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Multidocking Studies on 2-Phenyl-4h-Chromen-4-One Hybrid and Evaluation of Anti-Diabetic Activity

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Abstract

The aim of the study was synthesis of ten hybridised flavones and evaluated for anti-diabetic activity. Those compounds were subjected for *insilico* studies on multi targeted enzymes such as aldose reductase, protein tyrosine phosphatase and alpha amylase for prediction of their biological activity. Based on that result, the titled compounds were revealed for *in-vitro* and *in-vivo* anti-diabetic activity. The compound HF_d possess high significant ($p < 0.01$) results and restored the blood glucose level, liver enzymes and renal parameters. Based on docking result, the compound HF_d with 2, 4-dimethoxy group on ring C and 7-hydroxy substitution on ring A showed binding interactions with amino acid residues of alpha amylase as Arg 61, Pro 44, His 299, Gln 41 & Asp 96. Hence this article summarised that these scaffolds were acts as a navigator in the management of diabetic mellitus.

Keywords: Anti-diabetic Activity; Aldose reductase; Alpha amylase; Docking Study; Protein Tyrosine Phosphatase 1B.

INTRODUCTION

Diabetic mellitus is a metabolic disorder, which is characterised by improper secretion or utilisation of insulin, results in hyperglycaemia [1]. As per WHO report, diabetic mellitus is a one of the leading cause of death in 2030 [2]. According to the current scenario, the development of hypoglycemic drugs in the management of diabetes, as well as in the prevention of diabetic complication should be a challenging one in clinical importance. Digestion of dietary carbohydrate to maltose and glucose by intestinal enzymes such as pancreatic amylase, alpha glucosidase which result in increased level of post prandial glucose in blood. Inhibition of these enzymes will lead to suppression of carbohydrate digestion [3]. Hence these enzymes are considered as target for developing the newer and potent anti-diabetic agent. There are many herbal extracts having reported anti-diabetic potentials [4]. Among these phytochemicals, flavonoids and their related natural compounds are known to possess anti-diabetic activity, established in various animal models [5]. Flavonoids are the most common polyphenolic compounds used as medicaments for diabetes mellitus since ancient times and in that some of the classes of flavonoids may exhibit excellent alpha-glucosidase and alpha-amylase inhibitory properties [6, 7].

From the above facts, the attempt was made to develop novel derivatives of flavones scaffolds. Besides, literature survey revealed that the flavones derivatives posses antioxidant, anti-inflammatory and anti-diabetic activities. So the present study motivated towards the synthesis of flavones. Furthermore the flavones derivatives were docked with digestive enzymes using Autodock tool. Based on the result of Molecular docking with those enzymes these hybridized flavones were evaluated for *in-vitro* and *in-vivo* anti-diabetic activity. By which blood glucose level, liver and renal parameters were evaluated and compared with standard drug on treated rats. Hence the present study deals on development of potent drug in the management of diabetes mellitus.

MATERIAL AND METHODS

Chemical and Reagents

Substituted acetophenones, aromatic aldehydes and streptozotocin (STZ) were purchased from SRL Pvt. Ltd, Mumbai, Hi-media Pvt. Ltd, Mumbai and Loba chemicals, Cochin. The solvents and other reagents and kits were purchased commercially and were of analytical grade. The silica gel plates were used for monitoring the reaction progress obtained from Merck and by using hexane and ethyl acetate (4:1) as mobile phase. The melting point of the titled compounds was determined by open capillary method by using Sona melting point apparatus. FTIR were recorded on Schimadzu Fourier Transform Infrared Spectrophotometer in the range of 4000 cm^{-1} – 400 cm^{-1} using KBr pellet technique. Mass fragmentation for the hybridised compounds was recorded using JEOL GC mate (GC-MS) spectrometer. Proton NMR Spectra were recorded using BRUKER Advance III - 500 MHz FT NMR Spectrometer using the solvent DMSO. Chemical shifts were recorded in parts per million and Trimethylsilane used as an internal standard. The Mass and ^1H NMR spectrum of synthesised compounds were carried out from Indian institute of Technology, Chennai.

Experimental Methods

Firstly, chalcones were prepared by condensing an equimolar mixture of substituted acetophenone and aromatic aldehyde in presence of strong base under warm condition. Secondly, titled compound F_a-F_e & HF_a-HF_e were synthesised by cyclising chalcones using oxidising agent hydrogen peroxide in presence of strong base under the temperature below 50 - 70°C [8-10]. The scheme of synthesis and their substitution pattern were mentioned on Fig.1. and Table.1.

Molecular Docking Studies [11]

AutoDock 4.2 was used to identify the binding modes of titled compounds responsible for the activity on the receptor sites. The ligands were drawn using Chem Sketch software. The mol form of each ligand was converted to PDB format by using Open Babel, prior to the submission for the docking. The preparation of receptor was processed through downloading the crystal structure of enzymes such as Aldose reductase (PDB: 3m4h), Protein Tyrosine Phosphatase 1B (PDB: 1een) and α –Amylase (PDB: 3ole) from Protein Data Bank (http://www. rcsb.org/pdb). The pdb file was imported to Accelrys studio viewer where receptor preparation module was used to prepare the protein. All the bound water molecules and hetero atom were removed from the complex, both polar and non-polar hydrogens were added and 3D structure of protein was corrected. Ten conformations were generated for each ligand. For the discussion of active compounds, the top-ranked conformation was considered.

Evaluation of Alpha Amylase Inhibitory Assay [12]

The evaluation of alpha amylase inhibitory activity of synthesised flavones (F_a-F_e & HF_a-HF_e) and the standard drug (Acarbose) in the concentration of 10, 20, 40, 80 and 160 µg/ml respectively were prepared. From which, 0.5 ml were mixed with 1 ml of pancreatic alpha amylase enzyme (0.5 mg/ml) and incubated for 10 min at 25ºC. The preincubated 1 % starch solution (0.5 ml) in 0.02 M sodium phosphate buffer with sodium chloride (pH 6.9) were added in each tube and incubated for 10 min at 25ºC. The

reaction was retarded by adding a 1 ml of DNSA coloring reagent [3,5-dinitro salicylic acid] [13], incubated for 7 min followed by the addition of 1 ml of sodium potassium tartarate (18.2 % w/v) and finally the reaction mixture were further diluted to 10 ml with distilled water and the absorbance were measured at 540 nm against blank. All the *in-vitro* methods were done by triplicate and percentage inhibitions of alpha amylase were calculated by the following formula.

% Inhibition = $A_c - A_t / A_c \times 100$; Where A_t = Absorbance of Test, A_c = Absorbance of Control

Determination on Anti-Diabetic Activity by STZ induced method

Animals

Male albino Wister rats (150 – 200 g body weight) were obtained from College of Veterinary and Animal Sciences, Thrissur, India and maintained under a constant 12 Hr light and dark cycle at 21 – 23 °C. The animals were maintained in accordance with the guidelines of the National Institute of Nutrition, Hyderabad, India. The study was approved by the Institutional Ethics Committee Reg.no: 688/PO/Re/S/02/CPCSEA. Throughout the experimental period, all four groups of animals were fed with a normal laboratory chow standard pellet diet (Sai feeds, Bangalore, India) and water *ad libitum*.

Induction of Diabetes

In male wister rats, hyperglycemia were induced by administration of freshly prepared streptozotocin (STZ) at the concentration of 60 mg/kg bodyweight [14], i.p. in 0.1 mol/L cold citrate buffer, pH 4.5 to the fasted rats. The STZ-treated animals were allowed to drink 5 % glucose solution overnight to overcome drug-induced hypoglycemia. Rats having persistent glycosuria and hyperglycaemia with a fasting blood glucose >250 mg/dL on the third day after the STZ injection were considered as diabetic and use for further experimentation.

Experimental design

Animals were divided into 8 groups each consisting of a minimum of five animals, Group I-control rats received 0.1 mol/L citrate buffer (pH 4.5); Group II-diabetic control; Group III-VII diabetic rats were administered with 40 µg/ml of synthesized compounds (HF_a-HF_e) for 21 days. Group VIII, diabetic rats were administered 5 mg/kg glibenclamide solution orally per day for 21 days. The dose of the compounds was selected on the basis of previous literatures and *in-vitro* study of alpha amylase inhibitory assay.

2.7 ***Biochemical Estimations***

Blood was collected from the tail vein of the overnight fasting rat at 0^{th} (before the start of the experiment), 4^{th} day, 7^{th} day, 14^{th} day and 21^{st} day. The glucose levels were estimated by using Accu-Check Active glucometer. Weight of individual animals was measured gravimetrically on 0^{th} and 21^{st} days of the experiment. After the experimental regimen, the blood was collected through the retro-orbital puncture of eye of animals under mild diethyl ether anaesthesia in Eppendorff's tube (1 ml) containing 50 µl of anticoagulant (10 % trisodium citrate) and serum was separated by Centrifugation at 3000 rpm for 15 min. The biochemical parameters of liver such as SGPT, SGOT, SALP and Serum bilirubin were determined by using the Commercial kit available [15] (Ecoline, manufactured by Merck specialities, private Limited, Ambernath) and renal parameters such as Protein [16], creatinine [17] and serum urea [18] were measured the values using auto analyzer.

Statistical analysis

Data obtained from pharmacological experiments, are expressed as mean ± SD. Differences between control and treated groups were tested for significance using ANOVA followed by Dunnett's t-test, with $P < 0.05$ were considered as significant.

RESULT AND DISCUSSION

Chemistry

The targeted compounds were synthesised as per the protocol described in scheme.1. All the hybridised flavones were posses their percentage yield in the range of 47–70 % w/w. Those compounds afforded their melting point in the range of 178 – 199 ºC. The R_f values of the compounds were observed which confirmed their purity and its reaction progress by showed one spot on the TLC plate from their base line. Further all the titled compounds were characterised by various UV, FTIR, GCMS, H^1NMR spectroscopic techniques. All the compounds produced their maximum absorbance in the range of 230 – 358 nm. In FTIR, the compounds showed their presence of sharp band at 1789 – 1705 cm^{-1} (CO stretching) for the confirmation of lactone ring or coumarin ring. The new stretching vibration appeared at 1145 – 1065 cm^{-1} (C-O-C Str), which confirms the formation of benzopyrone ring for all compounds. The presence of hydroxyl group (OH Str) in the region between 3682 – 3510 cm^{-1} were confirmed the compounds (HF_a-HF_e). Along with these, the distinctive peaks in the region 1510 - 1460 cm^{-1} (aromatic C=C), 748 – 773 cm^{-1} (C-H bending) were confirmed those functional groups in all compounds. Incase of compound (F_c, F_d, HF_c & HF_d) showed their characteristic peaks 1115 - 1105 cm^{-1} observed on IR spectrum which holding their methoxy substitution on the titled compound. In ^{1}H NMR, the common peak for targeted compounds were confirmed by the presence of one proton in ring B as a singlet were confirmed in the range of δ 5.6 – 7.2. The aromatic protons of all synthesised derivatives were revealed in the range of δ 6 – 8.5 as multiplets. In certain compounds their substituents were confirmed as δ 2.5 – 3.2 ($N(CH_3)_2$) as singlet for F_e, in case of compounds F_c, F_d, HF_c & HF_d as δ 3.5-3.9 (OCH_3) and $(OCH_3)_2$ as singlets respectively. The different types of protons for all compounds were also depicted on proper multiplicities on expected signals. The mass spectrum of all compounds revealed their respective (M^+) parent ion peak for the corresponding calculated molecular mass, which accountable for further confirmation of the synthesised compounds.

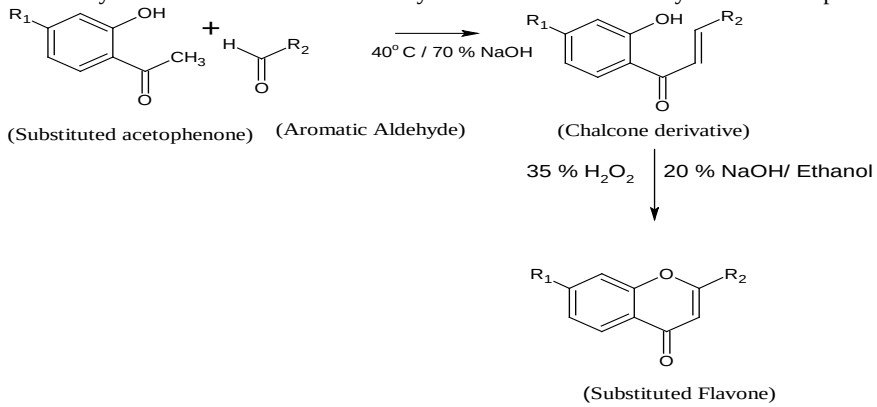


Fig.1. General synthetic scheme of flavones

Spectral Analysis

All the hybridised flavones were characterized by various spectroscopic techniques such as UV, IR, ^{1}H-NMR and mass spectrometry.

F_a: *2-(2-hydroxyphenyl)-4H-chromen-4-one*

MP: 185-188 ºC; R_f = 0.58; % yield = 47.2 % w/w; UV λmax: $CHCl_3$, nm: 248; IR (KBr cm^{-1}): 1734 (lactone), 1683 (CO str), 1558, 1541 (C=C Arom.str), 1139, 1093 (COC str), 765 (C-C bending) 3566, 3547 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.5 (m, 1H, ArH), 7.1 – 7.9 (m, 8H, ArH); m/z: 238 (m+1), 121 $(C_7H_6O_2)^+$, 106 $(C_7H_6O)^+$, 104 $(C_8H_8)^+$, 78 $(C_6H_6)^+$.

F_b: *2-(4-hydroxyphenyl)-4H-chromen-4-one*

MP: 181-183 ºC; R_f = 0.41; % yield = 47.5 % w/w; UV λmax: CH_3OH, nm: 287; IR (KBr cm^{-1}): 1772 (lactone), 1691 (CO str), 1560, 1543, 1516 (C=C Arom.str), 1047, 1139 (COC str), 748 (C-C bending) 3300, 3545 (OH str); ^{1}H NMR(500 MHZ, DMSO): δ 6.8 (m, 1H, ArH), 7.7 -7.4 (m, 8H, ArH); m/z: 238 (m+1), 121$(C_7H_6O_2)^+$, 118 $(C_8H_7O)^+$, 92 $(C_6H_6O)^+$, 76.9 $(C_6H_6)^+$.

F_c: *3-(4-methoxyphenyl)-4H-1-benzopyran-4-one*

MP: 182-185 ºC; R_f = 0.43; % yield = 49.2 % w/w; UV λmax: DMSO, nm: 265; IR (KBr cm^{-1}): 1658 (CO str), 1597, 1550 (C=C Arom.str), 1126, 1064 (COC str), 727 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 3.8 (s, OCH_3, ArH), 6.4 (m, 1H, ArH), 7.0, 7.8 (m, 8H, ArH); m/z: 252 (m+1), 134.9$(C_9H_{10}O)^+$, 107 $(C_7H_6O)^+$, 120.6$(C_7H_5O_2)^+$, 77.0 $(C_6H_5)^+$.

F_d: *2-(2,4-dimethoxyphenyl)-4H-chromen-4-one*

MP: 178-180 ºC; R_f = 0.6; % yield = 46.4 % w/w; UV λmax: CH_3OH, nm: 250; IR (KBr cm^{-1}) : 1660 (CO str), 1597, 1550 (C=C Arom.str), 1124, 1066 (COC str), 752 (C-C bending); ^{1}H NMR(500 MHZ, DMSO): δ 3.8 (s, OCH_3, ArH), 6.9 (m, 1H, ArH), 7.0 -7.7 (m, 7H, ArH) ; m/z: 283 (m+1), 121$(C_7H_6O_2)^+$, 92.7 $(C_6H_6O)^+$, 164 $(C_{10}H_{11}O_2)^+$, 137.5 $(C_8H_{10}O_2)^+$, 76.7 $(C_6H_5)^+$.

F_e: *2-[4-(dimethylamino)phenyl]-4H-chromen-4-one*

MP: 178-181 ºC; R_f = 0.71; % yield = 48.4 % w/w; UV λmax: DMSO, nm: 295; IR (KBr cm^{-1}): 1795 (lactone), 1658 (CO str), 1548, 1537 (C=C Arom.str), 1124, 1064 (COC str), 727 (C-C bending); ^{1}H NMR(500 MHZ, DMSO): δ 2.4 (s, 6H, $N(CH_3)_2$), 6.3 - 6.5 (m, 1H, ArH), 7.5 - 7.9 (m, 8H, ArH); m/z: 265 (m+1), 222$(C_{15}H_{10}O_2)^+$, 121 $(C_7H_6O_2)^+$, 104.9 $(C_8H_8)^+$, 77 $(C_6H_5)^+$.

HF_a: *2-(4-fluorophenyl)-7-hydroxy-4H-chromen-4-one*

MP: 195 - 198 ºC; R_f = 0.72; % yield = 67.2 % w/w; UV λmax: $CHCl_3$, nm: 234; IR (KBr cm^{-1}): 1685 (CO str), 1529, 1587 (C=C Arom.str), 1085, 1163, 1242 (COC str), 756 (C-C bending), 3072 (C-H str), 3463 (OH str); ^{1}H NMR(500 MHZ, DMSO): δ 6.3 (m, 1H, ArH), 7.2 - 8 (m, 7H, ArH), 10.7 (s, 1H, OH); m/z: 256 (m+1), 136.5 $(C_7H_6O_3)^+$, 122.6 $(C_8H_7F)^+$, 94.7 (C_6H_6O), 74.8 $(C_6H_5)^+$.

HF_b: *7-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one*

MP: 181-183 ºC; R_f = 0.54; % yield = 57.5 % w/w; UV λmax: DMF, nm: 315; IR (KBr cm^{-1}): 1716 (lactone), 1649 (CO str), 1521, 1556 (C=C Arom.str), 1166, 1184, 1218 (COC str), 771 (C-C bending), 3461 (OH str); ^{1}H NMR(500 MHZ, DMSO): δ 6.5 – 6.6 (m, 1H, ArH), 7.3 - 7.8 (m, 7H, ArH), 9.8 (s, 2H, OH); m/z: 254 (m+1), 137.1 $(C_7H_6O_3)^+$, 121.2 $(C_8H_8O)^+$, 93.3 (C_6H_6O), 79 $(C_6H_5)^+$.

HF_c: *6-hydroxy-3-(4-methoxyphenyl)-4H-1-benzopyran-4-one*

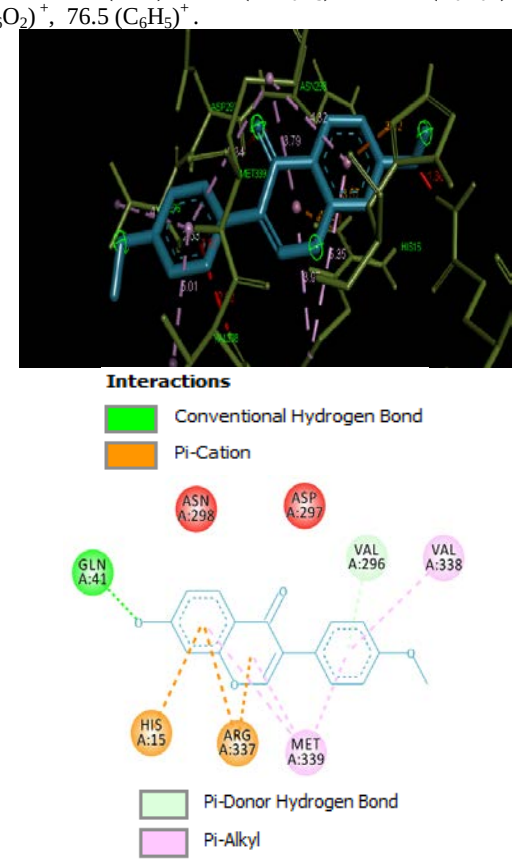
MP: 185-189 ºC; R_f = 0.62; % yield = 62.2 % w/w; UV λmax: $CHCl_3$, nm: 253; IR (KBr cm^{-1}): 1712 (lactone), 1635 (CO str), 1556, 1587 (C=C Arom.str), 1078, 1112 (COC str), 757 (C-C bending), 3097 (CH str), 3485 (OH str); ^{1}H NMR(500 MHZ, DMSO): δ 3.8 (s, 3H, OCH_3) 6.6 – 6.9 (m, 1H, ArH), 7.0 - 7.8 (m, 7H, ArH), 9.8 (s, 1H, OH); m/z: 268 (m+1), 136.9 $(C_7H_6O_3)^+$, 134.9 $(C_9H_{10}O)^+$, 110 $(C_6H_6O_2)$, 92.9 (C_6H_6O), 78 $(C_6H_5)^+$.

HF_d: *2-(2,4-dimethoxyphenyl)-7-hydroxy-4H-chromen-4-one*

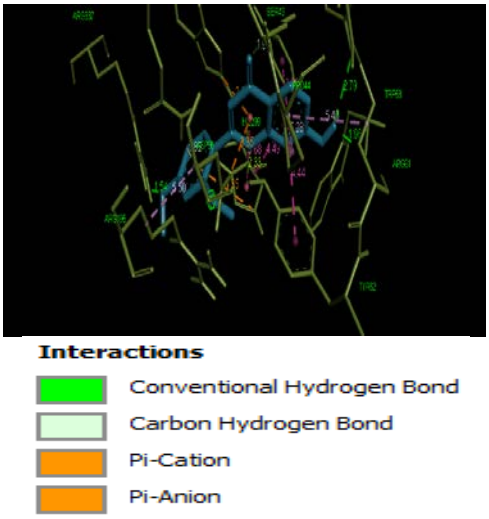
MP: 178-180 ºC; R_f = 0.47; % yield = 58.3 % w/w; UV λmax: CH_3OH, nm: 293; IR (KBr cm^{-1}): 1735 (lactone), 1683 (CO str), 1523, 1573 (C=C Arom.str), 1091, 1111, 1130 (COC str), 761 (C-C bending), 3093 (CH str), 3424 (OH str); ^{1}H NMR(500 MHZ, DMSO): δ 3.8 (s, 6H, OCH_3), 7.0 (m, 1H, ArH), 7.0 – 7.5 (m, 6H, ArH), 12.6 (s, 1H, OH); m/z: 298 (m+1), 139 $(C_7H_6O_3)^+$, 165.8 $(C_{10}H_{11}O_2)^+$, 149.9 $(C_9H_{11}O_2)$, 95.12 (C_6H_6O), 78 $(C_6H_5)^+$.

HF_e: *7-hydroxy-2-(thiophen-2-yl)-4H-chromen-4-one*

MP: 194-199 ºC; R_f = 0.76; % yield = 45.4 % w/w; UV λmax: DMF, nm: 326; IR (KBr cm^{-1}): 1772 (lactone), 1681 (CO str), 1541, 1558 (C=C Arom.str), 1043, 1101, 1134 (COC str), 702 (C-C bending), 3089 (CH str), 3444 (OH str); ^{1}H NMR(500 MHZ, DMSO): δ 6.1 (m, 1H, ArH), 7.1 – 7.7 (m, 6H, ArH), 10.5 (s, 1H, OH); m/z: 244 (m+1), 139.2 $(C_7H_6O_3)^+$, 111.3 $(C_6H_6S)^+$, 123.3 $(C_6H_6O_2)^+$, 76.5 $(C_6H_5)^+$.



Docked pose of Alpha Amylase with the compound HF_d



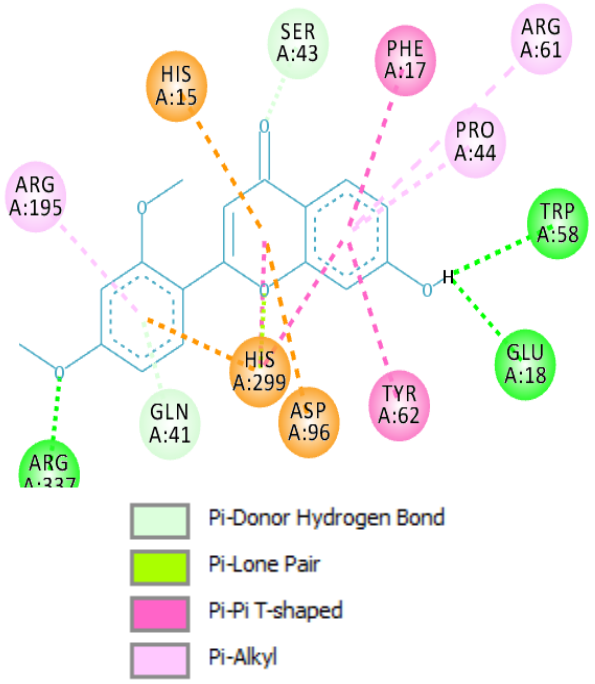


Fig.2. Visualization of synthesized ligands (HF_c & HF_d) interaction with α-amylase Inhibitor Docked pose of alpha amylase with the compound HF_c

Table.1. Different substitution patterns on basic nucleus (γ-benzopyrone ring)

Compound code	R1	R2
F_a	-H	$-C_6H_4$-2(OH)
F_b	-H	$-C_6H_4$-4(OH)
F_c	-H	$-C_6H_4$-4(OCH_3)
F_d	-H	$-C_6H_3$-2,4(OCH_3)
F_e	-H	$-C_6H_4$-N(CH_3)$_2$
HF_a	-H	$-C_6H_4$-4(F)
HF_b	-H	$-C_6H_4$-4(OH)
HF_c	-H	$-C_6H_4$-4(OCH_3)
HF_d	-H	$-C_6H_3$-2,4(OCH_3)
HFe	-H	$-C_4H_3S$

Table.2: Overall Docking score of synthesised flavones

Ligand	Docking score (kcal/mole)		
	Aldose reductase	PTP1B	α-Amylase
F_a	-7.03	-6.24	-9.09
F_b	-7.87	-7.91	-8.31
F_c	-7.16	-7.70	-9.56
F_d	-8.11	-7.21	-9.88
F_e	-7.22	-7.16	-8.16
HF_a	-3.51	-7.94	-9.38
HF_b	-8.65	-7.75	-9.41
HF_c	-5.03	-8.52	-9.15
HF_d	-8.33	-7.72	-9.90
HF_e	-7.28	-8.89	-9.86
Fidarestat	-10.35		
Ertiprotafib		-8.72	
Acarbose			-7.61

Table.3: Effect of synthesised flavones on Alpha amylase inhibitory assay

Compounds	% Inhibition of Alpha amylase					IC_{50} Values
	10 (µg/ml)	20 (µg/ml)	40 (µg/ml)	80 (µg/ml)	160 (µg/ml)	
F_a	29.5±1.47	42.3±2.38	59.7±2.77	73.3±2.86	86.2±1.93	38.3
F_b	30.8±2.14	43.7±2.22	59.6±3.29	71.9±1.62	84.8±2.37	37.05
F_c	32.6±2.28	45.5±2.47	60.3±2.81	73.4±2.77	86.4±2.24	32.52
F_d	33.5±2.34	46.1±2.56	59.7±2.65	74.8±2.51	88.3±2.54	30.9
F_e	31.7±2.08	44.8±2.15	57.3±3.11	72.1±2.68	85.8±2.98	36.8
HF_a	32.1±2.45	46.8±1.88	59.5±3.48	74.1±2.56	86.2±2.41	32.0
HF_b	34.9±2.18	45.8±1.67	58.6±3.36	70.6±2.29	82.3±2.32	32.9
HF_c	36.3±1.98	47.5±1.54	60.8±3.59	75.3±1.73	87.4±2.76	25.8
HF_d	36.8±2.31	48.5±1.72	62.7±3.28	73.6±2.28	88.2±2.63	23.6
HF_e	33.7±2.57	46.8±2.29	59.3±3.22	73.2±2.14	87.3±2.54	31.1
Acarbose	36.9±2.68	48.9± 1.84	63.7±3.43	78.2± 2.62	94.3±2.57	21.2

All values are Mean ± SEM, n = 3. One way Analysis of Variance (ANOVA) followed by Dunnett's test was performed as the test of significance.

Table.4: Effect of synthesized flavones on blood glucose level on STZ induced diabetic rats.

GROUPS	Fasting Blood Glucose Level (mg/dl)			
	1st day	7th day	14th day	21st day
Normal Control	94.2±7.03	97.2±7.65	96.6±7.65	95.8±7.16
Diabetic Control	267.6±7.93[a]	282.8±7.80[a]	311.6±7.91[a]	318.4±7.83[a]
Glibenclamide	246.6±8.18[b]	187.8±8.03[b]	148.2±8.46[b]	112.8±8.98[b]
HF_a	260.8±8.33	272.6±8.96[c]	299.8±8.09[c]	307.2±7.90
HF_b	259.8±8.60[c]	233.4±8.85[b]	182.2±7.48[b]	151.6±7.73[b]
HF_c	253.8±8.53[b]	215.8±8.48[b]	162.2±8.33[b]	130.8±8.57[b]
HF_d	248.6±8.97[b]	208.8±9.22[b]	159.2±8.27[b]	122.6±8.74[b]
HF_e	250.4±8.46[b]	223.2±8.31[b]	171.6±7.45[b]	145.2±8.72[b]

Values are mean ± SD for n = 5; One way Analysis of Variance (ANOVA) followed by Dunnett's test. [a]P < 0.01 = significant as compared with normal, [b]P < 0.01, [c]P < 0.05 = Significant as compared with diabetic control.

Table.5. Effect of synthesized flavones in Liver biomarker enzymes and total protein on STZ induced diabetic rats**.**

GROUPS	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	TOTAL BILIRUBIN (mg/dl)	TOTAL PROTEIN (g/dl)
Normal control	54.6±4.50	36.6±4.77	104.6±4.77	0.56±0.04	6.95±0.14
Diabetic control	97.6±5.72[a]	79.2±5.40[a]	229.6±4.62[a]	1.04±0.04[a]	4.84±0.16[a]
Glibenclamide	57.6±4.50[b]	41.6±4.72[b]	119.2±5.40[b]	0.63±0.04[b]	6.51±0.15[b]
HF_a	87.4±4.21[c]	71.4±5.32	221.2±4.60	0.95±0.03[c]	4.94±0.19[c]
HF_b	76.4±4.61[b]	60.2±5.44[b]	170.2±5.07[b]	0.85±0.05[b]	5.89±0.21[b]
HF_c	64.2±5.78[b]	53.6±5.12[b]	163.4±4.64[b]	0.76±0.05[b]	6.28±0.17[b]
HF_d	60.4±4.61[b]	44.2±5.63[b]	141.8±4.97[b]	0.68±0.05[b]	6.40±0.12[b]
HF_e	71.4±5.94[b]	55.8±5.45[b]	153.4±5.84[b]	0.71±0.04[b]	6.02±0.15[b]

Values are mean ± SEM for n = 5; One way Analysis of Variance (ANOVA) followed by Dunnett's test. [a]P < 0.01 = significant as compared with normal, [b]P < 0.01, [c]P < 0.05 = Significant as compared with diabetic control.

Table.6. Effect of synthesised flavones in renal parameters on STZ induced diabetic rats

GROUPS	BLOOD UREA (mg/dl)	SERUM CREATININE (mg/dl)
Normal control	15.7±0.53	0.66±0.03
Diabetic control	30.8±1.01[a]	0.97±0.04[a]
Glibenclamide	19.4±0.92[b]	0.71±0.05[b]
HF_a	28.6±1.12	0.92±0.04
HF_b	24.1±1.35	0.89±0.04[c]
HF_c	23.2±1.07[c]	0.80±0.04[b]
HF_d	21.5±1.56[b]	0.74±0.04[b]
HF_e	22.8±1.20[c]	0.86±0.05[b]

Values are mean ± SEM for n = 5; One way Analysis of Variance (ANOVA) followed by Dunnett's test.
[a]P < 0.01 = significant as compared with normal, [b]P < 0.01, [c]P < 0.05 = Significant as compared with diabetic control.

Molecular Docking Studies

Binding mode of ligands to the enzyme was explored by docking studies. Binding affinity of hybridised flavones with active site of enzymes was shown that the compounds posses better interaction. Docking score of the compounds were given in the table.2. Docking studies were validated by doing control docking of native inhibitors such as Fidarestat for aldose reductase (3m4h), Ertiprotafib for PTP1B (1een) and Acarbose (3ole) for α-amylase enzymes.

Docking analysis of Aldose reductase Inhibitor (3m4h)

In docking study, one of the targeted enzymes was aldose Reductase (AR), which is an monomeric small protein which composed of 315 amino acids. It is a cystolic reduced NAD-NADPH dependent enzyme and also a family of aldo-ketoreductase. It is essential for polyol pathway for the conversion of glucose into sorbitol and further convert into fructose in presence of sorbitol dehydrogenase by the usage of NADPH as an cofactor [19, 20]. The polyol pathway was discovered by Her's [21] and Van Heyningen [22] reported that the increased level of aldose reductase were noted on diabetic rat lens during cataractogenesis, also they derived the presence of sorbitol and galactitol were accumulated on the ocular lens of the rat. They clearly depicted that the polyol pathway is an alternate mechanism for utilisation of glucose upto 3 % in normal healthy adults but in case of diabetes, which can reached upto 30 % [23-24]. Due to high acceleration of polyol pathway, increases the accumulation of sorbitol which leads to tissue injury results in major diabetic complications such as retinopathy, neuropathy and nephropathy [25-26]. The inhibition of aldose reductase is a positive approach to overcome the diabetic complication on long term therapy [27]. By which, docking studies of the titled compound revealed that HF_b & HF_d (-8.65 & -8.33 kcal/mole) afforded their high binding score compared with respective standard Fidarestat (-10.35 kcal/mole), the binding affinity over the receptor site were exhibited by electron donating substituent's over those compounds. The presence of hydroxyl group and di-methoxy group on the compound HF_d created an electron flow and making the compound more active, polarizable and potent. This proves that, flavones showed the comparable potential with fidarestat as antidiabetic agent.

Docking analysis of Protein thyrosine phosphatase 1 B (PTP 1B) Inhibitor (1een)

Another target was Protein tyrosine phosphatise (PTP) is an responsive enzyme, acts as an negative regulator of insulin signalling it's clearly reported that PTP 1B is the reason for insulin resistance and obesity in type 2 diabetes mellitus [28]. In the pathway of insulin signalling, this enzyme PTP 1B undergoes dephosphorylation of insulin receptor and its substrate. Due to this reason, the essential enzyme tyrosine kinase on the insulin receptor was inactivated, which results in slow down the utilization of glucose on our body cells. Further study reported that by inhibition of PTP 1B in the mouse, reflects in enhancing the insulin sensitivity which was confirmed by the determination of glucose clearance and in insulin tolerance test [29, 30]. Hence, this research work clearly indicates that by deletion of PTP 1B is an ideal approach in the management of diabetes and to control the obesity by reducing the storage of adipose tissues of triglycerides on overload of nutrition during the diabetes. On docking study, in case of PTB 1B inhibitor, the compounds HF_c & HF_e (-8.52 & -8.89 kcal/mole respectively) posses the high potent inhibitory activity compared with standard ertiprotafib (-8.72 kcal/mole) a known PTP1B enzyme inhibitor. The compound HF_c showed hydrogen bond affinity towards the methoxy group of oxygen on the residues aswell as thiophenyl ring (HF_e) posses pibond and pialkyl interactions over the enzyme which confirmed their potential towards this enzyme.

Docking analysis of alpha – amylase inhibitor (3ole)

Another approach was Alpha-amylase (α-1,4 glucan-4-glucanohydrolase) is one of the important enzyme, present in the salivary gland as well as in the pancreas 5–6 % [31]. Along with alpha glucosidase, it is also present in the border of small intestine and helpful in the digestion of carbohydrates. Initially the digestion of carbohydrates starts by breakdown of oligosaccharides into disaccharides, which also converted into monosaccharides for absorption [32-35]. One of the therapeutic approaches to treat diabetes is inhibition of alpha amylase, which results in inhibiting the breakdown of carbohydrates. By which, it decreases the insulin demand and also the stimulation of cells to secrete insulin [36]. This approach helpful in decreasing the post prandial glucose level by slow down the carbohydrate digestion, results in slow down the glucose absorption [37]. The docking results revealed the best accommodation of HF_c & HF_d (docking score = -9.15 & -9.90 kcal/mol) respectively more than the value compared with their standard Acarbose (-8.61 kcal/mol). On this multi targeted docking study, while compared the binding scores

over three enzymes, the titled compounds afforded their high affinity towards alpha amylase (PDB: 3ole). The former two enzymes were responsible for conversion of glucose into sorbitol (AR) and related with insulin sensitivity for utilization of glucose (PTP 1B). But alpha amylase was used as a primary cause for formation of glucose by breakdown of carbohydrate, by inhibiting this enzyme were hidden the role of Aldose reductase and protein tyrosine phosphatase. Hence, the docking pose of highly active compounds HF_c & HF_d and their binding interactions over the alpha amylase were mentioned in the fig.2.

Invitro anti-diabetic activity

On the basis of docking score (Table.2), among the three enzymes alpha amylase afforded maximum binding score with the flavones scaffolds, due to that *in-vitro* anti-diabetic activity on alpha amylase inhibitory assay would be evaluated. In this assay, the pancreatic alpha amylase was added to the starch solution which enhances the reduction of starch into maltose. This reduced product was identified by DNSA by formation of colored product which can be measured at 540 nm. Hence in presence of hybridised flavones or standard acarbose which reduces the formation of maltose due to their inhibitory action towards the pancreatic amylase. Hence the intensity of color was inversely proportional to the drug concentration. Among the synthesised compounds, the compounds with hydroxyl substitution of flavones (HF_a-HF_e) showed their significant IC_{50} values were (32, 32.9, 25.8, 23.6 & 31.1 µg/ml respectively) compared with the standard (21.2 µg/ml) were mentioned in the Table.3.

Anti-Diabetic Activity by (STZ induced Model)

Based on the docking score and alpha amylase inhibitory assay, highly active derivatives were selected (HF_a-HF_e) and screened for anti-diabetic activity by streptozotocin induced rat model. The blood glucose level in rats was showed in the Table.4. The blood glucose level was highly significant ($p < 0.01$) compared to normal rats. After oral administration of synthesised flavones for 21 days were significantly reduced the blood glucose level compared with diabetic control rats. On 14th and 21st day, the compounds such as HF_c, HF_d & HF_e were significantly decreases ($p < 0.01$) the blood glucose level compared with diabetic control. It was evident from the table that diabetic control rats had elevated blood glucose level and the synthesised flavones were able to improve the metabolism significantly were compared with untreated rats.

Biochemical Parameters

The liver and kidney plays an important role in elimination of metabolite and some toxic moieties. Liver and kidney dysfunction may lead to increase the biochemical substances in the blood stream due to administration of certain drugs. In diabetic condition, the level of transaminase in liver such as SGOT, SGPT and SALP were increases, which are highly active in absence of insulin. This is because of availability of amino acids in diabetic blood, which increases gluconeogenesis and ketogenesis those were observed in diabetes. As per the above phenomena, the diabetic rats had significantly ($p < 0.01$) increased in transaminase and decreased in protein content than normal rats. After treatment with synthesised flavones had moderate significant decreases ($p < 0.01$) in liver enzyme activities and blood urea nitrogen aswell as serum creatinine were significantly increases by compared with diabetic rats were mentioned in the table.5 & 6.

CONCLUSION

In silico library were performed and designed the titled compound phenyl-4H-chromen-one on multi targeted diabetic enzymes. Those compounds were synthesized and its anti-diabetic activity was checked. The docking result clearly depicted that these compounds were well correlated with the prediction of anti-diabetic activity. Among the three enzymes, alpha amylase enzyme posses high binding accommodation over the synthesized compounds and afforded their binding score. The affinity of compounds over this enzyme by three common residues they are His 15, Arg 337 & Gln 41 which enhances the potent anti-diabetic activity of these hybrid derivatives. Further *invitro* and *invivo* results were correlated with the above concept which strongly depicted the potency of anti-diabetic activity over the titled compounds. The present study concludes that by small changes over the substituent pattern over the flavone moiety can enhances the inhibitory activity over these diabetic enzymes. Even though focusing on precise mechanism over the various enzyme inhibition study and changes over the evaluation of anti-diabetic activity is essential for further development of studies.

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REFERENCES

1. Mealey, B. L., Ocampo, G. L., Diabetes mellitus and periodontal disease *Periodontology*. 2007, *44*(1), 127-153.
2. Helaine, E., Resnick, M., In This Issue of Diabetes Care. *Diabetes Care*. 2013, *36*(6),1431-1432.
3. Unnikrishnan, P.S., Suthindhiran, K., Jayasri, M.A., Alpha-amylase inhibition and
4. antioxidant activity of marine green algae and its possible role in diabetes management. *Pharmacogn Mag.* 2015, *11*, 511-515.
5. Balaji, R.M., Jeyaram, C., Sundaram, K.M., Ramasamy, M.S., Studies on Activity of Indian Medicinal Plants Using α-Amylase and α-Glucosidase Inhibitory Activity-A Pathway to Antidiabetic Drugs. *World. J. Med. Sci*. 2015, *12*, 207-212.
6. Mohan, S., Nandhakumar, L., Role of various flavonoids: Hypotheses on novel approach to treat diabetes. *J. Med. Hypo. Id.* 2014, *8*, 1-6.
7. Garcia, L.A., Guillamon, E., Villares, A., Rostagno, M.A., Martinez, J.A., Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflamm Res.* 2009, *58*(9), 537-552.
8. Liu, S., Li, D., Huang, B., Chen, Y., Lu, X., Wang, Y., Inhibition of pancreatic lipase, α-glucosidase, α-amylase, and hypolipidemic effects of the total flavonoids from *Nelumbo nucifera* leaves. *J. Ethnopharmacol.* 2013, *149*(1), 263-269.
9. Wagner, H., Farkas, L., *Synthesis of flavanoids*, The flavanoids. Springer US, 1975; 127-213.
10. Core, A.L., Hossain, S., Cole, A.M., Phansteil, O., Synthesis and bioevaluation of substituted chalcones, coumaranones and other flavonoids as anti-HIV agents. *Bioorg. Med. Chem.* 2016, *24*, 2768-2776.
11. Pramod, S., Dasharath, D., Ravi, V., Pudukulathan, K., Cyclization of 2′-hydroxychalcones to flavones using ammonium iodide as an iodine source – an eco-friendly approach. *J. Serb. Chem. Soc.* 2013, *78* (7), 909 – 916.
12. Molecular Graphics Laboratory, The Scripps research Institute Available from: www.scripps.edu, graphics laboratory (MGL) tools and AutoDock4.2, downloaded on 25 July 2011.
13. Shetty, S., Secnik, K., and Oglesby, A., (2005). Relationship of glycemic control to total diabetes-related costs for managed care health plan members with type 2 diabetes. *Jl of Managed Care Pharm. 11*, 559–564.
14. Miller, G. L.(1959). Use os dinitrosalicylic acid reagent for determination of reducing sugar, *Anal.Chem*. *31*(3), 426-428.
15. Ghasemi, A., Khalifi, S., Jedi, S., Review - Streptozotocin-nicotinamide-induced rat model of type 2 diabetes. *Act. Physio. Hung*, **2014**, *101*(4), 408-420.
16. Dhanabal, S.P., Koate, C.K., Ramanathan, M., Elango, K., Suresh, B., The hypoglycemic activity of *Coccinia indica Wight* and its influence on certain biochemical parameters. *Indian. J. Pharmacol.* 2004, *3*(4), 244-250.
17. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., The original method. *J. Biol. Chem.* 1951, *193*, 265-275.
18. Folin, O., Wu, H. A., system of blood analysis. *J. Bio. Chem.* 1919, *38*, 81 -110.
19. Talke, H., Schubert, G.E., Enzymatic Determination of Urea Using the Coupled Urease-GLDH Enzyme System. *Mediat. Inflamm.* 1965, *43*, 174-176.

20. Ravindranath, T.M., Mong, P.Y., Ananthakrishnan, R., Li, Q., Quadri, N., Schmidt, M.A., Ramasamy, R., Wang, Q., Novel Role for Aldose Reductase in Mediating Acute Inflammatory Responses in the Lung. *J. Immunol.* 2009, *183*, 8128-8137.
21. Hwang, Y.C., Shaw, S., Kaneko, M., Aldose reductase pathway mediates JAK-STAT signaling: a novel axis in myocardial ischemic injury. *J. Fed. Am. Soci. Exp. Biol.* 2005, *19*, 795-797.
22. Hers, H.G., Le Mechanisme de la transformation de glucose en fructose par les vesicules seminales. *Biochim. Biophys. Acta.*, 1956, *22*, 202-203.
23. Van Heyningen, R., Formation of polyols by the lens of the rat with sugar cataract. *Nature*, 1959, *468*, 194-195.
24. Morrison, A.D., Clements, R.S., Travis, S.B., Oski, F., Winegrad, AI., Glucose utilization by the polyol pathway in human erythrocytes. *Biochem. Biophys. Res. Commun.* 1950, *40*, 199-205.
25. Gonzalez, R.G., Barnett, P., Aguayo, J., Cheng, H.M., Chylack, L.T.J., Direct measurement of polyol pathway activity in the ocular lens. *Diabetes*. 1984, *33*, 196-199.
26. Kinoshita, J.H., Nishimura, C., The involvement of aldose Reductase in diabetic complications. *Diab. Metab. Rev.* 1998, *4*, 323-337.
27. Pugliese, G., Tilton, G.T., Williamson, J.R., Glucose induced metabolic imbalances in the pathogenesis of diabetic vascular disease. *Diab. Metab. Rev.* 1991, *7*, 35-59.
28. Uma Maheswari, M., Aji, C. S., Asokkumar, K., Sivashanmugam, T., Subhadradevi, V., Jagannath, P., Madeswaran A., *In Silico* Docking studies of Aldose Reductase Inhibitory activity of selected Flavonoids. *Int. J. Drug Dev. & Res.* 2012, *4*(3),
29. 328-334.
30. Pazhinskuy, E., Ren, J., Nair, S., Pharmacological inhibition of protein tyrosine phosphate 1B: A promising strategy for the treatment of obesity and Type 2 diabetes mellitus. *Curr. Med.Chem.* 2013, *21*, 2609-25.
31. Elchebly, M., Payette, P., Michaliszyn, E., Cromlish, W., Collins, S., Loy, A.L., Normandin, D., Cheng, A., Himms-Hagen, J., Chan, C.C., Ramachandran, C., Gresser, M.J., Tremblay, M.L., Kennedy, B.P., Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatise 1B gene. *Science*. 1999, *283*, 1544-8.
32. Klaman, L.D., Boss, O., Peroni, O.D., Kim, J.K., Martino, J.L., Zabolotny, J.M., Moghal, N., Lubkin, M., Kim, Y.B., Sharpe, A.H., Stricker-Krongrad, A., Shulman, G.I., Neel, B.G., Kahn, B.B., Increased energy expenditure, decreased adiposity and tissue specific insulin sensitivity in protein tyrosine phosphatase 1B deficient mice. *Mol. Cell. Biol.* 2000, *20*(15), 5479-89.
33. Whitcomb, D.C., Lowe, M.E., Human Pancreatic Digestive Enzymes. *Dig. Dis. Sci.* 2007, *52*, 1-17.
34. Van de Laar, F.A., Lucassen, P.L.B.J., Akkermans, R.P., Van de Lisdonk, E.H., Rutten, G.E.H.M., Van Weel, C., Alpha-glucosidase inhibitors for type 2 diabetes mellitus, Cochrane Database of Systematic Reviews. 2005, Issue 2, Art. No: CD003639, DOI: 10.1002/14651858.CD003639.pub2.
35. Inzucchi, S.E., Oral antihyperglycemic therapy for type 2 diabetes. JAMA, 2002, *287*(3), 360-372.
36. Goke, B., Herrmann-Rinke, C., The evolving role of alpha-glucosidase inhibitors. *Diab. Metab. Rev.* 1998, *14*, S31-S38.
37. Lebowitz, H.E., Alpha-glucosidase inhibitors as agents in the treatment of diabetes. *Diab. Rev.* 1998, *6*(2), 132-145.
38. Funke, I., Melzing, M.F., Traditionally used plants in diabetes therapy – phytotherapeutics as inhibitors of *a*-amylase activity. *Rev. Bras. Farmacogn.* 2006, *16*, 1-5.
39. Cheng, A.Y.Y., Fantus, I.G., Oral antihyperglycemic therapy for type 2 diabetes Mellitus. *Can. Med. Assoc. J.* 2005, *172*, 213-226.