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Inhibition of monoamine oxidase A and B by demethoxycurcumin and bisdemethoxycurcumin

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Abstract Two curcumin derivatives, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), isolated from *Curcuma longa* were analyzed for their inhibitory activities against two isoforms of monoamine oxidase (MAO), which is involved in the catalysis of neurotransmitting monoamines. In the study, DMC and BDMC potently inhibited human MAO-B, with IC_{50} values of 2.45 and 2.59 μM, respectively, and both compounds showed effective inhibitory activities against human MAO-A, with IC_{50} values of 3.24 and 3.09 μM, respectively. The inhibitory activities of the two compounds were higher than those of curcumin. The removal of the methoxy or dimethoxy groups in curcumin might increase the inhibitory activities against human MAO-A and MAO-B. The inhibited activities were recovered to almost the values of the reversible references in the dialysis experiments with DMC and BDMC. DMC and BDMC showed competitive inhibition for MAO-A and MAO-B, respectively, with K_i values of 0.91 and 0.80 μM, respectively. These results suggest that the two curcumin derivatives may be useful or lead compounds in the treatment of related disorders as potent reversible MAO inhibitors.

Keywords Bisdemethoxycurcumin · Demethoxycurcumin · Monoamine oxidase · Reversible competitive inhibitor

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Introduction

Curcumin is a plant alkaloid in *Curcuma longa* and the major curcuminoid found in turmeric [1]. It has potential activities as an antidepressant [1,2] and a pharmaceutical for the treatment in Parkinson's disease (PD) [3]. Recently, various biological activities of curcumin have been studied in terms of its therapeutic effect on cancer [4], as well as the ameliorative effect on olanzapine-induced obesity [5]. Curcuminoids are a mixture of the three following principal compounds, in decreasing order: curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) [6,7].

Monoamine oxidases (MAOs) catalyze the oxidative deamination of monoamine neurotransmitters, acting as two isoforms, i.e., MAO-A and MAO-B [8]. In terms of substrate specificities, MAO-A prefers the deamination of serotonin, whereas MAO-B prefers the deamination of phenylethylamine and benzylamine [9]. MAO-A and MAO-B are targeted to treat depression and PD/Alzheimer's disease (AD) [8,10]. Therefore, considerable efforts have been continued in the exploration of potent MAO-A and/or MAO-B inhibitors from natural and synthetic compounds [11].

The inhibitory activity of MAO by pyrazoline analogues synthesized based on curcumin has been reported [12]. Inhibition of MAO activities by curcumin was observed using mitochondria isolated from the rat brain [6]. However, the neuroprotective effects of curcumin are still not fully understood. Furthermore, no information about the inhibitory activities of MAO by DMC and BDMC has been reported. In this study, we report higher inhibitory activities by the two derivatives than curcumin using recombinant human MAO-A and MAO-B and describe their inhibition patterns.

Materials and methods

Compound isolation

Curcuma longa (dry rhizomes, 200 g) was extracted with 1 L of

ethyl alcohol. The crude extract (29.5 g) was partitioned with ethyl acetate, and subjected to silica-gel column eluting with dichloromethane/methanol for 10 fractions. The fifth fraction (2.3 g) was further fractionated by repeating the silica-gel procedure. The second fraction (200 mg) from the fifth fraction was further purified using a C18 column (Phenomenex Luna C18 (2), 250×10.00 mm, 5 μm, 2.0 mL/min) with an isocratic solvent system (H_2O:CH_3CN =35:65 with 0.1% TFA, UV =420 nm) to yield curcumin (40.5 mg; **1**). The fraction containing curcuminoids was further purified (UV =390 nm) to yield DMC (20.7 mg; **2**) and BDMC (10.2 mg; **3**). These three compounds were identified by comparing the data to those of previous reports.

Enzyme assays

The initial rates of MAOs were measured using the continuous method at 25 °C, as described previously [13], except with 0.3 mM instead of 0.6 mM of benzylamine for the MAO-B assay. The K_m of kynuramine and benzylamine were 0.036 and 0.14 mM, respectively. Enzymes and chemicals used were also described previously [13].

Analysis of reversibility of the inhibitors

The reversibilities of the effective inhibitors were investigated using the dialysis method at ~2× IC_{50} concentrations, as previously described, with slight modifications [13], by adding 0.06 mM of kynuramine or 0.3 mM of benzylamine, respectively, as well as the reference inhibitors.

Analysis of inhibitory activities and enzyme kinetics

The IC_{50} values and the time-dependences by DMC and BDMC were investigated as previously described [14], using modified concentration of benzylamine (0.3 mM). The K_i values of the potent compounds were determined as previously described [13].

Results and Discussion

Compound isolation

Ethyl acetate soluble layer of the crude extract from *Curcuma Longa* was separated into three peaks in HPLC chromatography (Fig. S1). The ^{1}HNMR spectrum of **1** showed two set of 1,3,4 tri-substituted aromatic protons [δ 6.82 (dd, 2H, J=8.0 Hz,), 7.09 (dd, 2H, J=8.0 Hz), δ 7.20 (d, 2H, J=2.0 Hz), two set of olefinic protons [δ 6.61 (d, 2H, J=15.8 Hz), 7.56 (d, 2H, J=15.8 Hz)], and one methylene singlet at δ 5.95 (s, 2H). By comparing the NMR spectroscopic data of **1** with the previously reported data in the literature, **1** was identified as curcumin [15]. Based on the comparison of NMR data for **2** and **3** to those of reported in the literature [16], the two compounds were identified as DMC and BDMC, respectively. The chemical structures of the curcumin and its derivatives are shown in Fig. 1.

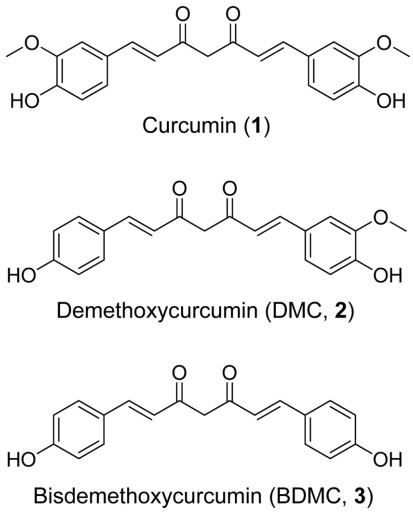

Fig. 1 Structure of curcumin (**1**), demethoxycurcumin (DMC, **2**) and bisdemethoxycurcumin (BDMC, **3**) from *Curcuma longa*

Inhibitory activities of DMC and BDMC

The compounds showed more potent inhibitory activities against MAO-B than MAO-A (Table 1). The IC_{50} values of BDMC (**3**) for MAO-A and MAO-B were 3.24 and 2.45 μM, respectively; those of DMC (**2**) were 3.09 and 2.59 μM, respectively. The values of BDMC and DMC were lower than those of curcumin (**1**). These results suggest that the removal of the methoxy or dimethoxy groups in curcumin increase the inhibitory activities against human MAO-A and MAO-B.

Reversibility of DMC and BDMC

In time dependency analyses, no delay were observed in the interactions between MAO-A and DMC or MAO-B and BDMC (data not shown).

For recovery experiments, the residual activities of the undialyzed control (~2×IC_{50}) and dialyzed sample by DMC for MAO-A were 34.5 and 68.9%, respectively (Fig. 2A). For the reference reversible MAO-A inhibitor (toloxatone), the values were 31.3 and 88.0%, respectively, while the values were 23.5 and 26.5% for the reference irreversible MAO-A inhibitor (clorgyline). Therefore, MAO-A inhibition by clorgyline was not recovered by dialysis, whereas inhibition by toloxatone was highly recovered. The results indicated the inhibitory activity by DMC was almost recovered to near the value of the reversible reference; thus, the findings showed that DMC is a reversible inhibitor of MAO-A.

In terms of BDMC for MAO-B, the patterns were similar to that of DMC for MAO-A (Fig. 2B). Like in the case of MAO-A, MAO-B inhibition by pargyline was not recovered, but inhibition by lazabemide was greatly recovered. These results suggested BDMC is also a reversible inhibitor of MAO-B.

Table 1 Inhibitions of human MAO-A and MAO-B by curcumin and its two derivatives

Compounds	IC_{50} (μM)		K_i (μM)	
	MAO-A	MAO-B	MAO-A	MAO-B
Curcumin (**1**)	3.64±0.035	3.36±0.162	-	-
DMC (**2**)	3.09±0.075	2.59±0.100	0.91±0.063	0.86±0.035
BDMC (**3**)	3.24±0.121	2.45±0.040	1.40±0.14	0.80±0.007
Toloxatone[a]	1.10±0.0085	-		
Lazabemide	-	0.039±0.001		
Clorgyline[a]	0.0062±0.0003	> 2.0		
Pargyline	>2.0	0.082±0.01		

[a]Values for toloxatone and clorgyline were from the reference [13]

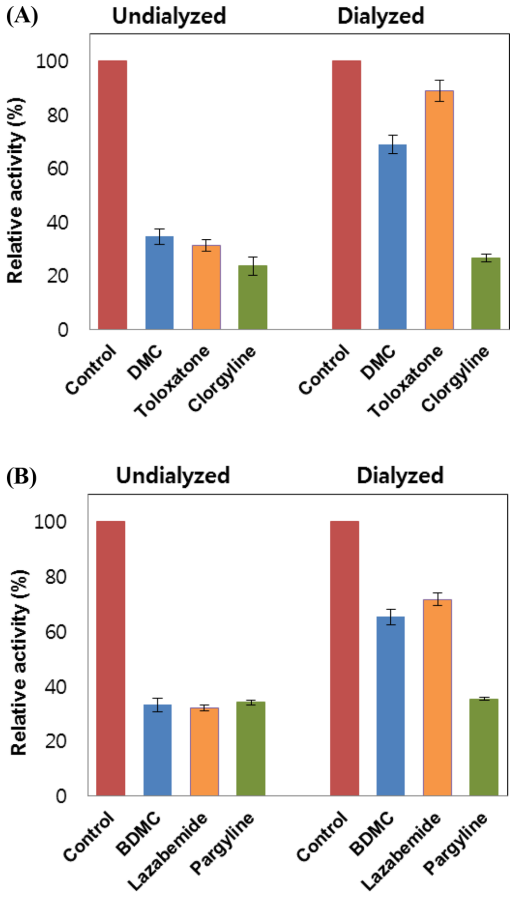

Fig. 2 Inhibitions of MAO-A by DMC (**A**) and MAO-B by BDMC (**B**), and recovery of activities by dialysis. The concentrations of inhibitors were used at approximately 2×IC_{50}. (**A**) Toloxatone (0.2 μM) and clorgyline (0.013 μM) were used as references for reversible and irreversible MAO-A inhibitors, respectively. (**B**) Lazabemide (0.3 μM) and pargyline (0.25 μM) were used as references for reversible and irreversible MAO-B inhibitors, respectively

Inhibition kinetics of DMC and BDMC

The Lineweaver-Burk plots of the MAO-A inhibitions by DMC showed linear lines and exactly intersected the *y*-axis at one point (Fig. 3A). The secondary plot showed that the K_i value of DMC for the inhibition of MAO-A was 0.91±0.063 μM (Fig. 3B). Similar patterns were observed for the inhibition of MAO-B by BDMC in the plots (Figs. 3C, D). The plot showed that the K_i value of BDMC for MAO-B was 0.80±0.007 μM. K_i values of DMC for MAO-B and BDMC for MAO-A were 0.86±0.035 and 1.40±0.14 μM, respectively (Table 1).

The potencies of DMC or BDMC for MAOs were lower than those of acacetin (IC_{50} values for MAO-A and MAO-B =0.19 and 0.17 μM, respectively), which is an extremely potent inhibitor in natural compounds [17]. However, they were slightly higher than those of genistein (IC_{50} for MAO-A and MAO-B =3.9 and 4.1 μM, respectively) [14]. Compared with the MAO-A selective inhibitors, the potencies of DMC and BDMC were lower than those of hispidol (0.26 μM) [13] and decursin (1.76 μM) [18]; however, they were comparable to or higher than those of purpurin (2.5 μM) [19] and wogonin (5.80 μM) [18], while they were also comparable to that of the marketed drug toloxatone (1.10 μM). In the comparison with the MAO-B selective inhibitors, the potencies of DMC and BDMC were lower than or comparable to those of maackiain (0.68 μM) [14] and the piloquinone (1.21 μM) [20]. Recently, several natural heterocyclic compounds, including curcumin, have been used in computational development for their MAO inhibitory profiling [21].

The results indicate that DMC and BDMC may be a useful or lead compounds for the treatment of related disorders, such as depression, PD and AD.

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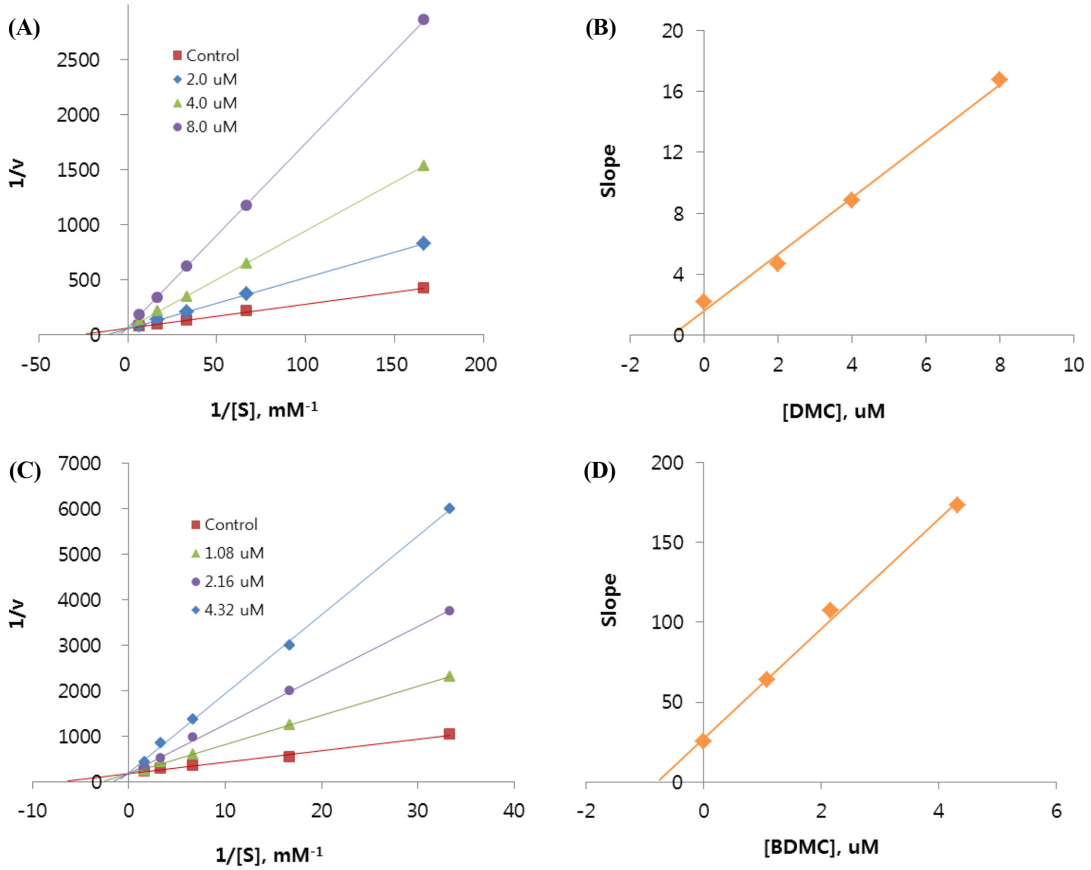

Fig. 3 Lineweaver-Burk plots of MAO-A inhibitions by DMC (**A**) and MAO-B by BDMC (**C**) and their respective secondary plots of MAO-A (**B**) and MAO-B (**D**)

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