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 amylose 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 HF2
possess high significant (p < 0.01) results and restored the blood glucose level, liver enzymes and renal parameters. Based on docking result,
the compound HF2 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 hybridised 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 Fx-Fy & HF2-HF2 were synthesized 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–Amylace (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
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 amylose inhibitory activity of synthesised
flavones (Fx-Fy & HF2-HF2) 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 amylose enzyme (0.5 mg/ml) and incubated for
10 min at 25°C. 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 25°C. The
reaction was retarded by adding a 1 ml of DNA 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 × 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 ºC. 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-HFe) 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 0th (before the start of the experiment), 4th day, 7th day, 14th day
and 21st day. The glucose levels were estimated by using Accu-
Check Active glucometer. Weight of individual animals was
measured gravimetrically on 0th and 21st 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
biliurbin 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’NMR 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 (O=C-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-HFe). 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 e, F d, HF e & HF d)
showed their characteristic peaks 1115 - 1105 cm -1 observed on IR
spectrum which holding their methoxy substitution on the titled
compound. In 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(CH3)2) as singlet for F e, in case
of compounds F c, F b, HF c & HF b as δ 3.5-3.9 (OCH3) and
(OCH3)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

(Substituted acetocephone)  (Aromatic Aldehyde)  
\[
\text{OH} + \text{CH}_3 + \text{H}_2\text{R}_2 \\
\text{40}^\circ\text{C} / 70\% \text{ NaOH} \\
\text{40°C} / 70\% \text{ NaOH}
\]

(Chalcone derivative)  (Substituted Flavone)
\[
\text{35\% H}_2\text{O}_2 \quad 20\% \text{ NaOH/ Ethanol} \\
\text{35\% H}_2\text{O}_2 \quad 20\% \text{ NaOH/ Ethanol}
\]
Spectral Analysis

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

Fα: 2-(2-hydroxyphenyl)-4H-chromen-4-one
MP: 185-188 °C; Rf = 0.58; % yield = 47.2 % w/w; UV λmax: CHCl3, 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); 1H NMR (500 MHZ, DMSO): δ 6.5 (m, 1H, ArH), 7.1 – 7.9 (m, 8H, ArH); m/z: 238 (m+1), 121 (C₇H₈O₂)⁷, 106 (C₆H₈O)⁴, 104 (C₇H₁₈O); 78 (C₆H₅O)⁴.

Fb: 2-(4-hydroxyphenyl)-4H-chromen-4-one
MP: 181-183 °C; Rf = 0.41; % yield = 47.5 % w/w; UV λmax: CH₃OH, 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, 3549 (OH str); 1H NMR (500 MHZ, DMSO): δ 6.8 (m, 1H, ArH), 7.7 - 7.4 (m, 8H, ArH); m/z: 238 (m+1), 121 (C₇H₆O₂)⁺, 118 (C₇H₆O)⁺, 92 (C₇H₈O₂)⁷, 76.9 (C₆H₅O)⁴.

Fc: 3-(4-methoxyphenyl)-4H-1-benzopyran-4-one
MP: 182-185 °C; Rf = 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) 3093, 3485 (OH str); 1H NMR (500 MHZ, DMSO): δ 3.8 (s, OCH₃, ArH), 6.4 (m, 1H, ArH), 7.0, 7.8 (m, 8H, ArH); m/z: 252 (m+1), 134.9 (C₉H₁₀O)⁺, 107 (C₇H₆O)⁺, 120.6 (C₇H₅O₂)⁺, 77.0 (C₆H₅O)⁴.

Fd: 2-(2,4-dimethoxyphenyl)-4H-chromen-4-one
MP: 178-180 °C; Rf = 0.6; % yield = 46.4 % w/w; UV λmax: CH₃OH, nm: 293 (IR (KBr cm⁻¹): 1683 (CO str), 1558, 1541 (C=C arom.str), 1139, 1093 (COC str), 765 (C-C bending) 3566, 3547 (OH str); 1H NMR (500 MHZ, DMSO): δ 3.8 (s, OCH₃, ArH), 6.9 (m, 1H, ArH), 7.0 - 7.7 (m, 7H, ArH); m/z: 283 (m+1), 121 (C₇H₆O₂)⁺, 92.7 (C₆H₆O)⁺, 164 (C₁₀H₁₁O₂)⁺, 137.5 (C₈H₁₀O₂)⁺, 76.7 (C₆H₅O)⁴.

Fe: 2-[4-(dimethylamino)phenyl]-4H-chromen-4-one
MP: 178-181 °C; Rf = 0.71; % yield = 48.4 % w/w; UV λmax: DMSO, nm: 293 (IR (KBr cm⁻¹): 1795 (lactone), 1658 (CO str), 1548, 1537 (C=C arom.str), 1124, 1064 (COC str), 727 (C-C bending) 3093, 3485 (OH str); 1H NMR (500 MHZ, DMSO): δ 2.4 (s, 6H, N(CH₃)₂), 6.3 - 6.5 (m, 1H, ArH), 7.5 - 7.9 (m, 8H, ArH); m/z: 265 (m+1), 222 (C₁₀H₁₄O₂)⁺, 136.9 (C₇H₆O₃)⁺, 134.9 (C₉H₁₀O)⁺, 110 (C₆H₆O₂), 92.9 (C₆H₆O), 78.9 (C₆H₅O)⁴.

HFa: 2-(4-fluorophenyl)-7-hydroxy-4H-chromen-4-one
MP: 195 - 198 °C; Rf = 0.43; % yield = 49.2 % w/w; UV λmax: CHCl₃, nm: 234 (IR (KBr cm⁻¹): 1660 (CO str), 1597, 1550 (C=C arom.str), 1124, 1066 (COC str), 752 (C-C bending) 3093, 3485 (OH str); 1H NMR (500 MHZ, DMSO): δ 3.8 (s, OCH₃, ArH), 6.9 (m, 1H, ArH), 7.0 - 7.7 (m, 7H, ArH); m/z: 238 (m+1), 121 (C₇H₈O₂)⁺, 92.7 (C₆H₇O)⁺, 164 (C₆H₈O₂)⁺, 137.5 (C₆H₉O₂)⁺, 76.7 (C₆H₅O)⁴.

HFb: 7-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one
MP: 181-183 °C; Rf = 0.54; % yield = 57.5 % 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); 1H 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 (C₉H₁₀O³)⁺, 111.3 (C₆H₆S)⁺, 123.3 (C₉H₁₀O²)⁺, 76.5 (C₆H₅O)⁴.

HFc: 6-hydroxy-3-(4-methoxyphenyl)-4H-1-benzopyran-4-one
MP: 185-189 °C; Rf = 0.47; % yield = 58.3 % w/w; UV λmax: CH₂OH, nm: 293; IR (KBr cm⁻¹): 1735 (lactone), 1685 (CO str), 1523, 1573 (C=C arom.str), 1091, 1111, 1130 (COC str), 761 (C-C bending), 3093 (CH str), 3424 (OH str); 1H NMR(500 MHZ, DMSO): δ 3.8 (s, 6H, OCH₃), 7.0 (m, 1H, ArH), 7.0 – 7.5 (m, 6H, ArH), 12.6 (s, 1H, OH); m/z: 298 (m+1), 139 (C₇H₆O₃)⁺, 165.8 (C₉H₁₀O₃), 149.9 (C₉H₁₀O₂), 95.12 (C₆H₈O), 78 (C₆H₅O)⁴.

HFd: 2-(2,4-dimethoxyphenyl)-7-hydroxy-4H-chromen-4-one
MP: 194-199 °C; Rf = 0.76; % yield = 45.4 % w/w; UV λmax: DMF, nm: 234; 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); 1H 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 (C₉H₁₀O³)⁺, 111.3 (C₆H₆S)⁺, 123.3 (C₉H₁₀O²)⁺, 76.5 (C₆H₅O)⁴.
One way Analysis of Variance (ANOVA) followed by Dunnett’s test was performed as the test of significance.

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

<table>
<thead>
<tr>
<th>Compound code</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;a&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;(2OH)</td>
</tr>
<tr>
<td>F&lt;sub&gt;b&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;(4OH)</td>
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<tr>
<td>F&lt;sub&gt;c&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;-4(OCH&lt;sub&gt;3&lt;/sub&gt;)</td>
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<tr>
<td>F&lt;sub&gt;d&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;-2,4(OCH&lt;sub&gt;3&lt;/sub&gt;)</td>
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<tr>
<td>F&lt;sub&gt;e&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;-4(CH&lt;sub&gt;2&lt;/sub&gt;)</td>
</tr>
<tr>
<td>HF&lt;sub&gt;a&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;-4(F)</td>
</tr>
<tr>
<td>HF&lt;sub&gt;b&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;-4(4OH)</td>
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<tr>
<td>HF&lt;sub&gt;c&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;-4(OCH&lt;sub&gt;3&lt;/sub&gt;)</td>
</tr>
<tr>
<td>HF&lt;sub&gt;d&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;-2,4(OCH&lt;sub&gt;3&lt;/sub&gt;)</td>
</tr>
<tr>
<td>HF&lt;sub&gt;e&lt;/sub&gt;</td>
<td>-H</td>
<td>-C&lt;sub&gt;H&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;-4(S)</td>
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**Table 2.** Overall Docking score of synthesized flavones

<table>
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<tr>
<th>Ligand</th>
<th>Docking score (kcal/mole)</th>
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<tbody>
<tr>
<td></td>
<td>Aldose reductase</td>
</tr>
<tr>
<td>F&lt;sub&gt;a&lt;/sub&gt;</td>
<td>-7.03</td>
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<tr>
<td>F&lt;sub&gt;b&lt;/sub&gt;</td>
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<tr>
<td>Ertiprotifab</td>
<td>-8.72</td>
</tr>
<tr>
<td>Acarbose</td>
<td>-7.61</td>
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**Table 3.** Effect of synthesised flavones on Alpha amylase inhibitory assay

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% Inhibition of Alpha amylase</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; Values</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10 (µg/ml) 20 (µg/ml) 40 (µg/ml) 80 (µg/ml) 160 (µg/ml)</td>
<td></td>
</tr>
<tr>
<td>F&lt;sub&gt;a&lt;/sub&gt;</td>
<td>29.5±1.47 42.3±2.38 59.7±2.77 73.3±2.86 86.2±1.93</td>
<td>38.3</td>
</tr>
<tr>
<td>F&lt;sub&gt;b&lt;/sub&gt;</td>
<td>30.8±2.14 43.7±2.22 59.6±2.29 71.9±1.62 84.8±2.37</td>
<td>37.05</td>
</tr>
<tr>
<td>F&lt;sub&gt;c&lt;/sub&gt;</td>
<td>32.6±2.28 45.5±2.47 63.0±2.81 73.4±2.77 86.4±2.24</td>
<td>32.52</td>
</tr>
<tr>
<td>F&lt;sub&gt;d&lt;/sub&gt;</td>
<td>33.5±2.34 46.1±2.56 59.7±2.65 74.8±2.51 88.3±2.54</td>
<td>30.9</td>
</tr>
<tr>
<td>F&lt;sub&gt;e&lt;/sub&gt;</td>
<td>31.7±2.08 44.8±2.15 57.3±3.11 72.1±2.68 85.8±2.98</td>
<td>36.8</td>
</tr>
<tr>
<td>HF&lt;sub&gt;a&lt;/sub&gt;</td>
<td>32.1±2.45 46.8±1.88 59.5±3.48 74.1±2.56 86.2±2.41</td>
<td>32.0</td>
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<tr>
<td>HF&lt;sub&gt;b&lt;/sub&gt;</td>
<td>34.9±2.18 45.8±1.67 58.6±3.36 70.6±2.29 82.3±2.32</td>
<td>32.9</td>
</tr>
<tr>
<td>HF&lt;sub&gt;c&lt;/sub&gt;</td>
<td>36.3±1.98 47.5±1.54 60.8±3.59 75.3±1.73 87.4±2.76</td>
<td>25.8</td>
</tr>
<tr>
<td>HF&lt;sub&gt;d&lt;/sub&gt;</td>
<td>36.8±2.31 48.5±1.72 62.7±3.28 73.6±2.28 88.2±2.63</td>
<td>23.6</td>
</tr>
<tr>
<td>HF&lt;sub&gt;e&lt;/sub&gt;</td>
<td>33.7±2.57 46.8±2.29 59.3±3.22 73.2±2.14 87.3±2.54</td>
<td>31.1</td>
</tr>
<tr>
<td>Acarbose</td>
<td>36.9±2.68 48.9±1.84 63.7±3.43 78.2±2.62 94.3±2.57</td>
<td>21.2</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Fasting Blood Glucose Level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day 7&lt;sup&gt;th&lt;/sup&gt; day 14&lt;sup&gt;th&lt;/sup&gt; day 21&lt;sup&gt;st&lt;/sup&gt; day</td>
</tr>
<tr>
<td>Normal Control</td>
<td>94.2±7.03 97.2±7.65 96.6±7.65 95.8±7.16</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>267.6±7.93&lt;sup&gt;a&lt;/sup&gt; 282.8±7.80&lt;sup&gt;b&lt;/sup&gt; 311.6±7.91&lt;sup&gt;a&lt;/sup&gt; 318.4±7.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glisebenclamide</td>
<td>246.6±8.18&lt;sup&gt;b&lt;/sup&gt; 187.8±8.03&lt;sup&gt;a&lt;/sup&gt; 148.2±8.46&lt;sup&gt;b&lt;/sup&gt; 112.8±8.98&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF&lt;sub&gt;a&lt;/sub&gt;</td>
<td>260.8±8.33 272.6±8.96 299.8±8.09 307.2±7.90</td>
</tr>
<tr>
<td>HF&lt;sub&gt;b&lt;/sub&gt;</td>
<td>259.8±8.60&lt;sup&gt;a&lt;/sup&gt; 233.4±8.85&lt;sup&gt;b&lt;/sup&gt; 182.2±7.48&lt;sup&gt;b&lt;/sup&gt; 151.6±7.73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF&lt;sub&gt;c&lt;/sub&gt;</td>
<td>253.8±8.53&lt;sup&gt;b&lt;/sup&gt; 215.8±8.48&lt;sup&gt;b&lt;/sup&gt; 162.5±8.33&lt;sup&gt;b&lt;/sup&gt; 130.8±8.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF&lt;sub&gt;d&lt;/sub&gt;</td>
<td>248.6±8.97&lt;sup&gt;b&lt;/sup&gt; 208.8±9.22&lt;sup&gt;b&lt;/sup&gt; 159.2±8.27&lt;sup&gt;b&lt;/sup&gt; 122.6±8.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF&lt;sub&gt;e&lt;/sub&gt;</td>
<td>250.4±8.46&lt;sup&gt;b&lt;/sup&gt; 223.2±8.31&lt;sup&gt;b&lt;/sup&gt; 171.6±7.45&lt;sup&gt;b&lt;/sup&gt; 145.2±8.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD for n = 5; One way Analysis of Variance (ANOVA) followed by Dunnett’s test. <sup>a</sup>P < 0.01 = significant as compared with normal, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.05 = Significant as compared with diabetic control.
By which, docking studies of the titled compound revealed that to overcome the diabetic complication on long term therapy [27]. Complications such as retinopathy, neuropathy and nephropathy of sorbitol which leads to tissue injury results in major diabetic high acceleration of polyol pathway, increases the accumulation case of diabetes, which can reached up to 30% [23-24]. Due to for utilisation of glucose up to 3% in normal healthy adults but in clearly depicted that the polyol pathway is an alternate mechanism galactitol were accumulated on the ocular lens of the rat. They cataractogenesis, also they derived the presence of sorbitol and aldose reductase were noted on diabetic rat lens during presence of sorbitol dehydrogenase by the usage of NADPH as an enzyme.

Docking score of the compounds were given in the table.2. Studies. Binding affinity of hybridised flavones with active site of enzymes.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>BLOOD UREA (mg/dl)</th>
<th>SERUM CREATININE (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>15.7±0.53</td>
<td>0.66±0.03</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>30.8±1.01</td>
<td>0.97±0.04</td>
</tr>
<tr>
<td>Gilbenamide</td>
<td>19.4±0.92</td>
<td>0.71±0.05</td>
</tr>
<tr>
<td>HF₆</td>
<td>28.6±1.12</td>
<td>0.92±0.04</td>
</tr>
<tr>
<td>HF₅</td>
<td>24.1±1.35</td>
<td>0.85±0.04</td>
</tr>
<tr>
<td>HF₄</td>
<td>23.2±1.07</td>
<td>0.80±0.04</td>
</tr>
<tr>
<td>HF₃</td>
<td>21.5±1.56</td>
<td>0.74±0.04</td>
</tr>
<tr>
<td>HF₂</td>
<td>22.8±1.20</td>
<td>0.86±0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for n = 5; One way Analysis of Variance (ANOVA) followed by Dunnett’s test. *P < 0.01, **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.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>SGOT (IU/l)</th>
<th>SGPT (IU/l)</th>
<th>ALP (IU/l)</th>
<th>TOTAL BILIRUBIN (mg/dl)</th>
<th>TOTAL PROTEIN (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>54.6±4.50</td>
<td>36.6±4.77</td>
<td>104.6±4.77</td>
<td>0.56±0.04</td>
<td>6.95±0.14</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>97.6±5.72</td>
<td>79.2±5.40</td>
<td>229.6±4.62</td>
<td>1.04±0.04</td>
<td>4.84±0.16</td>
</tr>
<tr>
<td>Gilbenamide</td>
<td>57.6±4.50</td>
<td>41.6±4.72</td>
<td>119.2±5.40</td>
<td>0.63±0.04</td>
<td>6.51±0.15</td>
</tr>
<tr>
<td>HF₆</td>
<td>87.4±4.21</td>
<td>71.4±5.32</td>
<td>221.2±4.60</td>
<td>0.95±0.03</td>
<td>4.94±0.19</td>
</tr>
<tr>
<td>HF₅</td>
<td>76.4±4.61</td>
<td>60.2±5.44</td>
<td>170.2±5.07</td>
<td>0.85±0.05</td>
<td>5.89±0.21</td>
</tr>
<tr>
<td>HF₄</td>
<td>64.2±5.78</td>
<td>53.6±5.12</td>
<td>163.4±4.64</td>
<td>0.76±0.05</td>
<td>6.28±0.17</td>
</tr>
<tr>
<td>HF₃</td>
<td>60.4±4.61</td>
<td>44.2±5.63</td>
<td>141.8±4.97</td>
<td>0.68±0.05</td>
<td>6.40±0.12</td>
</tr>
<tr>
<td>HF₂</td>
<td>71.4±5.94</td>
<td>55.8±5.45</td>
<td>153.4±5.84</td>
<td>0.71±0.04</td>
<td>6.02±0.15</td>
</tr>
</tbody>
</table>

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

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

Another target was Protein tyrosine phosphatase (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 PTP 1B inhibitor, the compounds HF₆ & HF₂ (-8.52 & -8.89 kcal/mole respectively) posses the high potent inhibitory activity compared with standard eritiprotafib (-8.72 kcal/mole) a known PTP1B enzyme inhibitor. The compound HF₂ showed hydrogen bond affinity towards the methoxy group of oxygen on the residues aswell as thiophenyl ring (HF₂) 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 amyrase, 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 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 HF₆ & HF₂ (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 exhibited by electron donating substituent’s over those compounds. The presence of hydroxyl group and di-methoxy group on the compound HF₆ 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.
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 HF₆ & HF₄ 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 blood, which increases gluconeogenesis and ketogenesis those insulin. This is because of availability of amino acids in diabetic condition, the level of transaminase in liver such as SGOT, SGPT and AutoDock4.2, downloaded on 25 July 2011.

Anti-Diabetic Activity by (STZ induced Model)

Based on the docking score and alpha amylase inhibitory assay, highly active derivatives were selected (HF₆-HF₄) 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 HF₆, HF₄ & HF₃ 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 synthesised compounds and afforded their binding score. The affinity of compounds over this enzyme by three common residues they are His 15, Arg 337 & Gin 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


