RESEARCH ARTICLE

Hypoglycemic activity of curcumin synthetic analogues in alloxan-induced diabetic rats

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Abstract

The currently available therapies for type 2 diabetes have been unable to achieve normoglycemic status in the majority of patients. The reason may be attributed to the limitations of the drug itself or its side effects. In an effort to develop potent and safe oral antidiabetic agents, we evaluated the *in vitro* and *in vivo* hypoglycemic effects of 10 synthetic polyphenolic curcumin analogues on alloxan-induced male diabetic albino rats. *In vitro* studies showed 7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione (**4**) to be the most potential hypoglycemic agent followed by 1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (**10**). Structure activity relationship (SAR) of the tested compounds was elucidated and the results were interpreted in terms of *in vitro* hypoglycemic activities. Furthermore, oral glucose tolerance test (OGTT) with compounds **4**, **10** and reference hypoglycemic drug glipizide showed that compound **4** and glipizide had relatively similar effects on the reduction of blood glucose levels within 2 h. Thus, compound **4** might be regarded as a potential hypoglycemic agent being able to reduce glucose concentration both *in vitro* and *in vivo*.

Introduction

Diabetes mellitus is characterized by chronic hyperglycemia with abnormal metabolism of carbohydrate, fat and protein resulting from defects either in insulin secretion, insulin action or both. Currently available treatments for type 2 diabetes include insulin and various oral antidiabetic agents, such as sulfonylureas, biguanides and glucosidase inhibitors. Most of the oral antidiabetic agents have a number of serious adverse effects[1,2]. Hence, there is a growing interest in herbal antidiabetic remedies due to their effectiveness, minimal clinical side effects and relatively low costs[3]. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown.

World Health Organization (WHO) approved the use of plant drugs for different diseases including diabetes mellitus[4]. It has been well established that diabetes is associated with low level of antioxidants and many plants show hypoglycemic property due to their antioxidant potential[5]. Many medically used antidiabetic compounds are either derived directly from plants or as a synthesized form. It is also known that many of the synthetic products, such as polyphenols are originally natural products[6].

Curcumin is a member of the linear diarylheptanoid natural products family, in which two oxy-substituted aryl moieties are linked together through a seven-carbon chain. Curcumin exists as different tautomers: a 1,3-diketo form and two equivalent enol forms (Scheme 1). The enol is the prevalent form both in the solid phase and in solution[7].

The curcumin analogues can be divided into three groups: analogues from turmeric, analogues from Mother Nature and synthetic analogues[8]. Most of the curcumin analogues are not obtained from curcumin but rather have been synthesized from smaller synthons. Curcuminoids are usually assembled from arylaldehydes and acetylacetone, and this route enables synthesis of a diverse set of curcumin analogues starting from arylaldehydes. The curcumin derivatives are generally synthesized by derivatization, starting from curcumin. For example, the phenolic hydroxy group may be acylated, alkylated, glycosylated or amino acylated[9,10].

Besides synthetic features, curcumin has been reported to possess interesting biological activities. Various reports are available on curcumin and its natural and synthetics analogues as anticancer, anti-tumor, anti-arthritic, antiseptic, antibacterial, anti-inflammatory and also antidiabetic agents. The antioxidant activity of curcumin and related compounds has been investigated by a variety of assay systems, under both *in vitro* and *in vivo* conditions[8]. Curcumin has already been the subject of several clinical trials as a treatment in human cancers. Moreover,

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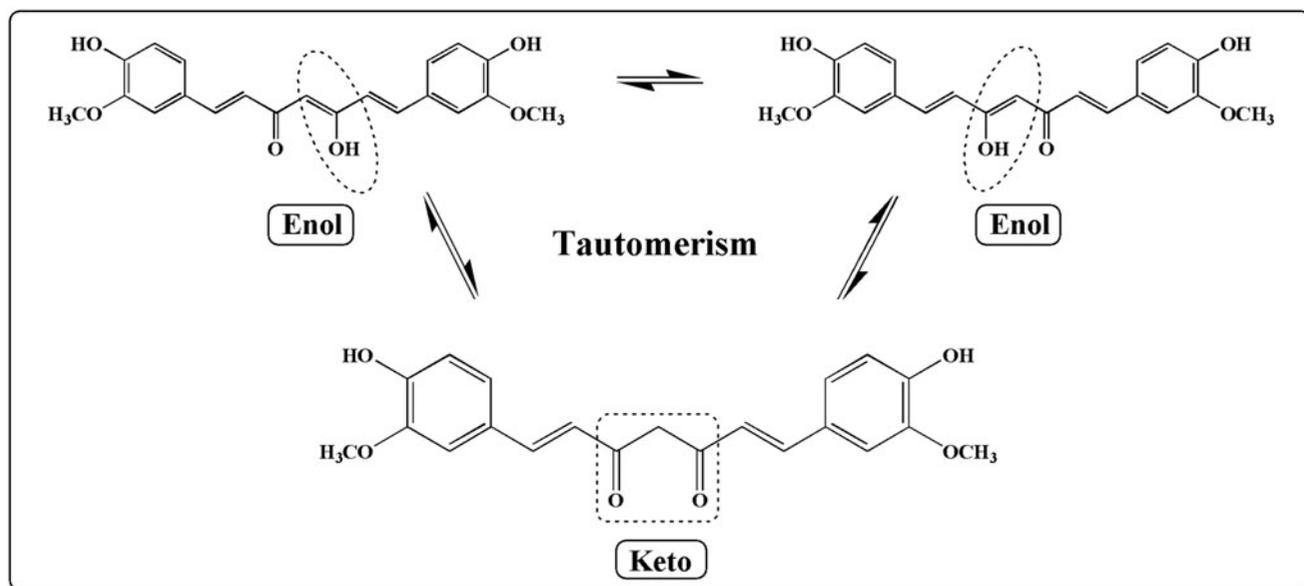
Scheme 1. Tautomeric structures of curcumin.

synthetic chemical modifications of curcumin have been studied intensively to find molecules endowed with better properties compared to those of curcumin[11].

Recent studies reported antidiabetic and hypoglycemic activities of curcumin and the underlying proposed molecular mechanism[12]. Due to our interest in developing modified curcumin structures with the aim of further extending the therapeutic applications of curcumin as privileged chemical scaffold, a few curcumin analogues were used in this study. In particular, curcumin analogues **1–10** (Figure 1) were synthesized and assayed for their hypoglycemic and antidiabetic effects. We selected the following compounds to cover a variety of structural features: (i) curcumin derivatives (**3–5**); (ii) curcuminoids **6** and **10**, characterized by a longer or shorter linker between the aromatic rings, respectively; (iii) curcuminoids **1**, **8** and **9**, in which the shorter linker of **10** is included in a ring; (iv) chalcone **7** representing a further shortening of the central linker; and (v) thiobarbiturate **2**, analog of **1**, **8** and **9**, lacking of the second aromatic moiety (Figure 1).

Materials and methods

Chemistry

Melting points were determined with a Buchi 530 Capillary Apparatus (Flawil, Switzerland) and were uncorrected. The purity of compounds was always >95%, determined by high pressure liquid chromatography (HPLC). HPLC analyses were carried out with a Shimadzu LC-10AD VP CTO-10AC VP instrument (Kyoto, Japan). The column used was generally Discovery Bio Wide Pore C18 (St. Louis, MO) (10 cm × 4.6 mm, 3 μm). Infrared (IR) spectra were recorded on a Perkin-Elmer Spectrum-One spectrophotometer (Waltham, MA). ^{1}H NMR spectra were recorded on a Bruker AC 400 spectrometer (Billerica, MA). Merck silica gel 60 F254 plates were used for analytical TLC. Developed plates were visualized by UV light. Column chromatographies were performed on silica gel (Merck, 70–230 mesh). Concentration of solution after reactions and extractions involved the use of a rotary evaporator operating at reduced pressure of approximately 20 Torr. The purity of newly synthesized compounds has been evaluated by elemental analysis. Analytical results agreed to within ±0.40% of the theoretical values, confirming the ≥95% purity. Dimethylsulfoxide-d_6 99.9% (code 44 139-2) and deuterochloroform 98.8% (code 41 675-4) of

isotopic purity (Aldrich, St. Louis, MO) were used. Solvents were of reagent grade and, when necessary, were purified and dried by standard methods. Organic solutions were dried over anhydrous sodium sulfate (Merck, Darmstadt, Germany).

Maximum wavelength values (λ_{max}) for each synthetic compound dissolved in dimethylsulfoxide was recorded within the range of 300–600 nm at pH 6.5 and room temperature (24.0 °C) (Table 1) by UV-Visible spectrophotometer SL-164, ELICO Ltd. (Hyderabad, India).

General procedure for the synthesis of curcumin analogues (*1–10*)

Compounds **1–6** and **8–10** were synthesized according to the literature[13,14] while derivative **7** was synthesized as reported below.

2,3-Bis(3,4-dihydroxyphenyl)acrylic acid (*7*)

Triethylamine (0.76 mL) was added to a well stirred mixture of 3,4-dihydroxybenzaldehyde (0.50 g, 3.62 mmol) and 3,4-dihydroxyphenylacetic acid in acetic anhydride (8 mL). The mixture was stirred at 40 °C for 12 h. Evaporation of the solvent gave a solid, which was treated with water (5 mL). The mixture was stirred under reflux for 2 h and then at room temperature overnight. The resulting mixture was diluted with water, acidified with 1N HCl and then extracted with ethyl acetate (3 × 50 mL). The organic layer was separated, washed with brine (3 × 100 mL) and dried. Evaporation of the solvent gave crude product which was purified by column chromatography on silica gel (chloroform/methanol, 10:2 as eluent) to obtain pure **7** (0.42 g, 55%). The spectroscopic data of compound **7** were checked and validated with those reported in the literature[15].

Biological assessment

In vitro *hypoglycemic effects*

In vitro hypoglycemic effects of the curcumin synthetic analogues were determined by using glucose oxidase-peroxidase method[16,17]. Dimethylsulfoxide was used to prepare 5×10^{-4} molar solutions of curcumin analogues. D-glucose, O-dianisidine, methanol, monosodium phosphate, disodium phosphate, horseradish peroxidase (Sigma life Sciences, St. Louis, MO), glucose oxidase (Merck Specialties Pvt Ltd, Mumbai, India),
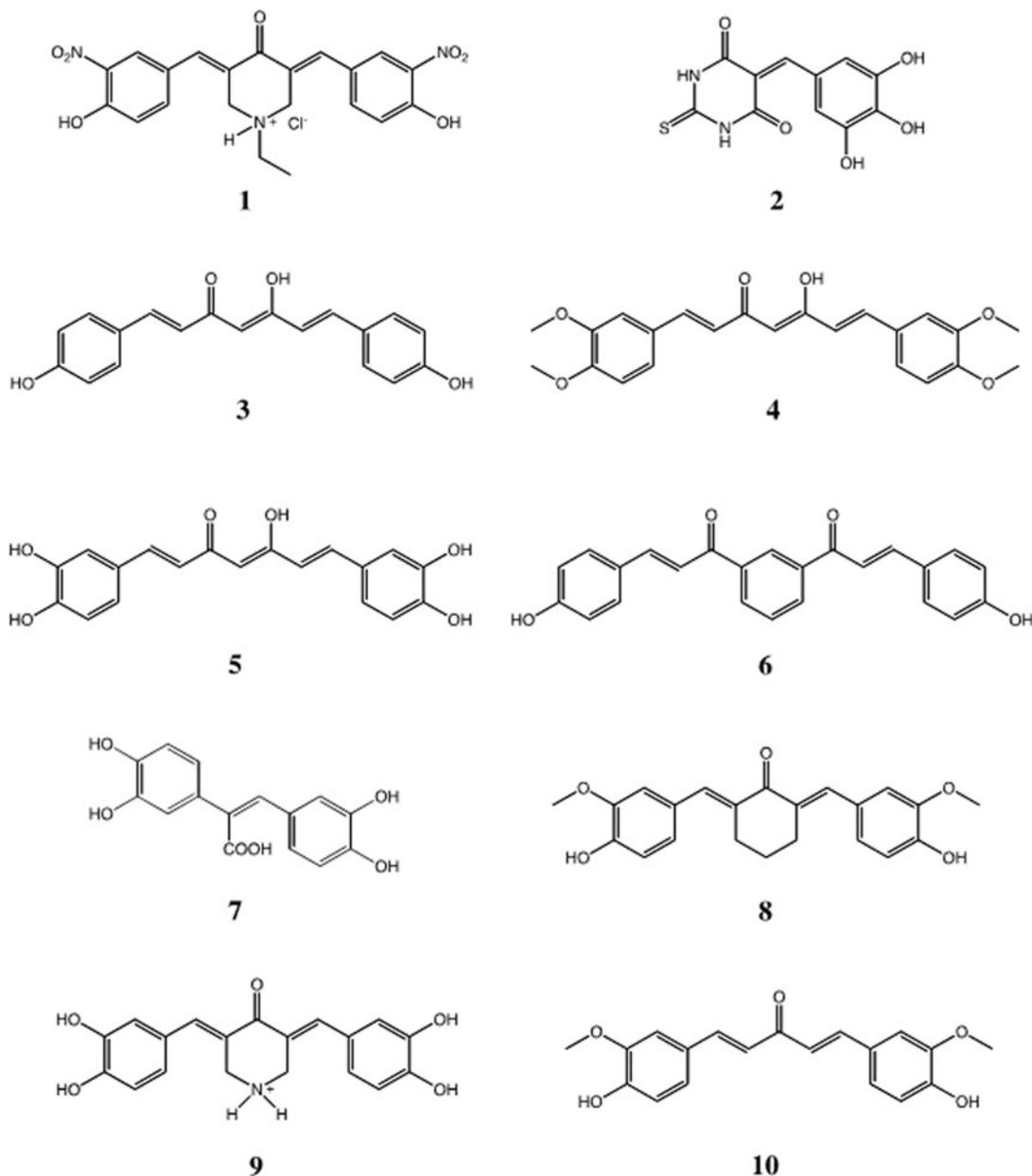
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Figure 1. Structures of synthetic curcumin analogues and curcuminoids used in this study.

Table 1. Peak wavelengths of key curcumin analogue synthetic compounds obtained by UV-VIS Spectrophotometer along with the bibliographic information on their hypoglycemic effect.

Comp. code	Known as hypoglycemic compound	Analogues known as hypoglycemic compound	λ_{max} (nm)
1	No	Yes[14]	417.0
2	No	No	490.2
3	Yes[14,15]	Yes[14]	472.5
4	Yes[15]	Yes[23]	463.0
5	No	Yes[14]	452.0
6	No	Yes[18]	348.0
7	No	No	341.5
8	Yes[14]	Yes[14]	436.5
9	Yes[14]	Yes[14]	432.0
10	Yes[13]	Yes[13]	432.0

λ_{max}: Peak wavelength.

hydrochloric acid (HCl) and triple distilled water were used for this assay.

Preparation of reagents

Glucose oxidase-peroxidase reagent has been prepared as follows: O-dianisidine (5 mg) was dissolved completely in methanol (0.2 mL) and then 9.8 mL of 0.1 M phosphate buffer (pH 6.5) was added to the solution. Finally, peroxidase (1 mg) and glucose oxidase (1 mg) were added to the above prepared O-dianisidine solution.

For the preparation of working standard glucose, firstly, stock solution was prepared by dissolving 100 mg of D-glucose in 100 mL distilled water. Then 1 mL of this stock was diluted up to 10 mL with distilled water.

Preparation of standard calibration graph

Zero (blank), 0.2, 0.4, 0.6, 0.8 and 1.0 mL of glucose working standard were transferred into the series of test tubes and total

volumes were made up to 1.0 mL with triple distilled water to get the glucose concentrations of 20, 40, 60, 80 and 100 mg/dL, respectively. A total of 0.5 mL dimethylsulfoxide and 1 mL of glucose oxidase-peroxidase reagent were added to the all standard solutions. In the next step, all the tubes were incubated at 35 °C for 40 min and the reactions were terminated by adding 6N HCl (2 mL). Finally, optical densities (OD) of the whole samples were recorded at 540 nm by UV-VIS spectrophotometer and standard curves of glucose concentrations (mg/dL) were plotted accordingly.

Glucose concentration of standard glucose solutions in the presence of curcumin analogues

Into a series of test tubes, 0 (blank 1), 0 (blank 2), 0.2, 0.4, 0.6, 0.8 and 1.0 mL of glucose working standard were pipetted out and total volumes were made up to 1.0 mL with triple distilled water. To each solution (except blank 2), 0.5 mL of sample solutions (including curcumin analogues) was added. To the blank 2, 0.5 mL of pure dimethylsulfoxide was added. Subsequently, 1 mL of glucose oxidase-peroxidase reagent was added to all of the tubes and the tubes were incubated at 35 °C for 40 min. The reactions were terminated by adding 6 N HCl (2 mL). Optical densities were recorded with regard to the blanks (blanks 1 and 2) as references at 540 nm using UV-VIS spectrophotometer. The glucose concentration was calculated using the following Equation (1):

$$\text{Glucose conc. of sample solution}$$
$$= \frac{\text{OD against blank 1(Including curcumin solution)} \times C}{\text{OD against blank 2(Including pure DMSO)}}$$
$$(1)$$

In the above equation, C represents known glucose concentration. The reduction of glucose concentration (%) in the presence of tested compounds (**1–10**) was determined for the whole compounds under study[18].

In vivo *hypoglycemic effects*

Rats were rendered diabetic and grouped as follows. Adult (aged 60 to 70 d) laboratory-bred male Wister strain rats (160 ± 5 g) were fed with laboratory stock diet and water *ad libitum* for 7 days and kept in an air-conditioned animal house under the condition of 22 °C to 24 °C and ∼70% relative humidity. The acclimatized animals were divided into five groups each including six animals. Group I was kept as control and all of the other remaining animals were induced diabetes. The rats were injected by alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight, intraperitoneally. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia[19]. After seven days, rats with fasting blood glucose level more than 175 mg/dL were considered to be diabetic and selected for studies.

Oral glucose tolerance test on diabetic rats

Oral glucose tolerance test (OGTT) for rats were performed according to the standard method[20]. Group I served as untreated control and the oral 2 h glucose tolerance test was carried out via estimating glucose of blood sample from tail vein by using glucometer (Accu-chek active, Roche Diagnostics, Mannheim, Germany) at 0, 30, 60, 90 and 120 min. An oral glucose load of 0.35 g/100 g body weight was administered for the test[21]. Group II

rats were diabetic control and similar OGTT was conducted on these animals. Group III diabetic rats were administered by compound **4** orally at a dose of 100 μg/kg body weight followed by OGTT. Group IV diabetic rats were administered by compound **10** orally at a dose of 100 μg/kg body weight followed by OGTT. Group V diabetic rats received a dose of 2.5 mg/kg of a well known antidiabetic drug glipizide (reference drug)[22].

All the experimental procedures were performed in accordance with the approval of the Institutional Animal Ethics Committee (1169/ac/08/CPCSEA) under strict compliance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the experimental studies. Data were expressed as mean ± standard deviation of mean (SD). Statistical comparisons were performed by the one-way ANOVA followed by *post-hoc t*-test and the values were considered statistically significant when $p < 0.05$.

Results and discussion

To further verify the synthetic curcumin analogues, peak wavelengths of compounds along with the bibliographic information on their hypoglycemic effect are summarized in Table 1.

In vitro **hypoglycemic studies**

In vitro hypoglycemic effects of the curcumin synthetic analogues (**1–10**) were estimated using the glucose oxidase-peroxidase method by determining the percentage reduction of glucose concentration (Table 2). Results of this study showed that at pH 6.5, a noticeable reduction in glucose concentration occurred in the presence of compound **4** and **10** (Table 2). It was also observed that the degree of hypoglycemic effect of the compounds depended on the glucose concentration in the samples. Compound **4** was found to be the most potential hypoglycemic compound followed by compound **10**.

Further results indicated the presence of active constituents in the solvents extracted from the compound material. Perhaps enolic form of heptadienone or pentadienone scaffolds of these synthesized compounds might act as an electron donor, which is very common mechanism for the scavenging activity of phenolic antioxidant to alter characteristics of glucose molecules[23]. Moreover, the hypoglycemic activities observed for these compounds were in agreement with previous findings on these analogues[24–27].

In vivo **hypoglycemic studies**

The two promising compounds **4** and **10** were further evaluated *in vivo* on alloxan-induced diabetic rats by OGTT. Relevant

Table 2. *In vitro* hypoglycemic effects of synthetic curcumin analogues.

| Comp. code | Glucose concentrations (mg/dL) | | | | |
	20	40	60	80	100
1	16.8%*	8.65%	5.73%	4.11%	3.5%
2	–	8.57%	4.6%	–	–
3	67.85%	36.92%	37.11%	19.18%	17.22%
4	93.35%	75.20%	58.90%	50.80%	50.97%
5	44.45%	35.62%	44.45%	44.83%	38.71%
6	14.30%	13.35%	–	1.41%	1.69%
7	–	7.5%	–	–	–
8	52.00%	85.00%	66.60%	51.75%	48.00%
9	18.20%	18.20%	12.50%	–	–
10	76.65%	81.4%	51.11%	38.46%	27.82%

*Data are shown as percent reduction of glucose concentrations *in vitro* by various curcumin analogue compounds using glucose oxidase-peroxidase method at pH 6.5.

Table 3. Oral glucose tolerance test on alloxan induced diabetic rats after treatment with curcumin analogues and glipizide.

Treatment groups	Period after glucose ingestion (h)				
	0 (FBS)[a]	0.5	1.0	1.5	2.0
I (control)	84.62 ± 2.54^b	124.50 ± 15.33^c (+47.13%)	95.00 ± 10.43^b (+12.26%)	88.32 ± 4.37^b (+4.37%)	85.50 ± 4.51^b (+1.03%)
II (diabetic)	180.66 ± 15.65^b	298.50 ± 26.57^c (+65.22%)	351.00 ± 34.86^e (+94.28%)	410.65 ± 25.45^e (+127.3%)	410.34 ± 43.54^e (+127.13%)
III (diabetic + comp. **4**)[f]	184.4 ± 20.64^b	222.34 ± 17.54^c (+20.54%)	186.00 ± 32.45^b (+0.84%)	133.50 ± 11.70^d (−27.62%)	102.45 ± 10.45^c (−44.45%)
IV (diabetic + comp. **10**)[g]	175.66 ± 13.26^b	276.33 ± 25.43^c (+57.30%)	300.45 ± 19.61^d (+71.04%)	280.16 ± 7.39^c (+59.48%)	280.45 ± 33.06^c (+59.65%)
V (diabetic + Glipizide)	187.50 ± 14.18^b	216.66 ± 12.75^c (+15.55%)	185.00 ± 11.41^b (−1.83%)	125.66 ± 9.00^d (−32.98%)	89.00 ± 4.49^e (−52.53%)
Statistical analysis	$F = 54.598$ $p = 0.0000$	$F = 66.25$ $p = 0.000$	$F = 108.36$ $p = 0.000$	$F = 582.21$ $p = 0.000$	$F = 204.21$ $p = 0.000$

[a]FBS: fasting blood sugar.
[b,c,d,e]Horizontal values are the mean ± SD of six observations in each group in every 0.5 h interval till 2 h. In each row, values with different superscripts (a, b, c, d) are significantly different from each other ($p < 0.05$). One-way ANOVA of different groups in every 0.5 h interval till 2 h is referred below vertically. Horizontal rows also indicate percentage variation of glucose concentrations in each 0.5 h interval till 2 h from baseline FBS (0h).
[f]Comp. **4**: 1,7-Bis(3,4 dimethoxyphenyl)hepta-1,6-diene-3,5-dione.
[g]Comp. **10**: 1,5-Bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one.

results are summarized in Table 3. We found that aqueous extract of 1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione (**4**) reduced the high blood glucose levels of diabetic rats during the OGTT. Glipizide was used as a reference drug in all diabetic models (positive control).

The observation of glucose percentage variation within OGTT study revealed that blood glucose level of group I (untreated control), group III (alloxan diabetic + compound **4**) and group V (alloxan diabetic + glipizide) rats reached its maximum after 0.5 h from FBS level followed by reduction of blood glucose levels till 2 h.

In the case of group II (alloxan diabetic) and IV (alloxan diabetic + compound **10**), maximum blood glucose levels were noticed after 1.5 h and 1 h, respectively, and then onwards till 2 h the levels of blood glucose steadily declined. This indicated that it took about 1 h for the active ingredient of **4** or its metabolites in the water extract to enter into the circulation and reach target tissues to bring about hypoglycemic effect[4].

From this OGTT study it might be concluded that derivative **4** decreased blood glucose level of alloxan-treated diabetic rats by 44.45% during 2 h (beginning from its baseline fasting blood sugar or FBS level). The reductions of blood glucose by **4** and glipizide were close to each other (52.53% reduction from baseline FBS level). It is noteworthy that the synthetic polyphenol 1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione (**4**) was nearly equally effective as the reference antidiabetic drug. Hence, it might be expected that the aqueous solution of **4** had some direct effect via increasing the tissue utilization of glucose[28], inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues[29].

The elevated blood glucose levels in the tested diabetic animals were in the range of 150–190 mg/dL, which resembled type-II diabetes (150 to about 250 mg/dL) with partially functional pancreas. From our OGTT results it could also be interpreted that compound **4** might enhance insulin release from partially functional pancreatic beta cells by the release of insulin stored in the granules.

The findings of this study also suggested that compound **4** exhibited activities similar to that of glipizide that stimulates the release of insulin from the surviving beta-cells[30]. It has been well-documented that many medicinal plants are enriched with phenolic compounds and flavonoids endowed with excellent antioxidant and antidiabetic properties. However, in the present study, we found that only compound **4** (100 µg/kg) could significantly reduce the blood sugar level in alloxan-treated hyperglycemic rats in OGTT. Compound **10** did not show similar hypoglycemic responses in the same assay, although *in vitro* studies had shown a decrease in glucose concentration.

Structure activity relationship elucidation

Considering the data summarized in Table 2, *in vitro* hypoglycemic activities of tested compounds may be prioritized as follows: **4** > **10** > **3** > **8** > **5** > **9** > **1** > **6** > **2**,**7**. Generally, curcumin analogues may be constructed from three building blocks as depicted in Figure 2.

Considering this structural pattern, the observed trend for biological activities may be interpreted as follows:

(1) Compound **4** showed higher *in vitro* hypoglycemic effect (Table 2). It is worthy to note that compound **4** and curcumin have the same linker (diketoeptadiene), while the aromatic (phenyl) substitution pattern is different: 3,4-dimethoxyphenyl for **4** and 4-hydroxy-3-methoxyphenyl for curcumin. This observation is in support of literature about the previous reports of this compound including its synthetic compound as hypoglycemic (Table 1).
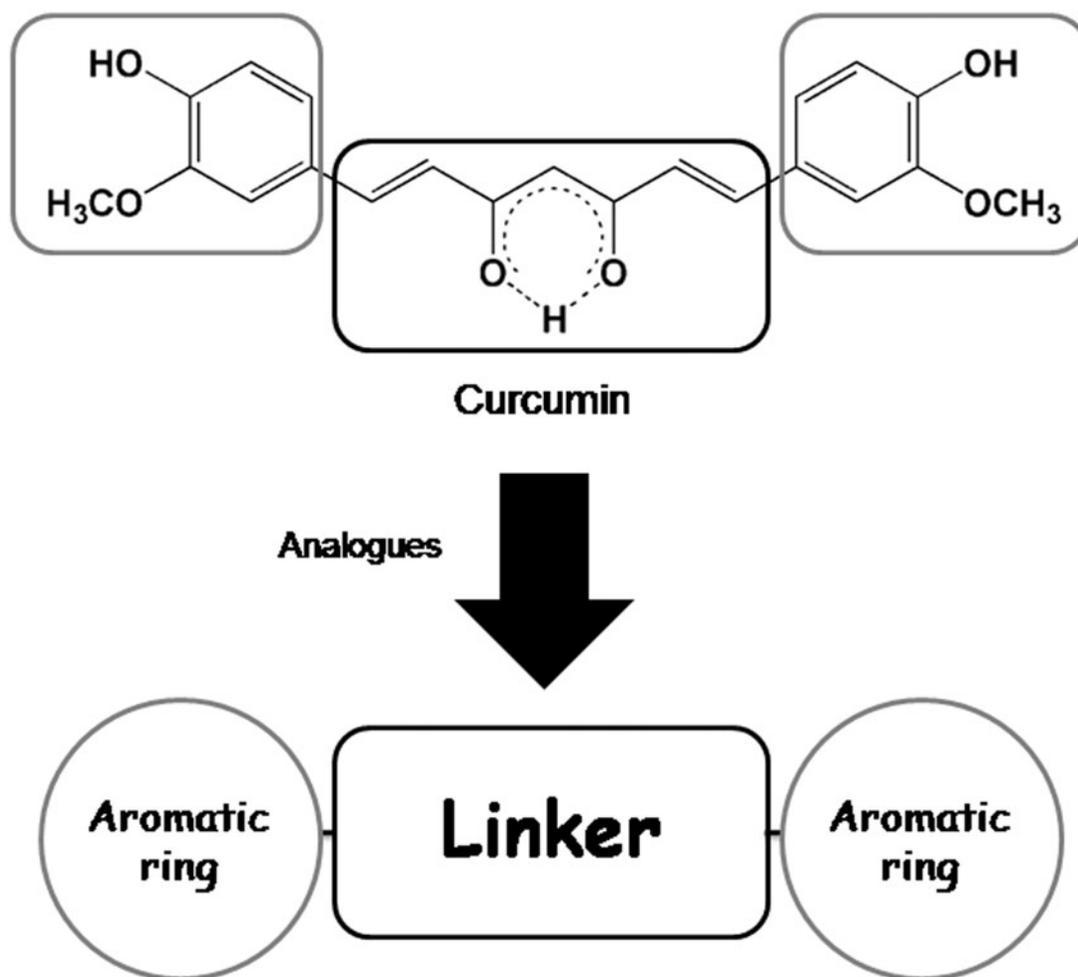
Figure 2. Constructive building blocks of curcumin derivatives.

(2) Compound **10** has the same aromatic substitution pattern when compared to curcumin, while the linker is shorter (5 carbon atoms compared to 7 of curcumin). This observation is also in support of literature about the previous reports of this compound including its synthetic compound as hypoglycemic (Table 1).

(3) Compound **3** is an analogue of curcumin in which the 3-methoxy substituents have been removed from the phenyl rings.

(4) Compound **8** is generated through cyclization of the linker of compound **10** and hence possesses more rigid structure. Data mining (Table 2) showed that more flexible linkers might show better glucose reducing activity (**10**; 76.65% and **8**; 52.00% glucose reduction at 20 mg/dL glucose concentration).

(5) Compound **5** is an analogue of curcumin with 3,4-dihydroxyphenyl substituents (bisdesmethyl curcumin).

(6) Hypoglycemic activities were decreased significantly for compound **9**. In this case, it would be useful to compare the structure of compounds **9** and **8**. Both of the structures are rigid in their linker segment but the type of cyclization differs. Compound **9** has a piperidinium-4-one ring while compound **8** possesses a cyclohexanone ring. Another difference is the substitution pattern since compound **9** is a biscatechol derivative.

(7) Compound **1** is similar to compound **9** in terms of linker; compound **1** possesses an ethyl group on N1 of the piperidinone ring which is absent in derivative **9**, and the substitution pattern on phenyl ring is different. In compound **1**, hydroxy groups of compound **9** have been changed into nitro groups. It seemed that hydroxy groups of **9** might be slightly better tolerated than nitro substituents of **1** (Table 2).

(8) In our opinion, significant changes of the curcumin linker might not be well tolerated since compound **6** (bearing a linker with 9 carbon atoms) exhibited weak hypoglycemic effect (14.30% glucose reduction at 20 mg/dL glucose concentration).

(9) Compounds **2** and **7** showed no *in vitro* hypoglycemic activity (Table 2). This observation might confirm our previous SAR and emphasized that significant variations of linker pattern would not lead to potent hypoglycemic agents. In compound **2**, the substitution pattern of aromatic rings was also changed. Moreover, the results of *in vivo* studies (Table 3) showed a better effect of **4** over **10** and confirmed our observed SAR that 7-carbon linker possessing two oxo groups might be a better molecular pattern than 5-carbon linker with one oxo group.

(10) Comparing the results of *in vitro* assessment, it would be expected that 3-methoxyphenyl substituents showed superior *in vitro* hypoglycemic effect than 3-hydroxyphenyl ones (**4**: 93.35% and **5:** 44.45% glucose reduction at 20 mg/dL glucose conc.). It should be noted that compound **3** possessed no 3-substituted pattern in its phenyl ring but exhibited higher activity than compound **5** and indeed was the borderline case when compared to compounds **4** and **5**

(**3**; 67.85% glucose reduction at 20 mg/dL glucose concentration).

Conclusion

In conclusion, compound **4** [1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione] was found to be a potential curcumin-based hypoglycemic agent (at dosage of 100 μg/kg), which reduced glucose concentration *in vitro*. This finding was further supported with *in vivo* studies on alloxan-induced diabetic animals. But in the case of another curcumin analogue [compound **10**; 1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one], *in vitro* effects did not directly correlate with *in vivo* results. However, we believe that *in vitro* tests may be very important as initial screening tools in the evaluation of antidiabetic herbs or compounds that may be further followed up to animal or human research. Further studies will be required to establish the exact hypoglycemic mechanism of compound **4**.

Declaration of interest

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