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Virtual Screening Based Synthesis of Analogs of Quercetin As Insulin Receptor Activators And In-Vivo Comparative Evaluation For Anti-Diabetic Activity

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ABSTRACT

Literature reveals that quercetin shows hypoglycaemic active by more than one way. In present work virtual docking study of quercetinand its probable analogs was carried out on insulin receptor PDB ID:1IR3by using biopredicta module of Vlife MDS 4.3.In results of virtual screening it was found that 2-(3, 4 dihydroxy phenyl)-3,5,7-trihydroxy-4H chromen-4-one(quercetin) and its probable analogs 3-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (analog 1), 3,7-dihydroxy-2-(3-methoxyphenyl)-4H-chromen-4-one(analog5)and 3-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4- one(analog7) showed promising results and hence were synthesized. The results of in- vivo screening confirm the results of virtual screening as analogs 1, 5 and 7 have shown better activity hypoglycamic activity than quercetin.

Keywords: Quercetine, synthetic analogs, Diabetes mellitus, Virtual screening

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INTRODUCTION

Diabetes mellitus, is a chronic disorder involving endocrine system and is related to metabolism of carbohydrate, proteins and fats due to absolute or relative deficiency of insulin secretion with or without varying degree of insulin resistance. Itis also defined as a disease where the body produce less insulin or become progressively resistance to its actions.(1)

World-wide occurrence and incidences of both type 1 and type 2 diabetes is on rise. It is estimated that, in the year 2000,171 million people had diabetes, and this is expected to double by 2030.

Currently in India the number of people with diabetes is around, 40.9 million and it is expected to rise to 60.9 million by 2025. India has emerged as the diabetic capital of the world. . (Joshi Unless urgent preventive steps are taken, it will become a major problem.(1,2)

Use of bioactives from natural sources as starting material in the drug development program is associated with few specific advantages such as mostly; the selection of a herb or plant species for investigations is done on the basis of long-term use by humans (ethnomedicine). This approach is based on an assumption that the active compounds isolated from such plants are likely to be safer. Natural resources as starting point has a bilateral promise of delivering the original isolated species or a semi-synthetic molecule development to overcome any inherent limitations of original molecule.(3)

Since ancient times natural compounds from various plants and several dietary constituents are in practice some of which have been reported to possess pronounced anti-diabetic properties. Different countries (India, America, and China) all over the world have been reported to use these plant based components for prevention or management of diabetes. These active ingredients from herbal sources not only possess therapeutic values, *i.e.* hypoglycaemic activity, antioxidant property but also can combat hyporglycaemic episode and other health problems of diabetes in a safe way. An effective control of blood sugar level is the fundamental step for prevention or reversing diabetic complications in patients of both type 1 and type 2, therefore improving the quality of life. Thus management of diabetes with traditional insulin therapy or synthetic oral hypoglycaemic drugs (OHDs) administration may result in serious side effects and sometimes fails to prevent typical diabetes-related health complications in many individuals. Thus the focus has now been shifted towards herbal remedies as a better alternative approach for effectively fighting diabetes. It should produce minimal or no side effects in clinical practice with long term cost effectiveness, compared to synthetic OHDs.(3,4)

With numerous pharmacological and biological functions bio-flavonoids have gained appreciable attention in diabetes and other therapeutic research. Among several beneficial flavonoids, quercetin exhibits impressive hypoglycaemic effects, with significant improvement, stabilization of long

sustaining insulin secretion and regeneration of human islets in the pancreas without producing serious health hazards.

Molecular docking study is now a days used as tool to identify exact mechanism of action of isolated bioactive. As docking interactions provide information about proper interaction between bioactive ligand and target, the semisynthetic modification can be brought in the structure of natural bioactive to improve its action.[5,6,7]The present work was designed to study interaction of naturally isolated quercetin on insulin receptor PDB ID:1IR3. On the basis of docking study semisynthetic analogs were synthesized and in-vivo study of comparative anti-diabetic was carried out using Albino Rat.

MATERIALS AND METHOD:

All the chemicals required for extraction of quercetine and synthesis of its analogs were purchased from Loba Chemi Pvt. Ltd. Mumbai.

The experimental animals were procured from Global BioResearch Solutions Pvt. Ltd., Pune (Reg. No. 1899/PO/Bt/S/16/CPCSEA)

Virtual Screening of Quercetine and Its Probable Analogs By Docking Study

Docking of molecules

Comparative docking study for quercetine and its analogs was carried out on Insulin receptor using Biopredicta module of Vlife MDS 4.3. PDB ID:1IR3 of insulin receptor was for docking study**.**

Synthesis of ANalogs

General Procedure for synthesis of quercetin analogs

- For synthesis of various analogs, 20 ml solution of sodium hydroxide having concentration range between 20 to 60%was placed in 50 ml RBF along with 10ml of rectified spirit. The flask was placed ice bath. The assembly was placed on magnetic stirrer.
- 5 ml of freshly distilled acetophenone having various substituents was added as reactant for synthesis of analogs to the flask as per the requirement.
- With stirring on magnetic stirrer at slow speed, addition of 4ml of pure benzaldehyde was done.
- The temperature of mixture was mentioned at about 25^0C and stirring was continued till the mixture became too thick so that stirring is no longer effective (2-8 hours).
- The stirrer was remove and the reaction mixture was kept in an ice bath or refrigerator overnight.

- The product was filtered and washed with cold water until washing was neutral to litmus. Further washing was done with 20ml of ice cold rectified spirit to get crud analogs.
- Recrystallization of crud analogs was carried out using rectified spirit.

Determination of Anti-diabetic Activity

In-vivo screening of quercetin and its semi synthetic analogs for Anti-diabetic Activity

Ten healthy Wistar Rat weighing between 120-150 gm were taken and fasted for 12 Hrs. before experimental work. They were divided. Alloxan Monohydrate was used to induce diabetes. Induction of diabetes was confirmed on the basis of blood sugar level. Oral administration of quercetin as standard and its synthetic analogs was done in a dose calculated as 75mg/kg body weight. The blood sugar level was checked by withdrawing of blood sample at 3, 7, 9 ,12 and 15 days interval.[8,9,10]

RESULTS AND DISCUSSION

Virtual Screening Study

The docking interactions shown by quercetine and its probable analogs done on Insulin Receptor (PDB ID - 1IR3) using Biopredicta module of Vlife MDS 4.3 are mentioned in table 2.

Table 1 Interaction with Insulin Receptors

Sr. No.	Name of Molecule	Energy	Hydrogen bonding interaction	Distance	Vanderwaal's interaction	Distance
1	3-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	961.1414	LYS1085A LYS1085A	1.911 2.022	VAL1010A ASP1083A GLY1005A GLY1003A	2.420 2.505 2.645 2.748
2	3-hydroxy-2-(3-methoxyphenyl)-4H-chromen-4- one	86.8117	LYS1030A	2.473	VAL1010A VAL1010A ASP1150A ASP1150A	2.626 2.706 2.526 2.739
3	2-(4-hydroxyphenyl)-3-methyl-4H-chromen-4one	45.2894	GLU1047A	1.464	GLY1003A GLY1003A	2.495 2.433
4	3,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4- one	54.3154	GLU1047A ASP1150A	2.129 2.239	GLY1003A GLY1005A	2.606 2.771
5	3,7-dihydroxy-2-(3-methoxyphenyl)-4H-chromen-4- one	74.1848	ASP1150A	1.933	VAL1010A VAL1010A	2.547 2.712
6	7-hydroxy-2-(4-hydroxyphenyl)- 3-methyl-4H-chromen-4-one	41.1678	LEU1002A ASP1150A	2.146 1.661	GLY1005A GLY1010A	2.517 2.778

7	3-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4- one	-8.1664	SER1006A SER1006A SER1006A	1.709 2.489 2.044	GLY1003A GLY1003A VAL1010A VAL1010A	2.631 2.828 2.537 2.887
8	3-hydroxy-7-methoxy-2-(3-methoxyphenyl)-4H-chromen-4-one	-9.5981	ASP1180A	1.654	TRP388A TRP388A	3.635 4.149
9	2-(4-hydroxyphenyl)-7- methoxy-3-methyl-4H- chromen-4one	-32.3050	LYS1030A LYS1030A	2.575 2.556	GLY1005A GLY1005A	2.875 2.440

On the basis of docking results analogs 1, 2 and 7 show better interaction with insulin receptor.

Docking interaction:

Representative docking interactions are shown in figure. 1 and 2

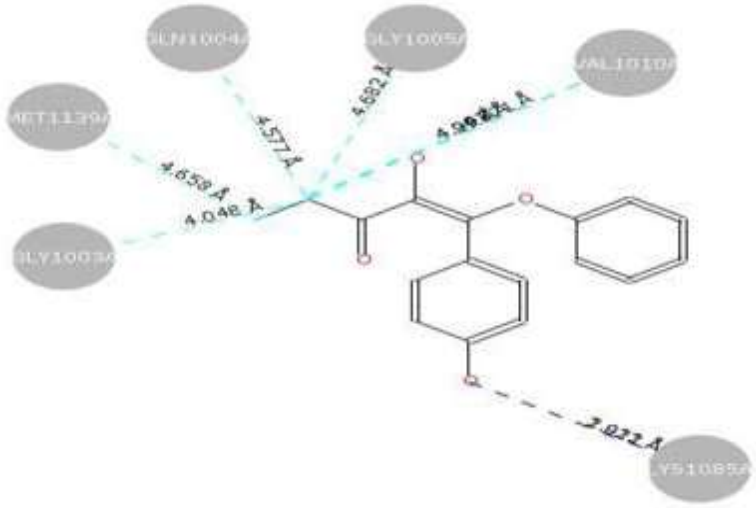


Figure. 1: Interactions shown by 3-hydroxy-2-(4-hydroxyphenyl)-4Hchromen- 4-one (analog-1) With Insulin Receptor

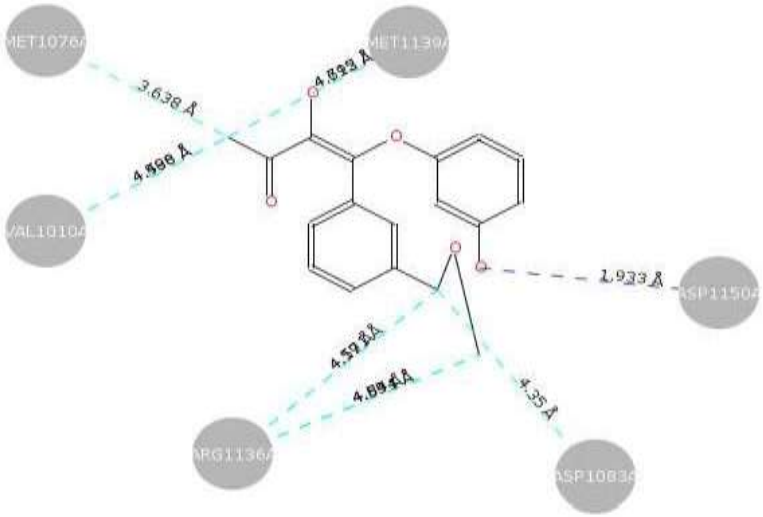
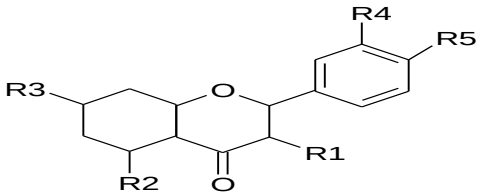


Figure 2: Interactions shown by 3-hydroxy-2-(4-hydroxyphenyl)-7- methoxy-4H-chromen-4-one

Synthesis of Quercetine Analogs:

Results of synthesis of quercetine analogs along with quercetine are presented in table no.3



General Structure

Table 3: Quercetine and its analogs

Sr. No.	Quercetine & analogs	R_1	R_2	R_3	R_4	R_5
	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one (Quercetine)	OH	OH	OH	OH	OH
Analogs						
1	3-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	OH	H	H	H	OH
2	3-hydroxy-2-(3- methoxyphenyl)-4H-chromen-4- one	OH	H	H	H	OCH_3
3	2-(4-hydroxyphenyl)-3-methyl-4H-chromen-4one	CH_3	H	H	H	OH
4	3,7-dihydroxy-2-(4- hydroxyphenyl)-4H- chromen-4- one	OH	H	OH	H	OH
5	3,7-dihydroxy-2-(3-methoxyphenyl)-4H- chromen-4- one	OH	H	OH	OCH_3	H
6	7-hydroxy-2-(4-hydroxyphenyl)-3-methyl-4H-chromen-4-one	CH_3	H	OH	H	OH
7	3-hydroxy-2-(4- hydroxyphenyl)-7-methoxy-4H-chromen-4- one	OH	H	OCH_3	H	OH
8	3-hydroxy-7-methoxy-2-(3-methoxyphenyl)-4H-chromen-4-one	OH	H	OCH_3	OCH_3	H
9	2-(4-hydroxyphenyl)-7- methoxy-3-methyl-4H- chromen-4one	CH_3	H	H	OCH_3	OH
8	3-Hydroxy-7-methoxy-2-(3-methoxy phenyl)-4-H-chromen-4-one	OH	H	OCH_3	OCH_3	H
9	2-(4-hydroxy phenyl)-7-methoxy-3-methyl-4-H-chromen-4-one	CH_3	H	OCH_3	H	OH

Antidiabetic effect

Induction of diabetes in rats was done by using single dose of 5% alloxan monohydrate (125 mg/kg, i.p.) after 24 h fasting. Induction of diabetes was confirmed after a week of alloxan treatment by estimation of fasting blood glucose level. Only those rats with blood glucose level between 200–300 mg/dl were included in the study. These rats were further divided into seven groups into 5 groups as three for testing analogs, one group as standard for giving standard quercetine and one group as positive control. Dose of 75mg/Kg body weight of quercetin and three analogs 1,2 and 7 was administered once daily for 4 weeks. The blood glucose levels

were measured by glucometer on day 0, 3,7,9,12 and 15. For testing blood sugar level the blood samples were obtained from tail vein puncture and blood glucose levels were analyzed.

Results of in-vivo screening of analogs along with quercetine for hypoglycaemic activity are shown in Table 4

Table 4: Results comparative hypoglycaemic activity shown by quercetine and its analogs

Group	Normal Blood Glucose(mg/dl)	Diabetic Blood Glucose(mg/dl)	Blood Glucose(mg/dl)				
			Day 3	Day 7	Day 9	Day 12	Day15
Standard (Quercetine)	66	276	259	246	221	211	202
Rat – 1	72	315	301	290	271	249	232
Rat – 2							
Control						75	
Rat – 1	75	------	81	79	74	75	76
Rat – 2	64	------	69	72	73		74
Test (analog 1)							
Rat – 1	82	277	256	238	210	195	182
Rat – 2	70	259	240	225	199	181	169
Test (analog 5)							
Rat – 1	68	273	261	239	212	195	179
Rat – 2	83	295	278	267	235	210	192
Test (analog 7)							
Rat – 1	79	269	251	232	205	190	180
Rat – 2	67	246	238	222	195	170	158

DISCUSSION

Optimization of the % solution of sodium hydroxide had to be done individually for synthesis of various analogs. Depending upon substitutions on acetophenone the time required for completion of reaction varied. Synthesis of analogs that showed good virtual screening results were only synthesized and used for comparative hypoglycaemic activity.

On the basis of results of virtual screening and hypoglycaemic activity the purpose of selective synthesis of analogs is justified and analogs have shown better activity as expected and proposed.

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