Scientia Iranica C (2012) 19 (6), 1580–1583

Sharif University of Technology

Scientia Iranica

Transactions C: Chemistry and Chemical Engineering

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Curcumin and its derivatives: Moderate inhibitors of acetylcholinesterase, butyrylcholinesterase and trypsin

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Received 18 September 2011; revised 5 March 2012; accepted 4 July 2012

KEYWORDS

Curcumin;
Synthetic derivatives;
Acetylcholinesterase;
Butyrylcholinesterase;
Lipoxygenase;
Trypsin.

Abstract Curcumin, a polyphenol yellow orange pigment present in Indian spice "turmeric", is a member of the ginger family (Zingiberaceae). The extract of turmeric was obtained by using soxhlet apparatus at 45–55 °C, using methanol as solvent and then curcumin was isolated from methanolic extract by column chromatography. In the present study, a series of four new derivatives of curcumin (3a–d) were synthesized by reacting curcumin (1) with different alkyl halides (2a–d) using DMF and lithium hydride. The structures of natural curcumin and its synthetic derivatives were confirmed with the help of their ^{1}H-NMR spectra and all these were screened against acetylcholinesterase, butyrylcholinesterase, lipoxygenase and trypsin enzymes. Curcumin and its derivatives exhibited moderate inhibitory potential against all aforesaid enzymes.

1. Introduction

Curcumin is a yellow orange dietary pigment present in Indian spice "turmeric" and is responsible for the yellow color of curry and good taste [1,2]. Curcumin, commonly called diferuloyl methane, is a hydrophobic natural polyphenol. It is also known as "Natural Yellow 3" [3,4]. It is effectively used for the recovery of many diseases including multiple myeloma, Alzheimer's disease, psoriasis, pancreatic cancer and myelodysplastic syndrome [5]. It is very effective against HIV (human immunodeficiency virus, the AIDS virus), because curcumin has ability to interfere with replication cycle of HIV [6,7]. Over the centuries it has been used as anti-inflammatory agent to relieve pain and inflammation in skin and muscle. It is also used for the treatment of jaundice and menstrual disturbance. It is an antioxidant, anti-inflammatory, anti-carcinogenic and antimicrobial agent [8]. It has been used for thousand years as a spice, coloring agent in foods, household medicines and insect repellent [2]. It is also used to minimize the risk of lung cancer associated with smoking. It reduces the effects of nicotine (a strong cancer-causing agent) as a carcinogen by 50% [9]. It showed efficient protective effect in reducing the chances of cancer in lungs, liver, duodenum, and kidneys [10,11]. In the present study, curcumin (1) was extracted from turmeric using Soxhlet apparatus and then its *O*-substituted derivatives were synthesized using different alkyl halides in DMF. This natural curcumin (1) and its synthetic derivates (3a–d) were screened against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), lipoxygenase (LOX), and trypsin enzymes to investigate their inhibitory potential against these enzymes.

2. Results and discussion

The synthesis of various derivatives of curcumin (1) has been outlined in Scheme 1. The structure of the extracted curcumin (1) was confirmed by it ^{1}H-NMR spectrum showing the signals at δ7.56 (d, J = 15.9 Hz, 2H, H-7, H-13), 7.19 (d, J = 1.2 Hz, 2H,

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Peer review under responsibility of Sharif University of Technology.

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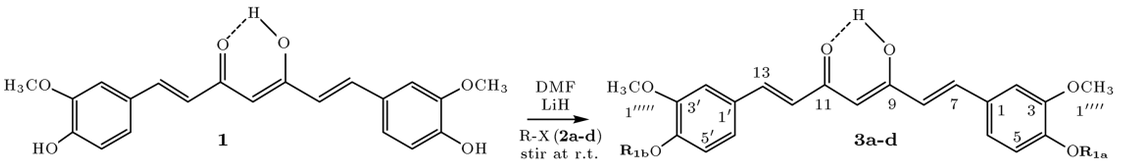
doi:10.1016/j.scient.2012.10.014



Compound no.	R_{1a}	R_{1b}
3a	$-CH(CH_3)_{2''}$	$-CH(CH_3)_{2'''}$
3b	$-CH_2-CH_2-CH_3$	$-CH_2-CH_2-CH_3$
3c	$-CH_2-CH_2-CH_2-CH_3$	$-CH_2-CH_2-CH_2-CH_3$
3d	$-CH_2-CH_2-CH_2-CH_2-CH_3$	$-CH_2-CH_2-CH_2-CH_2-CH_3$

Note: R_{1a} and R_{1b} are the same in each individual molecule but these are shown separately to avoid confusion in numbering in the molecule

Scheme 1: Synthesis of *O*-substituted derivatives of curcumin.

H-2 & H-2′), 7.08 (dd, $J = 1.5$, 8.1 Hz, 2H, H-6 & H-6′), 6.81 (d, $J = 8.1$ Hz, 2H, H-5 & H-5′), 6.59 (d, $J = 15.6$ Hz, 2H, H-8, H-12), 5.95 (br s, H-10) and 3.88 (s, 6H, OCH_3-1‴, OCH_3-1⁗) in agreement with reported literature [12]. In the ^{1}H-NMR spectrum of 3a, in addition to the similar signal of curcumin, the additional two proton multiplet at δ4.58 (m, 2H, CH-1″, CH-1‴), and a doublet at δ1.37 (d, $J = 6.0$ Hz, 12H, CH_3-2″, CH_3-3″, CH_3-2‴, CH_3-3‴) integrated for twelve protons, ascertained the substitution of two *O*-iso propyl group at available positions C-4 and C-4′ in the molecule. In 3b, the additional signals of 4.04 (t, $J = 7.1$ Hz, 4H, CH_2-1″, CH_2-1‴), 1.46 (sextet, $J = 7.4$ Hz, 4H, CH_2-2″, CH_2-2‴), and 0.96 (t, $J = 7.2$ Hz, 6H, CH_3-3″, CH_3-3‴) confirmed the substitution of two $O-n$-propyl groups at C-4 and C-4′ in this molecule. In 3c, the additional signals at δ4.00 (t, $J = 6.9$ Hz, 4H, CH_2-1″, CH_2-1‴), 1.79 (quintet, $J = 7.8$ Hz, 4H, CH_2-2″, CH_2-2‴), 1.48 (sextet, $J = 7.5$ Hz, 4H, CH_2-3″, CH_2-3‴) and 0.98 (t, $J = 7.5$ Hz, 4H, CH_3-4″, CH_3-4‴) established the substitution of two $O-n$-butyl groups at C-4 and C-4′ in this molecule. Similarly, relative to 1, the substitution of two $O-n$-pentyl groups was evident by the additional signals of 4.03 (t, $J = 7.0$ Hz, 4H, CH_2-1″, CH_2-1‴), 1.82 (m, 4H, CH_2-2″, CH_2-2‴), 1.41 (m, 4H, CH_2-3″, CH_2-3‴), 1.41 (m, 4H, CH_2-4″, CH_2-4‴), and 0.92 (t, $J = 7.3$ Hz, 6H, CH_3-5″, CH_3-5‴) at positions C-4 and C-4′ in 3d.

The screening of natural 1 and its synthesized derivatives 3a–d against AChE, BChE, LOX and protease enzymes revealed that almost all these were moderately active against all these enzymes except LOX (Table 1). The (1E,6E)-1, 7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (Curcumin, 1) showed better activity having IC_{50} values of 58.08 ± 0.89 μmol/L for AChE, and 41.25 ± 0.79 μmol/L for BChE, respectively, relative to its synthesized derivatives 3a–d. However, these derivatives exhibited better inhibitory potential against trypsin enzyme as in this case, the highest IC_{50} (54.89 ± 0.78 μmol/L) was shown by 1. The IC_{50} against AChE were found in the range between 58.08 ± 0.89 to 298.61 ± 0.95 μmol/L, and the highest being shown by 3c. Against BChE, it ranged between 41.25±0.79 to 143.2±0.93 μmol/L, and the highest being shown by 3a. However, against trypsin, 3b was found to be a better inhibitor with IC_{50} of 19.36 ± 0.88 μmol/L relative to all other studied molecules. None of the derivative exhibited antioxidant activity (maximum of 37.85%) or LOX inhibitory activity (maximum of 55%) at 0.5 mM concentration of test compound.

2.1. Conclusion

In conclusion, it was inferred from the aforesaid enzyme inhibitory results that the *O*-substituted synthetic derivatives of curcumin were moderately active against AChE, BChE and trypsin.

3. Experimental

3.1. General

TLC was performed on pre-coated silica gel G-25-UV_{254} plates. Purity was checked with different solvent systems using ethyl acetate and *n*-hexane giving single spot. ^{1}H NMR spectra were recorded in $CDCl_3$ and CD_3OD on a Bruker spectrometers operating at 300 MHz. Chemical shifts are given in ppm. The melting points were recorded on a Griffin & George melting point apparatus.

3.2. General procedure for the extraction of curcumin from turmeric

The extraction of curcumin (1) was carried out from turmeric spice (1 kg) with methanol using Soxhlet apparatus as it is simpler and more effective than other conventional extraction methods [12]. The extraction was continued until the solvent was found to be colourless. The dark brown methanolic extract was obtained and was filtered and concentrated by using a rotatory evaporator under reduced pressure at 50 °C. The dried crude methanolic extract was subjected to extensive column chromatography using various compositions

Table 1: Enzyme inhibition studies on curcumin and its derivatives.

Sample No.	DPPH[a]		AChE		BChE		LOX		Trypsin	
	(%) at 0.5 mM	(IC_{50})μmol/L	(%) at 0.5 mM	(IC_{50})μmol/L	(%) at 0.5 mM	(IC_{50})μmol/L	(%) at 0.5 mM	(IC_{50})μ mol/L	(%) at 0.5 mM	(IC_{50})μmol/L
1	85.70 ± 0.95	115.21 ± 0.87	96.50 ± 0.97	58.08 ± 0.89	97.80 ± 0.99	41.25 ± 0.79	41.21 ± 0.78	>500	55.23 ± 0.96	54.89 ± 0.78
3a	37.85 ± 0.83	–	67.91 ± 0.89	283.12 ± 0.93	71.58 ± 0.81	143.2 ± 0.93	34.73 ± 0.85	>500	57.32 ± 0.79	32.96 ± 0.83
3b	7.59 ± 0.94	–	68.84 ± 0.97	279.12 ± 0.98	73.90 ± 0.88	141.9 ± 0.97	55.45 ± 0.93	>450	72.91 ± 0.84	19.36 ± 0.88
3c	25.70 ± 0.77	–	66.79 ± 0.96	298.61 ± 0.95	45.70 ± 0.95	>500	50.42 ± 0.89	>450	62.34 ± 0.86	26.25 ± 0.82
3d	23.04 ± 0.89	–	82.13 ± 0.79	112.34 ± 0.92	76.94 ± 0.92	116.7 ± 0.95	50.22 ± 0.91	>450	51.45 ± 0.81	41.81 ± 0.79
Control	Quercetin	16.96 ± 0.14	Eserine	0.04 ± 0.001	Eserine	0.85 ± 0.001	Baicalein	22.4 ± 1.3	PMSF[b]	0.11 ± 0.02

[a] 1,1-diphenyl-2-picrylhydrazyl radical.
[b] Phenylmethanesulfonyl fluoride.

of *n*-hexane/dichloromethane and then dichloromethane/ethyl acetate to get the purified 1 (1.98 g). Its purity was checked on pre-coated thin layer chromatography (TLC) plate giving single spot. The purity of the isolated curcumin was also obvious from its clear ^{1}H-NMR spectrum.

3.3. General procedure for the synthesis of 4-O, 4′-O-Di-alkyl derivatives (3a–d) in DMF

Calculated amount of 1 (0.2 g; 0.000543 mol) was dissolved in dimethyl formamide (DMF) in a round bottom flask (50 mL), followed by the addition of lithium hydride (0.05 g). The mixture was stirred for 30 min at room temperature, then dimolar quantity (0.00109 mol) of the alkyl iodide, 2a–d, was added and the solution was further stirred for three hours. The progress of the reaction was monitored by TLC. After the completion of the reaction, excess cold water was added to the flask and it was shaken well. A semi-solid product formed was subjected to solvent extraction with chloroform. The lower organic layer was separated and dried to obtain the final product.

3.4. DPPH radical scavenging activity

Compounds were assayed for antioxidant activity using DPPH method. Ninty μL methanolic 0.1 mM DPPH solution contained 10 μL of 0.5 mM test solution to give total volume of 100 μL well^{-1}. After 30 min of incubation at 30 °C, the loss in absorbance was measured using Synergy HT (USA) 96-well plate reader. Percent DPPH scavenging activity was calculated by standard method.

3.5. AChE and BChE assay

The AChE and BChE inhibition activity were performed according to the method of Ellman et al. [13] with slight modifications. Total volume of the reaction mixture was 100 μL. It contained 60 μL $Na_2H\ PO_4$ buffer with concentration of 50 mM and pH 7.7. Ten μL test compound (0.5 mM well^{-1}) was added, followed by the addition of 10 μL (0.005 unit well^{-1}) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37 °C. The reaction was initiated by the addition of 10 μL of 0.5 mM well^{-1} substrate (acetylthiocholine iodide), followed by the addition of 10 μL DTNB (0.5 mM well^{-1}). After 15 min of incubation at 37 °C, the absorbance was measured at 405 nm using Synergy HT (USA) 96-well plate reader. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well^{-1}) was used as a positive control. The percent inhibition was calculated by the help of the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100.$$

IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

3.6. LOX assay

LOX activity was assayed according to the method of Tappel, Evans and Baylac and Racine [14–16] with slight modifications. A total volume of 200 μL assay mixture contained 150 μL sodium phosphate buffer (100 mM, pH 8.0), 10 μL test compound and 15 μL purified LOX enzyme (Sigma). The contents were mixed and pre-read at 234 nm and pre-incubated for 10 min at 25 °C. The reaction was initiated by the addition of 25 μL substrate solution. The change in absorbance was observed after 6 min at 234 nm using Synergy HT (USA) 96-well plate reader. All reactions were performed in triplicates. Baicalein (0.5 mM well^{-1}) was used as a positive control. The percentage inhibition (%) was calculated by formula given below. IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

$$\text{Inhibition(\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Control = Total enzyme activity without inhibitor

Test = Activity in the presence of test compound.

3.7. Trypsin inhibition assay

Trypsin inhibition assay was carried out according to the method of Jedinak et al. [17] with some modification. The stock solution of trypsin was prepared by dissolving 2 mg of trypsin in 10 mL of 1.0 mM HCl. N-a-benzoyl-DL-arginine paranitroanilide hydrochloride (BApNA) was dissolved in DMSO (20 mg/mL). Enzyme (0.3 mL) and inhibitor (100 μL) was incubated at 37 °C for 15 min then 0.6 mM substrate was added and final volume was made 2.5 mL with Tris buffer (100 mM, pH 7.5). The reaction mixture was incubated at 37 °C for 0.5 h. The reaction was quenched by adding 30% acetic acid and read at 410 nm using spectrophotometer. PMSF (phenylmethanesulfonyl fluoride) was used as positive reference. The percentage inhibition was calculated by using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100.$$

3.8. Structural characterization

(1E,6E)-1,7-*bis*(4-*hydroxy*-3-*methoxyphenyl*) *hepta*-1,6-*diene*-3,5-*dione* (*Curcumin*) (1)

Orange yellow powder, (1.98 g), ^{1}H-NMR (300 MHz, CD_3OD): δ7.56 (d, J = 15.9 Hz, 2H, H-7, H-13), 7.19 (d, J = 1.2 Hz, 2H, H-2 & H-2′), 7.08 (dd, J = 1.5, 8.1 Hz, 2H, H-6 & H-6′), 6.81 (d, J = 8.1 Hz, 2H, H-5 & H-5′), 6.59 (d, J = 15.6 Hz, 2H, H-8, H-12), 5.95 (br s, H-10) and 3.88 (s, $2OCH_3$, OCH_3-1′′′′, OCH_3-1′′′′′).

4-*O*,4′-*O*-*Di-isopropyl curcumin* (3a)

Dark brown oily solid, yield 69%, ^{1}H-NMR (300 MHz, $CDCl_3$): δ7.58 (d, J = 15.6 Hz, H-7), 7.10 (dd, J = 1.6, 8.4 Hz, 2H, H-6 & H-6′), 7.03 (d, J = 1.6 Hz, 2H, H-2 & H-2′), 6.85 (d, J = 8.4 Hz, 2H, H-5, H-5′), 6.47 (d, J = 15.9 Hz, H-8), 5.78 (br s, H-10), 4.58 (m, 2CH, CH_2-1′′, CH-1′′′), 3.88 (s, $2OCH_3$, OCH_3-1′′′′, OCH_3-1′′′′′), 1.37 (d, J = 6.0, 12H, CH_3-2′′, $CH_3$2′′′, CH_3. 3′′, CH_3-3′′′).

4-*O*, 4′-*O*-*Di-n-propyl curcumin* (3b)

Dark brown solid, yield 92%, ^{1}H-NMR (300 MHz, $CDCl_3$): δ7.65 (d, J = 15.3 Hz, H-7), 7.12 (dd, J = 1.7, 8.1 Hz, 2H, H-6 & H-6′), 6.95 (d, J = 1.7 Hz, 2H, H-2 & H-2′), 6.78 (d, J = 8.1 Hz, 2H, H-5 & H-5′), 6.59 (d, J = 15.6 Hz, H-8), 5.79 (br s, H-10), 4.04 (t, J = 7.1 Hz, 4H, CH_2-1′′, CH_2-1′′′), 3.88 (s, $2OCH_3$, OCH_3-1′′′′, OCH_3-1′′′′′), 1.46 (sextet, J = 7.4 Hz, 4H, CH_2-2′′, CH_2-2′′′), 0.96 (t, J = 7.2 Hz, 6H, CH_3-3′′, CH_3-3′′′).

4-*O*, 4′-*O*-*Di-n-butyl curcumin* (3c)

Brownish solid, yield 57%, ^{1}H-NMR (300 MHz, $CDCl_3$): δ7.65 (d, J = 15.3 Hz, H-7), 7.07 (dd, J = 1.5, 8.4 Hz, 2H, H-6 & H-6′), 6.96 (d, J = 1.5 Hz, 2H, H-2 & H-2′), 6.79 (d, J = 8.4 Hz, 2H, H-5 & H-5′), 6.59 (d, J = 15.6 Hz, H-8), 5.88 (br s, H-10), 4.00 (t, J = 6.9, $2CH_2$, CH_2-1′′, CH_2-1′′′), 3.84 (s, $2OCH_3$, OCH_3-1′′′′, OCH_3-1′′′′′), 1.79 (quintet, J = 7.8 Hz, $2CH_2$, CH_2-2′′, CH_2-2′′′), 1.48 (sextet, J = 7.5 Hz, 4H, CH_2-3′′, CH_2-3′′′), 0.98 (t, J = 7.5, 6H, CH_3-4′′, CH_3-4′′′).

4-*O*, 4′-*O*-*Di-n-pentyl curcumin* (3d)

Yellowish solid, yield 78%, ^{1}H-NMR (300 MHz, $CDCl_3$): δ7.65 (d, J = 15.3 Hz, H-7), 7.06 (dd, J = 1.6, 8.1 Hz, 2H, H-6 & H-6′), 6.97 (d, J = 1.6 Hz, 2H, H-2 & H-2′), 6.79 (d, J = 8.1 Hz, 2H, H-5 & H-5′), 6.59 (d, J = 15.6 Hz, H-8), 5.86 (br s, H-10), 4.03 (t, J = 7.0 Hz, $2CH_2$, CH_2-1′′, CH_2-1′′′), 3.85(s, $2OCH_3$, OCH_3-1′′′′, OCH_3-1′′′′′), 1.82 (m, 4H, CH_2-2′′, CH_2-2′′′), 1.41 (m, 4H, CH_2-3′′, CH_2-3′′′), 1.41 (m, 4H, CH_2-4′′, CH_2-4′′′), 0.92 (t, J = 7.3, 6H, CH_3-5′′, CH_3-5′′′).

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