target almost often goes along with increased molecular weight, however, compounds with higher weights are less likely to be absorbed and

to ever reach the place of action. Normal range is below 450), Drug likeness and Drug score.

RESULTS AND DISCUSSION

1. Structure of MIOX

The molecular structural features of Myo-inositol oxygenase (MIOX) from human is shown in figure 1 and the details of the active sites for this receptors obtained from "Active site predictor" from SCF bio are given in Table 1.

2. Docking energy result

Results of docking of the selected phytochemicals against MIOX are given in table 2. Hentriacontane had the least binding energy among the compounds tested in the present study (Table 2, Fig. 2)

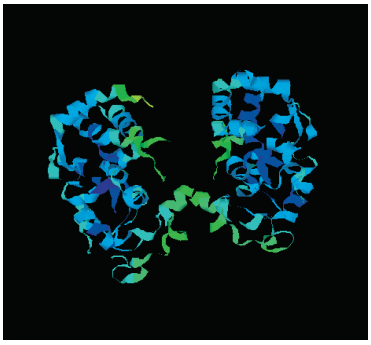

Figure 1. Structure Of Human MIOX [visualized by spdbv] PDB ID : "2IBN"

3. Ligand binding sites information from Q Site finder

The ligand binding sites for Hentriacontane with MIOX are given in Table 3.

Total Number of Predicted sites - 10

Table 2. Docking energy values of selected ligands with MIOX

S. No.	Receptor + Drug	Emin Kcal/Mol	Emax Kcal/Mol
1	2IBN+ Emodin	-131.97	-46.77
2	2IBN+Hentriacontane	-178.49	-69.50
3	2IBN + Kaempferol	-115.17	-27.46
4	2IBN +Betasitosterol	-95.26	-19.70
5	2IBN + Myrecetin	-105.00	-18.98
6	2IBN + Quercetin	-97.67	-26.66

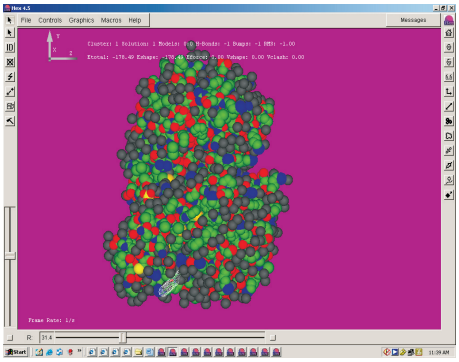

Figure 2. Docking of ligand Hentriacontane with Human MIOX

Table 1. Details of the active sites as (cavities) for this receptor MIOX as obtained from "Active site predictor" from SCF Bio (Total No. of Cavities: 17)

Cavities	Residues	Volume of Cavity	Cavity point		
1.	LNFDQVHSTWAIKGYERPC	1190	38.122	20.409	17.519
2.	YDTGARSVCNHPQLEFWKI	1094	32.547	32.965	12.181
3.	SYDTFAVCPQNRKHEWLGI	982	30.081	33.510	19.567
4.	QKHRYEDWVLFPGCTIASN	919	2.838	32.212	38.991
5.	RSFLEWHKVDYGATPQNC	899	4.917	35.568	30.074
6.	NLVYFQKCSIWAPERDGHT	864	1.176	19.134	38.103
7.	IKFPNLSGWTEYVHQCARD	843	-3.641	20.879	31.408
8.	LTNHYERFDPVWSAIKGQ	752	33.460	21.473	11.061
9.	LKNYRQFDGEVHSTWAIPC	700	32.876	19.677	18.920
10.	GHWRQLKVDTSFPNEYCA	686	10.531	29.280	29.635
11.	DSYRTGALFKVCNHPQEWI	612	26.218	27.681	16.438
12.	VFWALEGDIHRKQSNTPY	594	3.908	24.839	25.662
13.	SHTDFKGVQPWILRAEY	549	39.027	31.021	19.029
14.	HRLVFTYWDQGPSCNKEA	541	5.056	39.733	36.035
15.	YSWFQHVALEKRGDIPNT	530	-2.826	28.569	28.633
16.	DASVCFYTPQREKWHLGN	525	29.609	39.954	13.924
17.	QFEDTHAINLKVGYSRWPC	473	36.360	14.052	19.238

Table 3. Details of Hentriacontane binding sites with MIOX.

Site No.	Site Volume (Cubic Angstroms)	Residues
1	515	LEU,VAL, ASP, SER
2	330	LYS,GLU
3	234	PRO,PHE,TRP
4.	181	GLU,ALA,VAL,ASP
5	163	GLU,VAL,ASP
6	224	HIS,GLN,VAL,TRP
7	119	GLN,THR,VAL,PHE,PRO
8	152	PRO
9	165	TRP,PHE,VAL
10	149	TRP,PHE,VAL,ILE,ARG

4.Drug likeliness score for Hentriacontane ligand based on Lipinski rule of five

Hentriacontane which had the least e- value was subjected to further analysis and the drug likeliness scores are given in table 4.

Table 4. Drug likeness for the ligand Hentriacontane

Drug (Hentriacontane)	Values obtained	Normal Range
Molecular Weight	372.00	Less than 500 D
Hydrogen Bond Donor	0	Less than 5
Hydrogen Bond Acceptor	0	Less than 10
LogP	0.000	Less than 5
Molar Refractivity	0.000	40-130

5. Properties and bioactivity of Hentriacontane ligand [using "Molinspiration" and "Chemexper"]

The structure of Hentriacontane is shown in fig.3. Its molecular properties, bioactivity and bioproperty values are given in table 5 and its risk analysis values are given in table 6.

CONCLUSIONS

Based on the present bioinformatics analysis we conclude that

1. Totally 17 active sites were present in the Human MIOX.

2. Hentriacontane required a minimum energy for binding with Human MIOX [energy = -178.49 Kcal/ Mol] among the ligands studied.

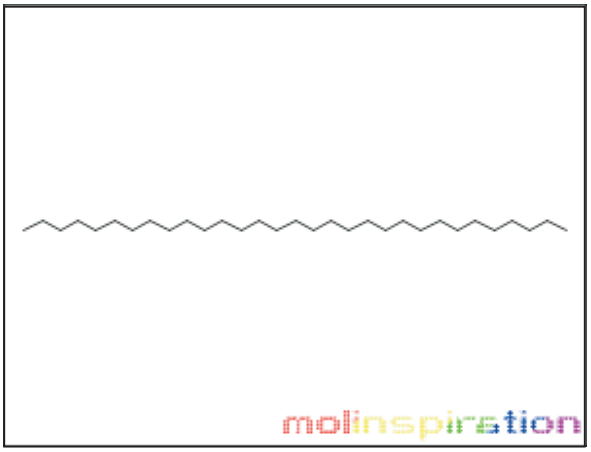

Figure 3. Structure of Hentriacontane[visualized by molinspiration]

Table 5. Physical properties, bioactivities and bioproperties values for Hentria contane

Physical Properties	
miLogP	10.229
TPSA	0.0
Natoms	31
Mol. Wt.	436.853
nON	0
nOHNH	0
Nviolations	1
n rot b	28
Volume	533.009
Bioactivities	
GPCR ligands	0.02
Ion channel modulator	-0.02
Kinase inhibitor	-0.06
Nuclear receptor ligand	0.04
Bioproperties	
Mutagenic property	Nil
Tumorigenic property	Nil
Irritant property	Nil
Reproductive property	Nil

Table 6. Risk analysis for Hentriacontane

S. No.	Properties	values
1	cLogp	14.83
2	solubility	-8.81
3	Mol Wt	436
4	Drug likeness	-20.4
5	Drug score	0.11

However, the efficacy of Hentriacontane needs to be evaluated further.

3. This ligand Hentriacontane satisfies 4/5 parameters of Lipinski rule. So it can act as a drug compound.

4. Totally 10 ligand binding sites were identified on the receptor.

5. Further analysis of the bioproperty and bioactivities by computational tools, showed that Hentriacontane possess moderate risk properties.

REFERENCES

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