Research Article

STUDY OF PLANTGLUCOSYLXANTHONE & ITS ANALOGS, AS DPPIV INHIBITOR FOR ANTI-DIABETIC ACTIVITY

Pavan Kumar K.V.T.S[1]*, Rama koteswararao.Chinta[2], Shriram Raghavan[1], P.M. Murali[1]
[1]Department of Plant Biotechnology, Dalmia Centre for Research & Development, Coimbatore, India.
[2]Department of Chemistry, College of Engineering, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, India.
***Corresponding author's E-mail:** kvtspavan@gmail.com

Accepted on: 18-05-2012; **Finalized on:** 30-06-2012.

ABSTRACT

Diabetes Mellitus, One of the life style disorders, has become common problem in current world scenario and creating health "Tsunami" due to un-availability of cost effective treatment. The role of Dipeptidyl peptidase IV receptor, an anti-diabetic drug target, involved in incretin metabolism has provided an opportunity to counteract type2 diabetes by inhibiting its action on glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Plants are one the major sources for natural products with various therapeutic activities and ancient Indian traditional medicine Ayurveda uses many of these plant ingredients to treat several human ailments. A Xanthone class compound, glucosylxanthone, is one of such natural compound from plant that has various biological activities. In the current effort glucosylxanthone and its analogues are studied for insilico inhibitory activity of DPPIV receptor along with known DPPIV inhibitors that includes molecules in various phases of drug discovery pipeline by using MOLA, tool for Virtual Screening using AutoDock4/Vina in a computer cluster using non-dedicated multi-platform computers. Glucosylxanthone and its analogues have shown good comparable insilico binding activity with FDA approved drugs and other molecules currently under development for DPPIV inhibitory activity as anti-diabetic therapy.

Keywords: Diabetes, Natural compounds, Xanthones, MOLA, Autodock vina, Virtual Screening, DPPIV.

INTRODUCTION

The multifactorial metabolic disorder, Diabetes Mellitus[1], is a chronic condition that occurs due to inability of the body to produce enough or effectively use insulin, has become common disorder. Out of three main types of Diabetes[2], NIDDM - Non Insulin Dependent Diabetes Mellitus occurs primarily due to defects in insulin secretion along with development of insulin resistance. The current available treatments for diabetes with insulin therapy including oral insulin are not cost effective and oral drugs that enhance insulin secretion showed undesirable side effects like hypoglycemia, cardiovascular abnormalities and pancreatic beta-cell apoptosis, failed to address the problem[3]. 5th edition of the Diabetes atlas released by International Diabetes federation (IDF) indicates people living with diabetes are expected to rise from 366 million in 2011 to 552 million in 2030[4]. The deadly disease caused 4.6 million deaths in 2011 with at least USD 465 billion dollars healthcare expenditures in 2011 which is 11% of total health care expenditures in adults (20-79 years), is undoubtedly one of the challenging health problems in 21st century and is going to create a health Tsunami if proper action is not taken.

Population-based diabetes studies shows many people remain undiagnosed due to few symptoms or symptoms may not be recognized related to diabetes. The role of DPP-IV inhibitor (Di Peptidyl Peptidase-IV) in incretin metabolism that prolongs in-vivo half-life of glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) from DPP-IV there by increased insulin gene expression, beta-cell proliferation, islets neo-genesis leading to the proposal that the DPP-IV inhibitors as oral drugs for treatment of Type-2 diabetes[5] and was supported by experimental results in DPP-IV knockout mice, diabetes mice models treated with DPP-IV inhibitors[6,7]. Though companies like Merck, Takeda developed molecules that belong to gliptin class of chemical compounds and several other companies are working on this target (table 1) there is no single molecule from natural source.

Table 1: DPPIV inhibitors - approved & under development

Drug Name	Company	Year of FDA approval
Sitagliptin	Merck	2006
Vildagliptin	Novartis	2008
Saxagliptin	BMS	2009
Alogliptin	Takeda	2010
Linagliptin	Eli Lilly	2011
Dutogliptin	Phenomix	Phase III
Teneligliptin	Mitsubishi Tanabe Pharma Corp	Phase III
SYR 472	Takeda	Phase III
KRP104	Kyorin	Phase II
Melogliptin	Glenmark	Phase II

Plants, one of the major sources of natural products with numerous therapeutic activities shows good efficacy with better safety profile and still remains unexplored to full extent. Varying degree of hypoglycemic and anti-hyperglycemic activity of plants (table 2) and the mechanism of action for these plant based formulations were not clearly understood. One of the world's ancient medicines, Indian traditional medicine Ayurveda uses

several of these plants to treat human ailments[8]. Drug discovery has reached important crossroads and organizations started realizing the importance of the approaches of ancient medicine and started focusing on natural products which have become a major source for lead molecules in current drug discovery efforts.

Figure 1: Plant based glucosylxanthone & its analogues

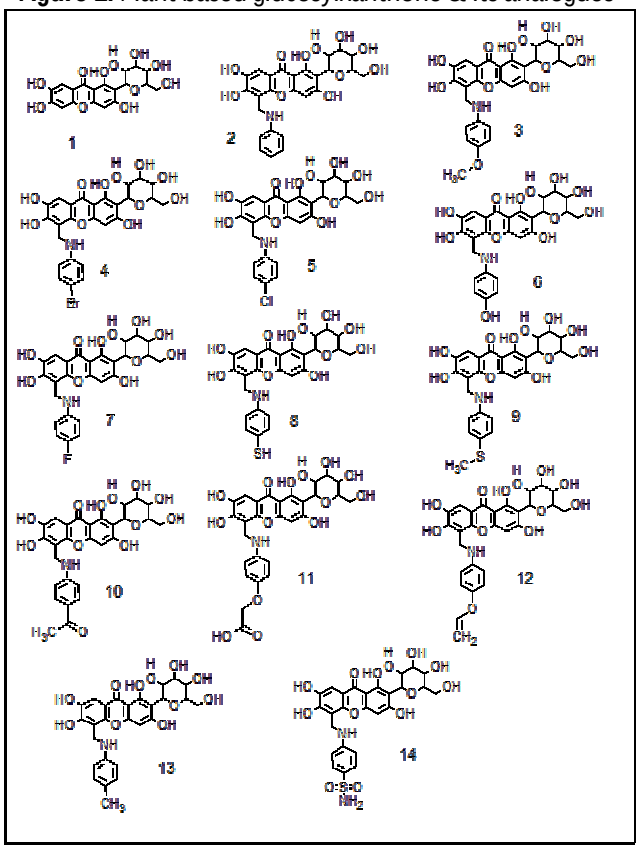

Table 2: Plants with hypoglycaemic activity

Plant Name	Plant Name
Annonasquamosa	*Artemisia pallens*
Areca catechu	*Beta vulgaris*
Boerhaviadiffusa	*Bombaxceiba*
Buteamonosperma	*Camellia sinensis*
Capparis decidua	*Caesalpiniabonducella*
Centellaasiatica	*Coccinia indica*
Emblicaofficinalis	*Eugenia uniflora*
Enicostemalittorale	*Ficusbengalenesis*
Gymnemasylvestre	*Hemidesmusindicus*
Hibiscus rosa-sinesis	*Ipomoea batatas*
Momordicacymbalaria	*Murrayakoenigii*
Musa sapientum	*Phaseolus vulgaris*
Pinuspinaster	*Punicagranatum*
Salaciareticulata	*Salvia miltiorrhiza*
Scopariadulcis	*Swertiachirayita*
Syzygium alternifolium	*Terminaliabelerica*
Terminaliachebula	*Tinosporacrispa*
Vincarosea	*Withaniasomnifera*

Considering these facts as a motivation, the current study is performed to understand the usefulness of DPP-IV inhibitory profile of plant based glucosylxanthone & its analogues (fig.1) using computational biology approach. Glucosylxanthone are major components of rhizomes, leaves, bark of some traditional Indian medicinal plants (table 3) and has shown several therapeutic activities including antioxidant, anti-diabetic, antihypertensive, anti-HIV, anticancer, analgesic, hepatoprotective, immunomodulatory properties[9].

Table 3: Indian Medicinal plants Producing Glucosylxanthone

Plant Name	Plant Name
Anemarrenaasphodeloides	*Arrabidaeasamydoides*
Bombaxmalabaricum	*Cratoxylumcochinchinense*
Cyclopiagenistoides	*Cyclopiaintermedia*
Cyclopiamaculata	*Cyclopiasessiliflora*
Cyclopiasubternata	*Gentianalutea*
Gnidiainvolucrata	*Hypericumperforatum*
Hypericumsampsonii	*Mangifera indica*
Salaciachinensis	*Salaciareticulata*
Swertiacorymbosa	*Swertiafranchetiana*
Swertiapunctata	

MATERIALS AND METHODS

Ligands preparation

ACD labs chemistry drawing tool Chemsketch version 12.01[10] was used to draw FDA approved DPPIV inhibitory drugs, DPPIV inhibitor molecules currently under development, Glucosylxanthone and its analogues. These molecules were converted to Structure data format files[11] using SDF plugin tool for Chemsketch. The Structure data files were converted to protein databank files[12] having 3D coordinates with open source tolls Balloon[13]& Open Babel applications[14] and energy minimized using MMF94 force field[15].

Protein preparation

Structure of DPPIV submitted by Kim D et al., to protein databank (PDB Code: 1X70) was obtained and employed as a virtual target. Auto Dock Tools[17] (ADT) was employed to select and separate the bound ligand file from target file initially downloaded from Protein Data Bank and each structure was restored as separate PDB files. Auto dock tools protein preparation wizard was used to prepare protein file and resulting structure was saved as a PDBQT file to use in docking studies. Grid maps were generated for all possible atom types (29 atom types - A BR Br C CA Ca CL Cl F FE Fe H HD HS I MG Mg MN Mn N NA NS OA OS P S SA ZN Zn) as we are using many compounds. The prepared protein-ligand complex of 1X70 was employed to build energy grids using the default value of protein atom scaling (1.0) within a cubic box of dimensions 30 Å X 30 Å X 30 Å centred around the centroid of the bound ligand pose. The bounding box dimensions were set to 14 Å X 14 Å X14 Å. No constraints were imposed on any of the active site protein atoms vis-à-vis their interactions with ligand atoms.

Docking Studies

In order to understand the binding affinity and interactions between protein and ligand molecules an easy-to-use graphical user interface tool, MOLA[18], which automates parallel virtual screening using AutoDock4[19]

and/or Vina[20] in bootable non-dedicated computer clusters was used. Several tasks needed for AutoDock4/Vina are automated with MOLA that includes ligand preparation, AutoDock4/Vina jobs distribution, result analysis and ligand ranking. To select the parameters for docking study using Autodock Vina and for handling output files A Graphical User Interface (GUI) provided by MOLA is used. UCSF Chimera version 1.5.3[21] was used to study and to generate images of the molecular interactions between docked protein and ligands molecules.

The molecular docking results of marketed drugs and glucosylxanthone analogues were presented in table 4. MOLA tool rank orders each docked ligand based on predicted binding energy thereby provides results in CSV format, ligand poses in PDBQ file. Ligand poses for best runs were obtained from output PDBQT file and separated out for protein ligand interaction study.

Table 4: Molecular docking results of DPPIV inhibitors

S. No	Molecule	Calculated ΔG Kcal/mole
1	gluxanth3D_1_1	-10.7
2	gluxanth3D_1_9	-10.6
3	gluxanth3D_1_10	-10.5
4	gluxanth3D_1_12	-10.5
5	gluxanth3D_1_2	-10.5
6	gluxanth3D_1_5	-10.5
7	gluxanth3D_1_11	-10.4
8	gluxanth3D_1_4	-10.4
9	gluxanth3D_1_8	-10.4
10	gluxanth3D_1_6	-10.3
11	gluxanth3D_1_7	-10.3
12	gluxanth3D_1_3	-10.2
13	gluxanth3D_1_13	-10.1
14	Linagliptin	-9.6
15	Sitagliptin	-8.7
16	ABT-297	-8.2
17	gluxanth3D_1_14	-8.2
18	Carmegliptin	-7.6
19	Denagliptin	-7.4
20	Saxagliptin	-7.3
21	NVPDPP728	-7.1
22	1X70_ligand	-7.0
23	Vildagliptin	-7.0
24	Alogliptin	-6.9

RESULTS AND DISCUSSION

Problems associated with drug resistance, undesirable side effects and emerging diseases making pharmaceutical scientists life difficult in identifying new lead molecules and novel scaffolds. Increasing adverse effects of synthetic drugs renewed the interest of scientific community towards natural product based drug discovery. The world Health Organization recognized the significance of traditional medicine and started working on providing guidelines for plant based medicines.[22]

Combining the knowledge base of traditional medicine with modern scientific methods provides better insights to the drug discovery efforts. Computational chemistry tools were well established in modern drug discovery that contributes towards activity profiling with better strategies, mode of action and in depth details on binding properties to select true positives in the lead screening process.

The major objective for anti-diabetic therapy is to reduce or control glycosylated haemoglobin (HbA_{1C}) levels in order to minimize complications associated with this disease such as retinopathy, nephropathy and neuropathy. Though some of the treatments able to address glycaemic control due to weight gain and increased risk of hypoglycaemia, Maintaining glycosylated haemoglobin (HbA_{1C}) levels <7% as per American Diabetic Association (ADA) guidelines is becoming difficult despite various treatments that are currently available emphasis on the need for better therapy to counteract diabetes that is prevailing like an iceberg.

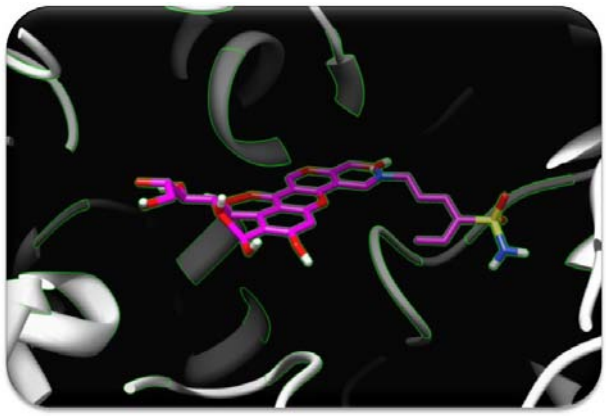

Figure 2: Docking pose for Gluxanth3D_1_1 in active site cavity

To address the problem various novel approaches were put forth by the scientists and one of such approaches is Dipeptidyl peptidase inhibitors which can be considered as second or third line of viable option in type 2 diabetes management. Inhibition of DPPIV would presumably increases serum Glucagon like Peptide-1 (GLP-1) that results in net anti hyperglycaemic effect. Animal studies conducted to evaluate the impact of DPPIV inhibition demonstrated improved glucose tolerance and enhance insulin secretion in Zucker diabetic fatty rats. Hence competitive, reversible inhibition of DPPIV would be one of the viable strategies to fight against type 2 diabetes.[23]

Xanthones represents a group of secondary metabolites normally found in higher plants, Fungi and lichens. Glycosylated xanthones were predominant from Gentianaceae and Polygalaceae families. These molecules represent a huge family of chemical compounds with wide diversity of biological and pharmacological properties. As natural products and traditional medicines are evolving as attractive options for drug discovery, traditional Indian medicines like Ayurveda etc. can show better approach & innovative strategies to drug

development process. The advent of using computational methodologies and tools to the emerging challenges combined with natural products as a source would certainly provide better results and the current approach is outcome of this. The study reveals that glucosylxanthone and its analogues have shown very good comparable *insilico* binding activity with FDA approved drugs and other molecules currently under development for DPPIV inhibitory activity. Binding poses of Auto dock Vina results shows that xanthone compounds interacts with backbone of GLU205, PHE 357 and ARG367 and is falling in to the definition of lead compound[24] proposed by opera *et al*.

CONCLUSION

Further optimization of these analogues can serve as good lead compounds in the development of new DPPIV inhibitors and the study presented in this article is an initial effort of the further study in development of Glucosylxanthone & its analogues as DPPIV inhibitors for anti -diabetes therapy.

Notes

† Electronic Supplementary Information (ESI) available:

1. ligands files used in this study

2. Protein file used in the study

3. Docking log files

REFERENCES

1 Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: Report of a WHO/IDF Consultation, World Health Organization, 2006, ISBN 92 4 1594934.

2 Harris M, Zimmet P. In Alberti K, Zimmet P, Defronzo R, editors. International Textbook of Diabetes Mellitus. Second Edition. Chichester: John Wiley and Sons Ltd; 1997, 9-23.

3 Cassia S Mizuno, Amar G Chittiboyina, Theodore W Kurtz, Harrihar A Pershadsingh, Mitchell A Avery,Type 2 diabetes and oral antihyperglycemic drugs, Current Medicinal Chemistry 2008, 15(1), 61-74.

4 IDF Diabetes Atlas, Fifth Edition, 14th November 2011.

5 Paul E. Wiedeman, DPPIV Inhibition: Promising Therapy for the Treatment of Type 2 Diabetes,Progress in Medicinal Chemistry, Edited by F.D. King and G. Lawton, 45, 2007, 63–109.

6 Marguet, D., Baggio, L., Kobayashi, T., Bernard, A.-M., Pierres, M., Nielsen, P.F.,Ribel, U., Watanabe, T., Drucker, D.J. and Wagtmann, N., Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26, Proc. Natl. Acad. Sci.U.S.A. 97, 2000, 6874–6879.

7 Nagakura, T., Yasuda, N., Yamazaki, K., Ikuta, H., Yoshikawa, S., Asano, O. and Tanaka, I.,Improved Glucose Tolerance via Enhanced Glucose-Dependent Insulin Secretion in Dipeptidyl Peptidase IV-Deficient Fischer RatsBiochem. Biophys. Res. Commun. 284, 2001, 501–506

8 Bhushan Patwardhan, Ashok D. B. Vaidya and Mukund Chorghade, Ayurveda and natural products drug discovery, current science, 86(6), 2004, 789-799.

9 Scartezzini, P. and Speroni, Review on some plants of Indian traditional medicine with antioxidant activityE. J. Ethnopharmacol. 71, 2000, 23–33.

10 ACD/ChemSketch Freeware, version 12.01, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2012.

11 Arthur Dalby, James G. Nourse, W. Douglas Hounshell, Ann K. I. Gushurst, David L. Grier, Burton A. Leland, John Laufer,Description of several chemical structure file formats used by computer programs developed at Molecular Design Limited J. Chem. Inf. Comput. Sci., 32 (3), 1992, 244–255, DOI: 10.1021/ci00007a012.

12 F.C.Bernstein, T.F.Koetzle, G.J.Williams, E.E.Meyer Jr., M.D.Brice, J.R.Rodgers, O.Kennard, T.Shimanouchi, M.Tasumi, The Protein Data Bank: a computer-based archival file for macromolecular structures, J. of. Mol. Biol., 112, 1977, 535.

13 Mikko J. Vainio and Mark S. Johnson, Generating Conformer Ensembles Using a Multiobjective Genetic Algorithm, Journal of Chemical Information and Modeling, 47, 2007, 2462 – 2474.

14 Noel M O'Boyle, Michael Banck, Craig A James,Chris Morley, Tim Vandermeersch and Geoffrey R Hutchison, Open Babel: An open chemical toolbox, Journal of Cheminformatics 3, 2011, 33, doi:10.1186/1758-2946-3-33.

15 Thomas A. Halgren, Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94, J. Comp. Chem.; 1996; 490-519.

16 Kim D, Wang L, Beconi M, Eiermann GJ, Fisher MH, He H, Hickey GJ, Kowalchick JE, Leiting B, Lyons K, Marsilio F, McCann ME, Patel RA, Petrov A, Scapin G, Patel SB, Roy RS, Wu JK, Wyvratt MJ, Zhang BB, Zhu L, Thornberry NA, Weber AE., (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes, J Med Chem. 48(1), 2005 Jan 13, 141-51.

17 Michel F. Sanner, *Python:* A Programming Language for Software Integration and Development, J. Mol. Graphics Mod., 17, 1999, 57-61.

18 Rui MV Abreu, Hugo JC Froufe, Maria JRP Queiroz and Isabel CFR Ferreira, MOLA: a bootable, self-configuring system for virtual screening using AutoDock4/Vina on computer clusters, Journal of Cheminformatics 2, 2010, 10.

19 Morris, G. M., Goodsell, D. S., Halliday, R.S., Huey, R., Hart, W. E., Belew, R. K. and Olson, A. J., Automated Docking Using a LamarckianGenetic Algorithm and and Empirical Binding Free Energy Function, J. Computational Chemistry, 19, 1998, 1639-1662.

20 O. Trott, A. J. Olson,AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading Journal of Computational Chemistry 31, 2010, 455-461.

21 Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE., UCSF Chimera--a visualization

system for exploratory research and analysis,J Comput Chem. 25(13), 2004 Oc, 1605-12.

22 Traditional Medicine Strategy 2002–2005, WHO, Geneva, 2002.

23 Sudre B, Broqua P, White RB:, Chronic Inhibition of Circulating DipeptidylPeptidaseIV by FE 999011 Delays the Occurrence of Diabetes in Male Zucker Diabetic Fatty Rats, Diabetes 51, 2002, 1461– 1469.

24 Tudor I. Oprea, Andrew M. Davis, Simon J. Teague, and Paul D. Leeson, Is There a Difference between Leads and Drugs? A Historical Perspective J. Chem. Inf. Comput. Sci., 41(5), 2001, 1308–1315.

About Corresponding Author: Mr. K.V.T.S. Pavan Kumar

K.V.T.S. Pavan Kumar obtained his Masters in biochemistry. He specializes in Bio-informatics, Trascriptome profiling for characterization of novel genes and *in silico*-biology. Currently working as a researcher in the field of Plant based natural products with Dalmia Centre for Research and Development, Coimbatore.