Development of Murrayanine-Chalcone hybrids: An effort to combine two privilege scaffolds for enhancing hypoglycemic activity

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Abstract

Murrayanine and chalcone are well known for their anti-diabetic potentials. The present research represents an effort is represented in which the two scaffolds wererationally integrated with an objective that the designed hybrid molecule will cumulatively produce higher pharmacological activity than their parents, and will also illustrate analogous or more pronounced activity with the marketed product. All the novel murrayanine-chalcone hybrids exhibited activity in the range of 6.27-30.87%. The molecules 3a, 3f, 3g, and 3h exhibited an effective reduction of blood glucose level (>18%). Chalcone 3g, having two lipophilic substituents at meta positions in the B-ring presented the highest anti-hyperglycemic activity (30.87% lowering of glucose level). From the study, it was evidenced that the positions of substitution and their number played an intense role in exhibiting anti-diabetic activity. A superior biological activity was observed with electrophilic halogen substituents at para and meta positions as compared to ortho position. However, a very well defined structure-activity-relationship (SAR) cannot be defined from this study. The study opened new avenues for utilizing the natural scaffolds in form of hybrids as anti-diabetic agents which may act by modulating various anti-diabetic targets. Still, further research is needed to elucidate the complete mechanism of action(s) of the synthesized hybrids.

Keywords: Murrayanine; Chalcone; Hybrid; Antihyperglycemic; Antidiabetic; Hypoglycemic.

Introduction

Diabetes Mellitus (DM) refers to a persistent metabolic disorder of carbohydrate, primarily exemplified by high blood sugar, caused due to lack of insulin level in the body (in Diabetes Mellitus Type-1, DMT1) or due to insulin resistance (in Diabetes Mellitus Type-2, DMT2). At present, in the case of DMT1, the only available therapy involved is insulin administration for the purpose of compensation.(1) In contrast, the drugs produce effectual management of hyperglycemic phases by modulating various molecular targets in DMT2 conditions. DMT2 is fast progressing in this century across the world. About 400 million individuals suffer from this disease and are predicted to invite nearly 200 million more by the ending of 2030. In DMT2, the metabolic disturbance originates due to postprandial surge.(2) Presently, USFDA has approved five prime classes of drugs for the management of hyperglycemia: dipeptidyl peptidase-4 (DPP-4) inhibitors, peroxisome proliferator activated receptor-γ (PPAR-γ), protein tyrosine phosphatase 1B (PTP1B) inhibitors, α-glucosidase inhibitors, and aldose reductase (ALR) inhibitors. However, they have several minor to major complications, demonstrate possible adverse effects, and generally produce an insufficient duration of hyperglycemic control lead to suffering and reduction in quality of life of individuals.(3) With the pace of time, as the technology become smarter and drugs are so. In today’s avenue, it is recommended that those compounds which have the perspective of increasing the sensitivity of muscular tissues and adipose tissues to insulin are preferred for therapeutics.

There is an imperative requirement of an agent that have the ability to act as anti-hyperglycemics with the similar degree of efficacy with the marketed product along with reduced therapeutic complications. Based on the fact that nature-based small molecular weight ligands have good recognition of glucose lowering properties.(4) Hybrid molecules have such reputation for a long time and right now are booming with great pace at present, owing to their combined attributes of the scaffolds. Therefore, in order to design such analogs with pronounced glucose lowering potentials, we initially inspired from nature and designed a series of hybrid molecules based on murrayanine and chalcone.

Murrayanine is a carbazole product present in traditional herb of Indian origin, Murraya koenigii L. or Curry tree (Rutaceae). The plant is known for centuries for its ethnopharmacological significance in treating elevated blood glucose levels. The aqueous, hydroalcoholic, and alcohol extracts of the leaves and stem bark have been reported to exhibit anti-diabetic activity due to the carbazole principles present in it. The carbazole alkaloids and other phytochemicals; murrayamine, mahanine, mahanimbine, mahaminbicine, murrayacine, koenoline, murrayafoline A, mahambilone, etc. are well known for their low to moderate anti-diabetic activity, as performed on various animal models.(5) Chalcones, chemically known as (E)-1,3-diphenyl-2-propene-1-one is a precursor of flavonoids and open
chain intermediates in the aurone synthesis of flavones. These are natural products with a benzylideneacetophenone scaffold in which two aromatic nuclei are connected via three carbon α, β unsaturated carbonyl bridge. They are well known for exhibiting multifarious pharmacological activities like MDRC inhibition, anti-arrhythmic, antiplatelet, anti-steroidal, anti-obesity, anti-nociceptive, hypolipidemic, anti-spasmodic, anti-diabetic, anxiolytic, anti-neoplastic, anti-ulcer, anti-angiogenic, immunosuppressant, anti-retroviral, anti-inflammatory, osteogenic, anti-histaminic, hypnotic, anti-oxidant, anti-tubercular, anti-invasive, antimarial, anti-bacterial, anti-fungal, antifilarial, anti-protozoal, and anti-gout. They are well known insulin sensitizer along with their property to modulate diverse anti-diabetic targets.\(^{(6-9)}\)

Therefore, an effort is represented in which the two scaffolds with already reported anti-diabetic activity; murrayanine and chalcone were rationally integrated with an objective that the designed hybrid molecule will cumulatively produce higher pharmacological activity than their parents, and will also illustrate analogous activity with the marketed product (Fig. 1).

![Murrayanine - Chalcone Hybrids](image)

**Fig. 1: Rational behind the development of murrayanine-chalcone hybrids**

**Materials and Method**

**Chemical and Instrumentation:** Merck, HiMedia, and Sigma-Aldrich remained the chief sources for procuring chemicals and solvents. Streptozotocin was obtained from HiMedia Ltd., India. The Glucose strips (One Touch\textsuperscript{TM}) were purchased from a local pharmacy. The prepared compounds were characterized by following techniques: melting point apparatus (Perfit), precoated thin layer chromatography plates (Merck), elemental analyses (Perkin-Elmer 240C analyzer), FT-IR spectra instrument (IRAffinity-1), \(^1\)H-NMR (400 MHz) spectra instrument (Bruker spectrospin NMR DPX-300) using TMS as internal standard, and mass spectra instrument (JEOL-JMS-DX 303).

**Animals:** Swiss albino rat (average weight 170-260 g, aged 5-6 weeks) were utilized for screening the anti-diabetic activity of the synthesized hybrids. After receiving approval from Department Ethical Committee, the animals were used from the animal house; kept in proper hygienic conditions, free access to water, fed standard rodent pellets, with 6 mice per cage enclosure and specified environment (24–25°C temperature, humidity 50–60%, 12 hr light and dark).

**Extraction of murrayanine:** The starting material, murrayanine was extracted from *Murraya koenigii* L.by soxhlation as per our previously reported protocol in which powdered *M. koenigii* stem bark was extracted with n-hexane.\(^{(5)}\) The silica gel-based column chromatography was utilized for isolation of desired product from the concentrated extract employing eluant mixtures in the following order: hexane, hexane/ethyl acetate, ethyl acetate, ethyl acetate/methanol and methanol. All the produced fractions were examined by thin layer chromatography in which murrayanine (1) appeared in the fractions B\textsubscript{21}-B\textsubscript{37} of hexane extract.

**Synthesis of target compounds:** The newly synthesized compounds were fabricated from the murrayanine (1), the active carbazole principle obtained from the stem bark of *M. koenigii* L. For developing the derivatives 3a-3f, the aldehydic (-CHO) portion of murrayanine (1) was exploited. The aldehydic part reacted successfully with the acetyl portion (-COCH\textsubscript{3}) of acetophenone. The mechanism of chalcone preparation involves aldol condensation in which an enol or an enolate ion reacts with a carbonyl compound to form β-hydroxyketone or β-hydroxyaldehyde with dehydration to form conjugated enone. **Scheme 1** describes the synthetic outline.
1-methoxy-9H-carbazole-3-carbaldehyde (1)
m.p.: 165-167°C, Rf: 0.47, hexane: ethyl acetate: methanol (7:2:1). FTIR (KBr) ν (cm⁻¹): 3250 (-NH), 3081 (C-H, aromatic), 1722 (C=O), 1295 (C=O). ¹H NMR (δ, ppm, DMSO-d₆): 10.4 (9, 1H), 9.81 (4, 1H), 7.2-8.8 (Aromatic, 6H). 3.86 (1, 3H). MS: M⁺ [M+225; 181 (30%)]. Anal. Calcd. for C₂₉H₁₉NO₂: C, 74.65; H, 4.92; N, 6.22. Found: C, 74.56; H, 4.88; N, 6.03.

Synthetic protocol for (E)-4-(4-substituted-phenyl)-2-(2-((1-methoxy-9H-carbazol-3-yl)carbonyl)hydrazinyl)thiazole (3a-3h)

Equal quantity (0.01 M) of murrayanine (1) and substituted acetonaphenones (2a-h) were stirred in 90% ethanol (25 mL) and then an aqeous solution of sodium hydroxide (20 mL) was added to it. The content was refluxed for a period of 4 hr and the reaction mixture was kept overnight. Next day, the crushed ice was poured into the mixture and the content was further acidified with dilute HCl. The hybrid products were precipitated out as a solid, which was then filtered and recrystallized suitably.

(E)-3-(2-fluorophenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one (3a)
77% yield; FTIR (KBr) ν (cm⁻¹): 3264 (-NH, stretch), 3105 (C-H, aromatic), 1724 (C=O), 1664 (C=C, aromatic), 1611 (C=C, aromatic), 1598 (-NH, bending), 1214 (C-O), 1058 (C-F). ¹H NMR (δ, ppm, CDCl₃): 10.13 (9, 1H), 7.2-8.3 (Aromatic, 10H), 3.90 (1, 3H). MS: M⁺345. Anal. Calcd. for C₂₉H₁₉FNO: C, 76.51; H, 4.67; N, 4.06. Found: C, 76.12; H, 4.33; N, 3.98.

(E)-3-(4-fluorophenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one (3b)
69% yield; FTIR (KBr) ν (cm⁻¹): 3286 (-NH, stretch), 3120 (C-H, aromatic), 1706 (C=O), 1652 (C=C, aromatic), 1627 (C=C, aromatic), 1569 (-NH, bending), 1199 (C-O), 1087 (C-F). ¹H NMR (δ, ppm, CDCl₃): 10.16 (9, 1H), 7.1-8.5 (Aromatic, 10H), 3.94 (1, 3H). MS: M⁺354. Anal. Calcd. for C₂₉H₁₉FNO: C, 76.51; H, 4.67; N, 4.06. Found: C, 76.23; H, 4.44; N, 3.94.

(E)-3-(2-iodophenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one (3c)
41% yield; FTIR (KBr) ν (cm⁻¹): 3243 (-NH, stretch), 3137 (C-H, aromatic), 1741 (C=O), 1684 (C=C, aromatic), 1646 (C=C, aromatic), 1566 (-NH, bending), 1232 (C-O), 726 (C-I). ¹H NMR (δ, ppm, CDCl₃): 10.19 (9, 1H), 7.1-8.4 (Aromatic, 10H), 3.92 (1, 3H). MS: M⁺453, M⁺+2 479. Anal. Calcd. for C₂₉H₁₉I₂NO: C, 58.30; H, 3.56; N, 3.09. Found: C, 57.87; H, 3.36; N, 2.99.

(E)-3-(4-iodophenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one (3d)
48% yield; FTIR (KBr) ν (cm⁻¹): 3224 (-NH, stretch), 3082 (C-H, aromatic), 1719 (C=O), 1647 (C=C, aromatic), 1618 (C=C, aromatic), 1613 (-NH, bending), 1246 (C-O), 774 (C-F). ¹H NMR (δ, ppm, CDCl₃): 10.18 (9, 1H), 7.2-8.6 (Aromatic, 10H), 3.88 (1, 3H). MS: M⁺453. Anal. Calcd. for C₂₉H₁₉I₂NO: C, 58.30; H, 3.56; N, 3.09. Found: C, 58.06; H, 3.34; N, 2.95.

(E)-3-(4-bromophenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one (3e)
39% yield; FTIR (KBr) ν (cm⁻¹): 3277 (-NH, stretch), 3135 (C-H, aromatic), 1734 (C=O), 1644 (C=C, aromatic), 1610 (C=C, aromatic), 1568 (-NH, bending), 1229 (C-O), 697 (C-Br). ¹H NMR (δ, ppm, CDCl₃): 10.14 (9, 1H), 7.2-8.5 (Aromatic, 10H), 3.90 (1, 3H). MS: M⁺405, M⁺+2 407. Anal. Calcd. for C₂₉H₁₉BrNO: C, 65.04; H, 3.97; N, 3.45. Found: C, 64.82; H, 3.88; N, 3.27.

(E)-1-(1-methoxy-9H-carbazol-3-yl)-3-(2-(trifluoromethyl)phenoxy)prop-2-en-1-one (3f)
52% yield; FTIR (KBr) ν (cm⁻¹): 3239 (-NH, stretch), 3121 (C-H, aromatic), 1703 (C=O), 1666 (C=C, aromatic), 1616 (C=C, aromatic), 1594 (-NH, bending), 1222 (C-O), 1074 (C-F). ¹H NMR (δ, ppm, CDCl₃): 10.11 (9, 1H), 7.2-8.4 (Aromatic, 10H), 3.89 (1, 3H). MS: M⁺395. Anal. Calcd. for C₂₉H₁₈F₂NO: C, 69.87; H, 4.08; N, 3.45. Found: C, 69.68; H, 3.97; N, 3.28.

(E)-3-(3,5-bis(trifluoromethyl)phenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one (3g)
66% yield; FTIR (KBr) ν (cm⁻¹): 3294 (-NH, stretch), 3114 (C-H, aromatic), 1712 (C=O), 1676 (C=C, aromatic), 1608 (C=C, aromatic), 1553 (-NH, bending), 1248 (C-O), 1065 (C-F). ¹H NMR (δ, ppm, CDCl₃): 10.23 (9, 1H), 7.3-8.4 (Aromatic, 9H), 3.91 (1, 3H). MS: M⁺416. Anal. Calcd. for C₃₀H₂₁F₂NO: C, 62.21; H, 3.26; N, 3.02. Found: C, 62.04; H, 3.08; N, 2.85.

(E)-3-(2,4-dichloro-5-fluorophenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one (3h)
47% yield; FTIR (KBr) ν (cm⁻¹): 3274 (-NH, stretch), 3128 (C-H, aromatic), 1737 (C=O), 1688 (C=C, aromatic), 1620 (C=C, aromatic), 1562 (-NH, bending), 1208 (C-O), 1026 (C-F), 693 (C-Cl). ¹H NMR (δ, ppm, CDCl₃): 10.18 (9, 1H), 7.2-8.5 (Aromatic, 8H), 3.93 (1, 3H). MS: M⁺413, M⁺+2 415. Anal. Calcd. for C₂₉H₁₈Cl₂NO: C, 63.79; H, 3.41; N, 3.38. Found: C, 63.53; H, 3.24; N, 3.19.

Anti-diabetic screening: The anti-diabetic study was performed according to the protocol described by Satyanarayana et al.¹⁰ The rats demonstrating blood glucose level of range 60-75 mg/dl were initially selected. Streptozotocin solution (prepared by dissolving 100 mM citrate buffer of pH 4.5) was administered to the fasted male albino rats at a dose of 60 mg/kg body weight i.p. After the lapse of 48 hr, the blood glucose was monitored by commercial glucose strips. Only those rats were selected further which presented elevated levels.
(200–300 mg/dl) of blood glucose. On the 4th day, the blood glucose level was re-measured to authenticate the steady state hyperglycemia. The chosen animals were divided into 2 groups, each consisting of 6 rats. The first group (control) received only 1% gum acacia (carrier). The second group involved the standard drug (glibenclamide) for comparing the hypoglycemic activity. The successive groups were fed orally with synthesized hybrid derivatives (100 mg/kg body weight dose in 1% gum acacia). The glucose profile of each rat was determined using the glucometer. Firstly, a sugar load of 5 g/kg was given to each animal orally and following that the test sample was administered after 30 min. The glucose lowering attribute of the analogs was scrutinized at time intervals of 0 hr, 1 hr, 3 hr, and 6 hr. The ability of murrayanine-chalcone hybrids to reduce the blood glucose level was calculated according to the AUC method and expressed in percentage (%).

**Result and Discussion**

**Chemistry:** The IR spectra reflected several essential information of the hybrid derivative. The amide stretching was predominantly located by the peaks in the range 3224-3294 cm\(^{-1}\) and the bending was detected in the range 1553-1613 cm\(^{-1}\). The aromatic C-H stretching was noticed in the range 3082-3135 cm\(^{-1}\) and aromatic C=C stretching was observed in the range 1644-1684 cm\(^{-1}\). The appearance of aliphatic C=C stretching confirmed that the aldehydes get converted into the chalcone product. The \(^1\)H NMR recommended that the proposed structure was synthesized splendidly. The carboxolic protons were chiefly noticed at the range of 10.1-10.4 ppm. The peaks of the aromatic protons were noticed at 7.1-8.9 ppm. The mass spectra portrayed that the base peak was matching or very similar to the molecular weight of the fabricated products. The halogen (particularly, chlorine and bromine) isotope forms were monitored as molecular mass +2 (plus two) mass, along with the appearance of several fragment peaks of m/z in the range of 100-200. The elemental analysis described that the elements (C, H, and N) were in a quite close agreement with that of theoretical value, which further confirmed the formation of desired hybrid compounds.

**Anti-diabetic activity:** The experimental attempt highlighted that the fabricated hybrids demonstrated impressive anti-hyperglycemic activity at a dose of 100 mg/kg. The molecules 3a, 3f, 3g, and 3h exhibited an effective reduction of blood glucose level (>18%). The results were found to be comparable with standard drug glibenclamide which displayed 38.49% hypoglycemic control. The hybrids also demonstrated better control of glucose level as compared to single parent molecules; murrayanine and chalcone. Chalcone 3g, having two lipophilic substituents at meta positions in the B-ring presented the highest anti-hyperglycemic activity (30.87% lowering of glucose level). The compounds 3b, 3d, and 3e, having substituents at para position showed moderate glucose lowering activity in range of 9.33-18.17%. The ortho substituted analog (3c) illustrated the lowest anti-diabetic activity (<10%), which is an indicative of the fact that the position of substituents played vital role in therapeutic target modulation. From these observations, a clear cut structure-activity-relationship (SAR) cannot be defined; however, it might be concluded that a superior biological activity was observed with electrophilic halogen substituents at para and meta positions as compared to ortho position. Although, a primary screening of the compounds was done, the detailed mechanism(s) of action needs to be elucidated. The % hypoglycemic control of the synthesized compounds is represented in **Table 1**.

**Table 1:** In vivo anti-hyperglycemic potential of murrayanine-chalcone hybrids (3a–h) in STZ rat models

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>% anti-hyperglycemic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>2-F</td>
<td>19.34</td>
</tr>
<tr>
<td>3b</td>
<td>4-F</td>
<td>13.51</td>
</tr>
<tr>
<td>3c</td>
<td>2-I</td>
<td>6.27</td>
</tr>
<tr>
<td>3d</td>
<td>4-I</td>
<td>9.33</td>
</tr>
<tr>
<td>3e</td>
<td>4-Br</td>
<td>18.17</td>
</tr>
<tr>
<td>3f</td>
<td>2-CF(_3)</td>
<td>21.29</td>
</tr>
<tr>
<td>3g</td>
<td>3.5-CF(_3)</td>
<td>30.87</td>
</tr>
<tr>
<td>3h</td>
<td>2.4-Cl; 5-F</td>
<td>26.74</td>
</tr>
<tr>
<td>Std.</td>
<td>-</td>
<td>38.49</td>
</tr>
</tbody>
</table>

Std. = Standard drug (glibenclamide); Control = 1% gum acacia

**Conclusion**

The present research highlighted the prospective of novel murrayanine-chalcone hybrids in producing hypoglycemic level in the range of 6.27-30.87%. From the study, it was evidenced that the positions of substitution and their number played an intense role in exhibiting anti-diabetic activity. A superior biological activity was observed with electrophilic halogen substituents at para and meta positions as compared to ortho position. However, a very well defined structure-activity-relationship (SAR) cannot be defined from this study. Still, further research is needed to elucidate the complete mechanism of action(s) of the synthesized hybrids.

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**Conflict of Interest**

Authors have no conflict of interest regarding the publication of this article.
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