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Original article

α-Glucosidase inhibition of natural curcuminoids and curcumin analogs

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Abstract

Natural curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**) isolated from *Curcuma longa* (turmeric), and synthetic curcumin analogs ($\mathbf{A_{1-7}}$, $\mathbf{B_{1-7}}$, $\mathbf{C_{1-6}}$ and $\mathbf{D_{1-7}}$) were evaluated in vitro for the α-glucosidase inhibitory activity via UV and circular dichroism (CD) spectroscopy. The results indicated that natural curcuminoid compound **3** showed a remarkable inhibitory effect with IC_{50} of 23.0 μM, and the synthetic compounds $\mathbf{A_2}$, $\mathbf{B_2}$, $\mathbf{C_2}$ and $\mathbf{D_2}$ showed potent inhibitory effects with IC_{50} of 2.8, 2.6, 1.6 and 8.2 μM, respectively. Kinetic study exhibited that the mechanism of α-glucosidase inhibition of both **3** and $\mathbf{C_2}$ was non-competitive. The structure activity relationship revealed that the *ortho* dihydroxyl groups could form a more tight interaction with α-glucosidase to exert more potential inhibitory activities.

Keywords: α-Glucosidase inhibitor; Curcuminoids; Curcumin analogs

1. Introduction

α-Glucosidases (α-D-glucoside glucohydrolase EC. 3.2.1.20) were membrane-bound enzymes located at the epithelium of the small intestine [1], and the key enzymes of carbohydrate digestion [2]. It specifically hydrolyzed the α-glucopyranoside bond, thereby releasing an α-D-glucose from the non-reducing end of the sugar. α-Glucosidase had been found to contribute to the glycosylation of human immunodeficiency virus type I (HIV-I) 120 [3] and inhibitors of α-glucosidase can block the viral infection [4,5]. Recently there had been widespread interest in these enzymes, partly because of their potential as therapeutic targets, especially, the inhibition of α-glucosidase had been found to help control postprandial blood glucose levels in diabetic patients [6,7]. Clinical trials showed that the α-glucosidase inhibitor improved long-term glycemic control as measured by decreased hemoglobin A1c(HbA1c) in patients with type II diabetes and delay the development of type II diabetes in patients with impaired glucose tolerance [8].

Curcuma longa Linn. or turmeric (Zingiberaceae) was a medicinal plant widely cultivated in tropical regions of Asia. The extract from *C. longa*, commonly called curcuminoids, was mainly composed of curcumin (75–90%) and together with a small amount of demethoxycurcumin and bisdemethoxycurcumin [9]. Curcumin was a yellow spice and pigment in food system, and well known for its antioxidant, anti-inflammatory, anticancer, and anti-HIV integrase activity [10–14]. *C. longa* was recommended to use in Chinese traditional medical prescriptions against the diabetic complications [15]. Recently, Lee [16] found the plant extract of *C. longa* could inhibit the activity of α-glucosidase resulting in lowering the high blood sugar. The interesting discovery of the α-glucosidase inhibitory activity of phenolic compounds like curcuminoids prompted us to study a series of curcumin analogs.

In the present study, the effects of the natural curcuminoids isolated from *C. longa* (Fig. 1) and the synthetic curcumin analogs (Fig. 2) on α-glucosidase were evaluated in vitro, and the inhibitory modes of action of curcuminoids and curcumin ana-

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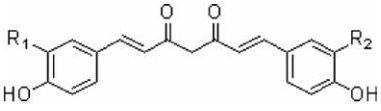
logs against α-glucosidase were described. The structure activity relationship was also discussed.

2. Chemistry

Natural curcuminoid compounds of **1**–**3** (Fig. 1) were isolated by the supercritical CO_2 liquid extractor from *C. longa* roots and purified by silica gel column chromatography. Curcumin analogs (**A**–**D**) were prepared through condensation of the appropriate ketone with a variety of aromatic aldehydes under acidic condition (Fig. 2).

3. Pharmacology

The natural curcuminoids (**1**–**3**) isolated from *C. longa* and the synthetic curcumin analogs ($\mathbf{A_{1-7}}$, $\mathbf{B_{1-7}}$, $\mathbf{C_{1-6}}$ and $\mathbf{D_{1-7}}$) were evaluated for their ability to inhibit α-glucosidase using spectrophotometric assay (Table 1). Enzyme inhibition data were expressed as IC_{50} values (the concentration of the inhibitor required to produce 50% inhibition of the test models). In the case of some compounds exerted too low inhibitory activity

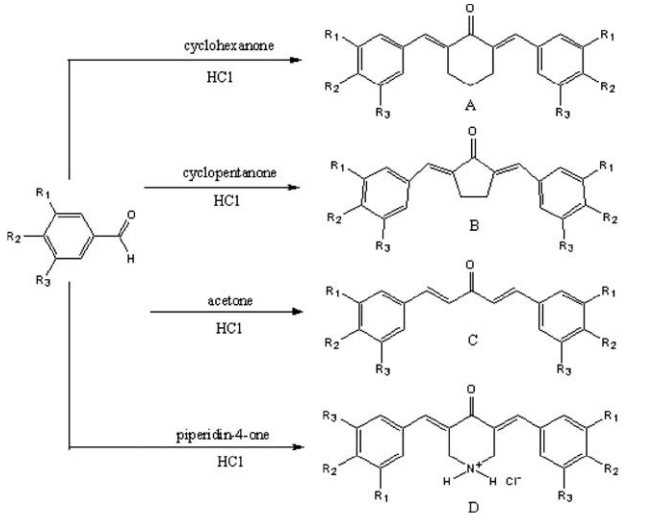


1: $R_1 = R_2 = OCH_3$ (curcumin)

2: $R_1 = OCH_3$, $R_2 = H$ (demethoxycurcumin)

3: $R_1 = R_2 = H$ (bisdemethoxycurcumin)

Fig. 1. Chemical structures of curcuminoids.



$\mathbf{A_1/B_1/C_1/D_1}$: $R_1 = R_3 = H$, $R_2 = OH$; $\mathbf{A_2/B_2/C_2/D_2}$: $R_1 = R_2 = OH$, $R_3 = H$;

$\mathbf{A_3/B_3/C_3/D_3}$: $R_1 = H$, $R_2 = OH$, $R_3 = OCH_3$; $\mathbf{A_4/B_4/C_4/D_4}$: $R_1 = R_3 = C(CH_3)_3$, $R_3 = OCH_3$;

$\mathbf{A_5/B_5/C_5/D_5}$: $R_1 = R_2 = OCH_3$, $R_3 = H$; $\mathbf{A_6/B_6/C_6/D_6}$: $R_1 = R_3 = OCH_3$, $R_2 = OH$;

$\mathbf{A_7/B_7/D_7}$: $R_1 = Br$, $R_3 = OCH_3$, $R_2 = OH$

Fig. 2. Synthesis of curcumin analogs.

Table 1
Inhibitory activities of curcuminoids and curcumin analogs on α-glucosidase

Compound	R1	R2	R3	IC_{50} (μM) [a]	Inhibition (%) [b]
1	OCH_3	OCH_3		37.2	ND
2	H	OCH_3		42.7	ND
3	H	H		23.0	ND
$\mathbf{A_1}$	H	OH	H	37.6	ND
$\mathbf{A_2}$	OH	OH	H	2.8	ND
$\mathbf{A_3}$	OCH_3	OH	H	47.0	ND
$\mathbf{A_4}$	$C(CH_3)_3$	OH	$C(CH_3)_3$	> 100	25.6
$\mathbf{A_5}$	OCH_3	OCH_3	H	> 100	NI [c]
$\mathbf{A_6}$	OCH_3	OH	OCH_3	> 100	35.8
$\mathbf{A_7}$	Br	OH	OCH_3	29.3	ND
$\mathbf{B_1}$	H	OH	H	32.5	ND
$\mathbf{B_2}$	OH	OH	H	2.6	ND
$\mathbf{B_3}$	OCH_3	OH	H	52.8	ND
$\mathbf{B_4}$	$C(CH_3)_3$	OH	$C(CH_3)_3$	> 100	28.6
$\mathbf{B_5}$	OCH_3	OCH_3	H	> 100	NI
$\mathbf{B_6}$	OCH_3	OH	OCH_3	> 100	37.4
$\mathbf{B_7}$	Br	OH	OCH_3	33.9	ND
$\mathbf{C_1}$	H	OH	H	21.8	ND
$\mathbf{C_2}$	OH	OH	H	1.6	ND
$\mathbf{C_3}$	OCH_3	OH	H	37.0	ND
$\mathbf{C_4}$	$C(CH_3)_3$	OH	$C(CH_3)_3$	> 100	27.5
$\mathbf{C_5}$	OCH_3	OCH_3	H	> 100	NI
$\mathbf{C_6}$	OCH_3	OH	OCH_3	> 100	31.3
$\mathbf{D_1}$	H	OH	H	61.3	ND
$\mathbf{D_2}$	OH	OH	H	8.2	ND
$\mathbf{D_3}$	OCH_3	OH	H	> 100	38.2
$\mathbf{D_4}$	$C(CH_3)_3$	OH	$C(CH_3)_3$	> 100	23.3
$\mathbf{D_5}$	OCH_3	OCH_3	H	> 100	NI
$\mathbf{D_6}$	OCH_3	OH	OCH_3	> 100	28.9
$\mathbf{D_7}$	Br	OH	OCH_3	38.1	ND
Acarbose				56.2	ND

[a] IC_{50} (95% CL).
[b] Percent inhibition at 100 μM concentration; ND, no determination.
[c] NI, non-inhibition.

to obtain IC_{50} values at the concentration requested in the assay, the activity of these compounds were then reported as inhibition (%) at certain concentration. Acarbose was used as reference compound.

4. Results and discussion

In the course of screening study on α-glucosidase inhibitors from plant sources, we found that the natural curcuminoids (**1**–**3**) showed different inhibitory activities against α-glucosidase. Among curcuminoids, **3** exhibited the highest inhibitory activity with IC_{50} value of 23.0 μM, which was approximately two-fold lower than that of acarbose.

The synthetic curcumin analogs with tetrahydroxyl groups ($\mathbf{A_2}$, $\mathbf{B_2}$, $\mathbf{C_2}$ and $\mathbf{D_2}$) exhibited much higher inhibitory activity with lower IC_{50} values than others, the inhibitory effects of $\mathbf{A_2}$, $\mathbf{B_2}$ and $\mathbf{C_2}$ were approximately 15-fold lower than that of curcumin, and about 20-fold lower than that of acarbose. Comparison among curcumin analogs, the introduction of bromic groups at the *ortho* positions of phenols ($\mathbf{A_7}$, $\mathbf{B_7}$, and $\mathbf{D_7}$) led to the activities higher than that of *p*-hydroxyl phenolic compounds ($\mathbf{A_1}$, $\mathbf{B_1}$, and $\mathbf{D_1}$). However, the introduction of methoxyl groups at the *ortho* positions of phenols ($\mathbf{A_{2,\ 6}}$, $\mathbf{B_{2,\ 6}}$, $\mathbf{C_{2,\ 6}}$ and $\mathbf{D_{2,\ 6}}$) led to the activities lower than that of *p*-hydroxyl

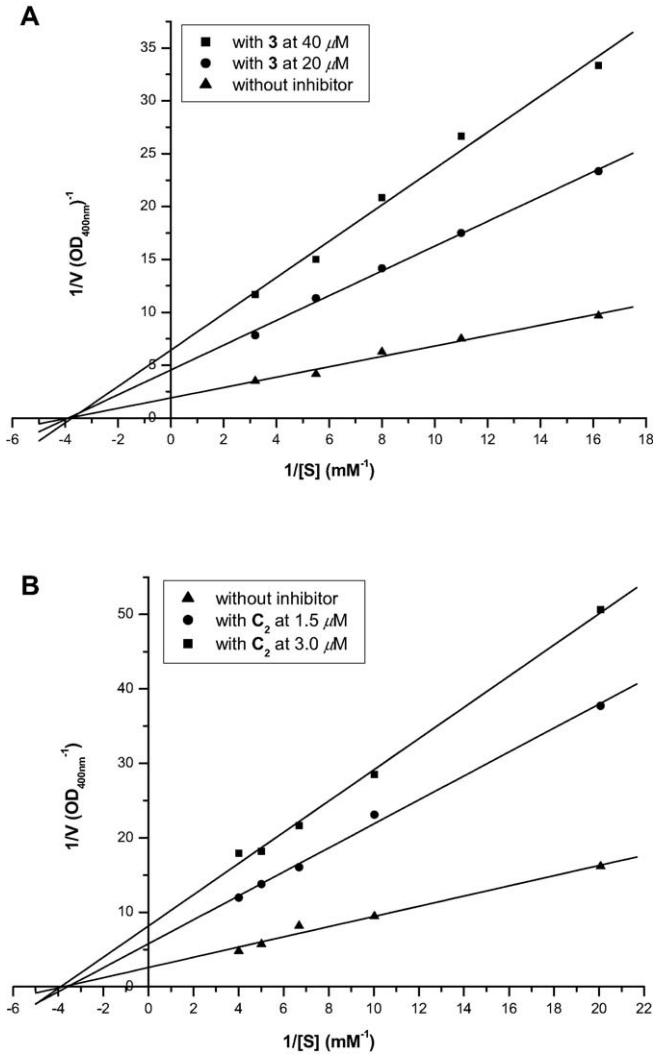


Fig. 3. Double-reciprocal plots of the inhibition kinetics of α-glucosidase by curcuminoid **3** and curcumin analog $\mathbf{C_2}$.
a. Without (▲) and with [(●) 20 μM, (■) 40 μM] **3**;
b. Without (▲) and with [(●) 1.5 μM, (■) 3.0 μM] $\mathbf{C_2}$.

phenolic compounds. The introduction of the bulky *ortho tert*-butyl groups ($\mathbf{A_4}$, $\mathbf{B_4}$, $\mathbf{C_4}$ and $\mathbf{D_4}$) also led to the activities decreased remarkably. Without hydroxyl group presence ($\mathbf{A_5}$, $\mathbf{B_5}$, $\mathbf{C_5}$ and $\mathbf{D_5}$), the activities almost disappeared. Therefore, we assumed that the *para* hydroxyl groups of curcumin analogs played a very important role on the enzyme inhibitory activity, and the *ortho* dihydroxyl groups could form a more tight interaction with enzyme to exert more potential inhibitory activities.

Double-reciprocal plots of the inhibition kinetics of yeast α-glucosidase were shown in Fig. 3. The kinetic results demonstrated that the mechanism of α-glucosidase inhibition of both **3** and $\mathbf{C_2}$ was non-competitive, indicated that the binding modes of curcuminoids and curcumin analogs towards α-glucosidase were similar.

In order to study the inhibitory mechanism of curcumin and curcumin analogs, the influence of **3** and $\mathbf{C_2}$ on the secondary structure of α-glucosidase was examined via circular dichroism (CD) spectroscopy. The results (Table 2) showed the decline in the percentage of α-helix of α-glucosidase when the enzyme was treated with increasing concentrations of inhibitor **3** or $\mathbf{C_2}$, indicated that the α-helix of α-glucosidase dominated the enzyme activity, and inhibition of α-glucosidase activity by curcuminoids or curcumin analogs might be due to the α-helix of α-glucosidase changed into other conformations of the secondary structure.

In conclusion, curcuminoids isolated from *C. longa* were found as potent α-glucosidase inhibitors, and the synthetic curcumin analogs with *ortho* dihydroxyl groups exhibited the strongest inhibitory activity on α-glucosidase. This study suggested that curcuminoids and curcumin analogs would be the lead compounds suitable for designing new patent α-glucosidase inhibitors of this kind.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Yanagimoto micro-melting apparatus and were uncorrected. The ^{1}H NMR spectra were measured on a Varian Gemini-2000 spectrometer using DMSO as solvent unless otherwise specified. Chemical shifts for ^{1}H NMR were expressed in a (ppm) unit with TMS as an internal standard. Multiplicities were recorded as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were obtained on a LC-MS-2010A spectrometer with ESI. Elemental analyses were performed on a Perkin Elmer 240C and within ± 0.4% of the theoretical values. Thin-layer chromatography was performed on Merk silica gel plates (DC-60 F_{254}). All regents were used as received unless otherwise stated.

Table 2
The influence of **3** and $\mathbf{C_2}$ on the secondary structure of α-glucosidase

Inhibitors	Concentration (μM)	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Random (%)
Without inhibitor		39.1	16.1	18.7	26.1
3	10	33.6	21.6	23.5	21.3
	20	28.9	24.2	22.6	24.3
	30	25.3	28.3	28.5	17.9
	40	23.8	34.6	18.9	22.7
$\mathbf{C_2}$	2.0	27.2	22.9	25.6	24.3
	5.0	24.0	31.7	16.5	27.8
	7.5	18.2	38.6	18.9	24.3
	12.5	3.2	46.9	17.6	32.3

*5.1.1. Isolation of compounds **1**–**3** from C. longa*

The *C. longa* extract was isolated from *C. longa* roots by supercritical CO_2 liquid extraction system. The extract (2.0 g) was chromatographed on a silica gel column (Merk 70–230 mesh, 80 g, 4.5 cm × 100 cm) using chloroform/methanol/acetic acid (95:4:1) as the eluent to give curcumin (1.32 g), demethoxycurcumin (0.12 g), and bisdemethoxycurcumin (0.25 g), respectively. R_f values of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were 0.68, 0.43, and 0.21 in hexane/chloroform/methanol (3:6:1) on TLC, respectively.

*5.1.1.1. 1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin, **1**).* Yellow needles. M.p. 183–184 °C (Kosuge et al. reported [17]: m.p. 183 °C). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.66 (brs, 2H), 7.53 (d, J = 15.9 Hz, 2H), 7.12–7.31 (m, 4H), 6.86 (d, J = 8.1 Hz, 2H), 6.75 (d, J = 15.9 Hz, 2H), 6.04 (s, 1H), 3.83 (s, 6H). LC–MS (*m*/*z*): 368. Anal. Calc. for $C_{21}H_{20}O_6$: C 68.47, H 5.47. Found: C 68.32, H 5.38.

*5.1.1.2. 1-(4-Hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (demethoxycurcumin, **2**).* Yellow needles. M.p. 180–181 °C (Kosuge et al. reported [17]: m.p. 181–182 °C). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 10.05 (brs, 1H), 9.66 (brs, 1H), 7.55 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 15.9 Hz, 2H), 7.31 (d, J = 1.5 Hz, 1H), 7.13 (d, J = 8.1 Hz, 1H), 6.80 (d, J = 8.1 Hz, 2H), 6.75 (d, J = 15.9 Hz, 1H), 6.68 (d, J = 15.9 Hz, 1H), 6.03 (s, 1H), 3.83 (s, 3H). LC–MS (*m*/*z*): 338. Anal. Calc. for $C_{20}H_{18}O_5$: C 70.99, H 5.36. Found: C 70.78, H 5.23.

*5.1.1.3. 1,7-bis (4-Hydroxyphenyl)-1,6-heptadiene-3,5-dione (bisdemethoxycurcumin, **3**).* Light red needles. M.p. 232–233 °C (Kosuge et al. reported [17]: m.p. 232–234 °C). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 10.05 (brs, 2H), 7.55 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 15.9 Hz, 4H), 6.80 (d, J = 8.1 Hz, 4H), 6.68 (d, J = 15.9 Hz, 2H), 6.02 (s, 1H). LC–MS (*m*/*z*): 308. Anal. Calc. for $C_{21}H_{20}O_6$: C 74.01, H 5.23. Found: C 73.87, H 5.16.

5.1.2. General procedure for the preparation of curcumin analogs

Curcumin analogs ($\mathbf{A_{1–7}}$, $\mathbf{B_{1–7}}$, $\mathbf{C_{1–6}}$ and $\mathbf{D_{1–7}}$) were synthesized as previously described in [18,19] with modification. A mixture of the appropriate aldehyde (0.01 mol) and the ketone (0.005 mol) was dissolved in glacial acetic acid saturated with anhydrous hydrogen chloride and heated in a water bath at 25–30 °C for 2 h. After standing for 2 d, the mixture was treated with cold water and filtered. The solid obtained was then washed and dried. The crude product was recrystallized from appropriate solvents (methanol or ethanol).

*5.1.2.1. 2,6-bis(4-Hydroxybenzylidene)cyclohexanone **A₁**.* Yield 95%. M.p. > 300 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.88 (brs, 2H, –OH), 7.52 (s, 2H, –CH=), 7.38 (d, J = 8.1 Hz, 4H, ArH), 6.82 (d, J = 8.1 Hz, 4H, ArH), 2.84 (t, J = 5.7 Hz, 4H, –CH_2–C–CH_2–), 1.72 (q, J = 5.7 Hz, 2H, –C–CH_2–C–). LC–MS (*m*/*z*): 306. Anal. Calc. for $C_{20}H_{18}O_3$: C 78.41, H 5.92. Found: C 78.28, H 5.83.

*5.1.2.2. 2,6-bis(3,4-Dihydroxybenzylidene)cyclohexanone **A₂**.* Yield 90%. M.p. > 300 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.43 (brs, 2H, –OH), 9.12 (brs, 2H, –OH), 7.43 (s, 2H, –CH=), 6.96 (s, 2H, ArH), 6.85 (d, J = 8.1 Hz, 2H, ArH), 6.78 (d, J = 8.1 Hz, 2H, ArH), 2.83 (t, J = 6.7 Hz, 4H, –CH_2–C–CH_2–), 2.04 (q, J = 6.7 Hz, 2H, –C–CH_2–C–). LC–MS (*m*/*z*): 338. Anal. Calc. for $C_{20}H_{18}O_5$: C 70.99, H 5.36. Found: C 70.76, H 5.27.

*5.1.2.3. 2,6-bis(4-Hydroxy-3-methoxybenzylidene)cyclohexanone **A₃**.* Yield 98%. M.p. 178–179 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.48 (brs, 2H, –OH), 7.53 (s, 2H, –CH=), 7.08 (s, 2H, ArH), 7.01 (d, J = 8.1 Hz, 2H, ArH), 6.82 (d, J = 8.1 Hz, 2H, ArH), 3.79 (s, 6H, OCH_3), 2.87 (t, J = 6.7 Hz, 4H, H_2C–C–CH_2), 1.71 (q, J = 6.7 Hz, 2H, C–CH_2–C). LC–MS (*m*/*z*): 366. Anal. Calc. for $C_{22}H_{22}O_5$: C 72.12, H 6.05. Found: C 72.03.48, H 6.02.

*5.1.2.4. 2,6-bis(3,5-Di-tert-butyl-4-hydroxylbenzylidene)cyclohexanone **A₄**.* Yield 65%. M.p. 188–190 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.77 (s, 2H, –C=), 7.35 (s, 4H, ArH), 5.45 (brs, 2H, –OH), 2.94 (t, J = 6.7 Hz, 4H, H_2C–C–CH_2), 1.83 (q, J = 6.7 Hz, 2H, C–CH_2–C), 1.47 (s, 36H, C–CH_3). LC–MS (*m*/*z*): 530. Anal. Calc. for $C_{36}H_{50}O_3$: C 81.46, H 9.49. Found: C 81.21, H 9.36.

*5.1.2.5. 2,6-bis(3,4-Dimethoxybenzylidene)cyclohexanone **A₅**.* Yield 95%. M.p. 138–140 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.58 (s, 2H, –CH=), 7.13 (d, J = 8.1, 4H, ArH), 7.02 (s, 2H, ArH), 3.79 (s, 12H, OCH_3), 2.91 (t, J = 6.7 Hz, 4H, –CH_2–C–CH_2–), 1.73 (q, J = 6.7 Hz, 2H, –C–CH_2–C–). LC–MS (*m*/*z*): 394. Anal. Calc. for $C_{24}H_{26}O_5$: C 73.08, H 6.64. Found: C 69.90, H 6.57.

*5.1.2.6. 2,6-bis(4-Hydroxy-3,5-dimethoxybenzylidene)cyclohexanone **A₆**.* Yield 62%. M.p. 134–135 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 8.89 (brs, 2H, –OH), 7.55 (s, 2H, –CH=), 6.83 (s, 4H, ArH), 3.71 (s, 12H, OCH_3), 2.93 (t, J = 6.7 Hz, 4H, –CH_2–C–CH_2–), 1.74 (q, J = 6.7 Hz, 2H, –C–CH_2–C–). LC–MS (*m*/*z*): 426. Anal. Calc. for $C_{24}H_{26}O_7$: C 67.59, H 6.15. Found: C 67.48, H 6.09.

*5.1.2.7. 2,6-bis(3-Bromo-4-hydroxy-5-methoxybenzylidene)cyclohexanone **A₇**.* Yield 51%. M.p. 218–220 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.94 (brs, 2H, –OH), 7.50 (s, 2H, –CH=), 7.27 (s, 2H, ArH), 7.13 (s, 2H, ArH), 3.86 (s, 6H, OCH_3), 2.88 (t, J = 6.7 Hz, 4H, –CH_2–C–CH_2–), 1.73 (q, J = 6.7 Hz, 2H, –C–CH_2–C–). LC–MS (*m*/*z*): 522. Anal. Calc. for $C_{22}H_{20}Br_2O_5$: C 50.41, H 3.85. Found: C 50.22, H 3.76.

*5.1.2.8. 2,5-bis(4-Hydroxybenzylidene)cyclopentanone **B₁**.* Yield 90%. M.p. > 300 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 10.01 (brs, 2H, –OH), 7.51 (d, J = 8.1 Hz, 4H,

ArH), 7.31 (s, 2H, –CH=), 6.85 (d, J = 8.1 Hz, 4H, ArH), 3.01 (s, 4H, –CH_2–CH_2–). LC–MS (m/z): 292. Anal. Calc. for $C_{19}H_{16}O_3$: C 78.06, H 5.52. Found: C 77.93, H 5.46.

5.1.2.9. 2,5-bis(3,4-Dihydroxybenzylidene)cyclopentanone ***B₂***. Yield 85%. M.p. > 300 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.53 (brs, 2H, –OH), 9.18 (brs, 2H, –OH), 7.23 (d, 2H, –CH=), 7.10 (s, 2H, ArH), 7.00 (d, J = 8.1 Hz, 2H, ArH), 6.82 (d, J = 8.1 Hz, 2H, ArH), 3.00 (s, 4H, –CH_2–CH_2–). LC–MS (m/z): 324. Anal. Calc. for $C_{19}H_{16}O_5$: C 70.36, H 4.97. Found: C 70.18, H 4.89.

5.1.2.10. 2,5-bis(4-Hydroxy-3-methoxybenzylidene)cyclopentanone ***B₃***. Yield 94%. M.p. 212–214 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.64 (brs, 2H, –OH), 7.34 (s, 2H, –CH=), 7.23 (s, 2H, arom), 7.15 (d, J = 8.1 Hz, 2H, ArH), 6.87 (d, J = 8.1 Hz, 2H, ArH), 3.83 (s, 6H, OCH_3), 3.06 (s, 4H, –H_2C–CH_2–). LC–MS (m/z): 352. Anal. Calc. for $C_{21}H_{20}O_5$: C 71.58, H 5.72. Found: C 71.50, H 5.68.

5.1.2.11. 2,5-bis(3,5-Di-tert-butyl-4-hydroxylbenzylidene)cyclopentanone ***B₄***. Yield 82%. M.p. 137–138 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.51 (s, 2H, –CH=), 7.43 (s, 4H, ArH), 7.35 (brs, 2H, –OH), 3.05 (4H, –H_2C–CH_2–), 1.45 (s, 36H, –C–CH_3). LC–MS (m/z): 516. Anal. Calc. for $C_{35}H_{48}O_3$: C 81.35, H 9.36. Found: C 81.28, H 9.30.

5.1.2.12. 2,5-bis(3,4-Dimethoxybenzylidene)cyclopentanone ***B₅***. Yield 85%. M.p. 188–190 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.35 (s, 2H, –CH=), 7.27 (d, J = 8.1 Hz, 4H, ArH), 7.06 (d, J = 8.1 Hz, 2H, ArH), 3.81 (12, O–CH_3), 3.10 (4H, –CH_2–CH_2–). LC–MS (m/z): 380. Anal. Calc. for $C_{23}H_{24}O_5$: C 72.61, H 6.36. Found: C 72.52, H 6.32.

5.1.2.13. 2,5-bis(4-Hydroxy-3,5-dimethoxybenzylidene)cyclopentanone ***B₆***. Yield 82%. M.p. 226–228 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.03 (brs, 2H, –OH), 7.36 (s, 2H, –CH=), 6.96 (s, 4H, ArH), 3.82 (s, 12H, OCH_3), 3.12 (t, J = 6.7 Hz, 4H, –CH_2–CH_2–). LC–MS (m/z): 412. Anal. Calc. for $C_{23}H_{24}O_7$: C 66.98, H 5.87. Found: C 66.83, H 5.82.

5.1.2.14. 2,5-bis(3-Bromo-4-hydroxy-5-methoxybenzylidene) cyclopentanone ***B₇***. Yield 45%. M.p. 280–281 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 10.09 (brs, 2H, –OH), 7.41 (s, 2H, –CH=), 7.32 (s, 2H, ArH), 7.27 (s, 2H, ArH), 3.89 (s, 6H, OCH_3), 3.07 (4H, –CH_2–CH_2–). LC–MS (m/z): 508. Anal. Calc. for $C_{21}H_{18}Br_2O_5$: C 49.44, H 3.56. Found: C 49.32, H 3.52.

5.1.2.15. 1,5-bis(4-Hydroxyphenyl)-1,4-pentadiene-3-one ***C₁***. Yield 95%. M.p. 243–245 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 10.01 (brs, 2H, –OH), 7.64 (d, J = 15.9 Hz, 2H, –CH=C–), 7.60 (d, J = 8.1 Hz, 4H, ArH), 7.08 (d, J = 15.9 Hz, 2H, –C=CH–), 6.82 (d, J = 8.1 Hz, 4H, ArH). LC–MS (m/z): 266. Anal. Calc. for $C_{17}H_{14}O_3$: C 76.68, H 5.30. Found: C 76.56, H 5.23.

5.1.2.16. 1,5-bis(3,4-Dihydroxyphenyl)-1,4-pentadiene-3-one ***C₂***. Yield 90%. M.p. 150–154 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.58 (brs, 2H, –OH), 9.11 (brs, 2H, –OH), 7.54 (d, J = 15.9 Hz, 2H, –CH=C–), 7.13 (s, 2H, ArH), 7.06 (d, J = 8.1 Hz, 2H, ArH), 6.98 (d, J = 15.9 Hz, 2H, –C=CH–), 6.78 (d, J = 8.1 Hz, 2H, ArH). LC–MS (m/z): 298. Anal. Calc. for $C_{17}H_{14}O_5$: C 68.45, H 4.73. Found: C 68.26, H 4.67.

5.1.2.17. 1,5-bis(4-Hydroxy-3-methoxphenyl)-1,4-pentadiene-3-one ***C₃***. Yield 98%. M.p. 99–100 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.60 (brs, 2H, –OH), 7.63 (d, J = 15.9 Hz, 2H, –CH=C–), 7.35 (s, 2H, ArH), 7.18 (d, J = 8.1 Hz, 2H, ArH), 7.12 (d, J = 15.9 Hz, 2H, –C=CH–), 6.81 (d, J = 8.1 Hz, 2H, ArH), 3.84 (s, 6H, OCH_3). LC–MS (m/z): 326. Anal. Calc. for $C_{19}H_{18}O_5$: C 69.93, H 5.56. Found: C 69.79, H 5.51.

5.1.2.18. 1,5-bis(3,5-Di-tert-butyl-4-hydroxylphenyl)-1,4-pentadiene-3-one ***C₄***. Yield 81%. M.p. 224–226 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.64 (d, J = 15.9 Hz, 2H, –CH=C–), 7.50 (s, 4H, ArH), 7.14 (d, J = 15.9 Hz, 2H, –C=CH–), 1.40 (s, 36H, C–CH_3). LC–MS (m/z): 490. Anal. Calc. for $C_{33}H_{46}O_3$: C 80.77, H 9.45. Found: C 80.63, H 9.40.

5.1.2.19. 1,5-bis(3,4-Dimethoxyphenyl)-1,4-pentadiene-3-one ***C₅***. Yield 98%. M.p. 89–90 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.68 (d, J = 15.9 Hz, 2H, –CH=C–), 7.38 (s, 2H, ArH), 7.31 (d, J = 8.1 Hz, 2H, ArH), 7.21 (d, J = 15.9 Hz, 2H, –C=CH–), 7.01 (d, J = 8.1 Hz, 2H, ArH), 3.84 (s, 6H, OCH_3), 3.81 (s, 6H, OCH_3). LC–MS (m/z): 354. Anal. Calc. for $C_{21}H_{22}O_5$: C 71.17, H 6.26. Found: C 71.02, H 6.14.

5.1.2.20. 1,5-bis(4-Hydroxy-3,5-dimethoxyphenyl)-1,4-pentadiene-3-one ***C₆***. Yield 65%. M.p. 164–166 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.00 (brs, 2H, –OH), 7.63 (d, J = 15.9 Hz, 2H, –CH=C–), 7.17 (d, J = 15.9 Hz, 2H, –C=CH–), 7.07 (s, 4H, ArH), 3.83 (s, 12H, OCH_3). LC–MS (m/z): 386. Anal. Calc. for $C_{21}H_{22}O_7$: C 65.28, H 5.74. Found: C 65.16, H 5.65.

5.1.2.21. 3,5-bis(4-Hydroxyphenyl)-4-piperidone ***D₁***. Yield 90%. M.p. > 300 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 10.23 (brs, 2H, –OH), 9.50 (brs, 2H, –N^+–H), 7.77 (s, 2H, –CH=), 7.38 (d, J = 8.1 Hz, 4H, ArH), 6.91 (d, J = 8.1 Hz, 4H, ArH), 4.45 (s, 4H, –CH_2–). LC–MS (m/z):343. Anal. Calc. for $C_{19}H_{18}ClNO_3$: C 66.38, H 5.28, N 4.07. Found: C 66.18, H5.20, N 4.04.

5.1.2.22. 3,5-bis(3,4-Dihydroxybenzylidene)-4-piperidone ***D₂***. Yield 90%. M.p. > 300 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.79 (brs, 2H, –OH), 9.60 (brs, 2H, –N^+–H), 9.37 (brs, 2H, –OH), 7.67 (s, 2H, –CH=), 6.90 (s, 2H, ArH), 6.82 (d, J = 8.1 Hz, 2H, ArH), 6.76 (d, J = 8.1 Hz, 2H, ArH), 4.43 (s, 4H, –CH_2–). LC–MS (m/z): 375. Anal. Calc. for $C_{19}H_{18}ClNO_5$: C 60.72, H 4.83, N 3.73. Found: C 60.62, H4.78, N 3.69.

5.1.2.23. 3,5-bis(4-Hydroxy-3-methoxybenzyliden)-4-piperidone ***D₃***. $C_{23}H_{24}O_7$:Yield 98%. M.p. 254–256 °C. ^{1}H NMR

(DMSO-d_6, 300 MHz) δ (ppm): 9.86 (brs, 2H, –OH), 9.62 (brs, 2H, $-N^+-H$), 7.79 (s, 2H, –CH=), 7.11 (s, 2H, ArH), 6.95 (d, $J = 8.1$ Hz, 2H, ArH), 6.89 (d, $J = 8.1$ Hz, 2H, ArH), 4.49 (s, 4H, $-CH_2-$). LC–MS (*m*/*z*): 403. Anal. Calc. for $C_{21}H_{22}ClNO_3$: C 62.45, H 5.49, N 3.47. Found: C 62.31, H5.46, N 3.42.

5.1.2.24. 3,5-bis(3,5-Di-tert-butyl-4-hydroxylbenzylidene)-4-piperidone ***D*****$_4$**. Yield 98%. M.p. 231–233 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.45 (brs, 2H, –OH), 9.40 (brs, 2H, $-N^+-H$), 7.81 (s, 2H, –CH=), 7.68 (s, 4H, ArH), 4.51 (s, 4H, $-CH_2-$), 1.42 (s, 36H, C–CH_3). LC–MS (*m*/*z*): 567. Anal. Calc. for $C_{35}H_{50}ClNO_3$: C 73.98, H 8.87, N 2.46. Found: C 73.79, H8.81, N 2.41.

5.1.2.25. 3,5-bis(3,4-Dimethoxybenzylidene)-4-piperidone ***D*****$_5$**. Yield 88%. M.p. 216–218 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.82 (brs, 2H, $-N^+-H$), 7.82 (s, 2H, –CH=), 7.14 (s, 2H, ArH), 7.10 (d, $J = 8.1$ Hz, 2H, ArH), 7.02 (d, $J = 8.1$ Hz, 2H, ArH), 4.50 (s, 4H, $-CH_2-$), 3.82 (s, 3H, O–CH_3), 3.83 (s, 3H, O–CH_3). LC–MS (*m*/*z*): 431. Anal. Calc. for $C_{23}H_{26}ClNO_5$: C 63.96, H 6.07, N 3.24. Found: C 63.78, H5.98, N 3.21.

5.1.2.26. 3,5-bis(4-Hydroxy-3,5-dimethoxybenzylidene)-4-piperidone ***D*****$_6$**. Yield 88%. M.p. 231–233 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.74 (brs, 2H, –OH), 9.20 (brs, 2H, $-N^+-H$), 7.78 (s, 2H, –CH=), 6.82 (s, 4H, ArH), 4.55 (s, 4H, $-CH_2-$), 3.82 (s, 6H, O–CH_3). LC–MS (*m*/*z*): 463. Anal. Calc. for $C_{23}H_{26}ClNO_7$: C 59.55, H 5.65, N 3.02. Found: C 59.42, H5.56, N 2.96.

5.1.2.27. 3,5-bis(3-Bromo-4-hydroxy-5-methoxybenzylidene)-4-piperidone ***D*****$_7$**. Yield 75%. M.p. 260–262 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 10.24 (brs, 2H, –OH), 9.73 (brs, 2H, $-N^+-H$), 7.75 (s, 2H, –CH=), 7.22 (m, 4H, ArH), 4.50 (s, 4H, $-CH_2-$), 3.86 (s, 6H, O–CH_3). LC–MS (*m*/*z*): 559. Anal. Calc. for $C_{21}H_{20}Br_2ClNO_5$: C 44.91, H 3.59, N 2.49. Found: C 44.82, H 3.52, N 2.44.

5.2. α-Glucosidase inhibitory assay

α-Glucosidase activity was assayed using 50 mM phosphate buffer at pH 7.0, and the appropriate PNP glycoside (at 1 mM) were used as substrates. The concentration of the enzyme was specified in each experiment. Curcuminoids at the designated concentration was added to the enzyme solution and incubated at 37 °C for 30 min, and the substrate was then added to initiate the enzyme reaction. The enzyme reaction was carried out at 37 °C for 30 min. Product (PNP) was monitored spectrophotometrically by measuring the absorbance ($\lambda = 400$ nm). One unit of α-glucosidase is defined as the amount of enzyme liberating 1.0 μmol of PNP per minute under the assay conditions specified.

The enzyme reaction was performed in the above reaction conditions with inhibitors of various concentrations. Inhibition types for the inhibitors were determined by Lineweaver–Burk plots and its replot of slope versus the reciprocal of the substrate concentration.

The characterization of secondary structure of α-glucosidase in the buffer solution with or without inhibitors was examined with CD spectroscopy. The data obtained from the experiments were dealt with the professional software *secondary structure estimation* and *origin* 6.0.

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