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Design, synthesis and anti-Alzheimer properties of dimethylaminomethyl-substituted curcumin derivatives

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ABSTRACT

Eight dimethylaminomethyl-substituted curcumin derivatives were designed and synthesized. The antioxidant test revealed that the synthesized compounds had higher free radical scavenging activity towards both 2,2-diphenyl-1-picrylhydrazyl free radicals (DPPH) (IC_{50} 1.5–29.9 μM) and galvinoxyl radicals (IC_{50} 4.9–41.1 μM) than the lead compound curcumin. Besides, compound **3a** could effectively inhibit the Aβ self-aggregation in vitro. Investigated in phosphate-buffered solutions (pH = 7.4) in the presence or absence of 0.1% FBS **3a** showed a good stability while curcumin did not. Furthermore, **3a** showed a good lipophilicity ($\log P = 3.48$), suggesting a potential ability to penetrate the blood–brain-barrier. The aqueous solubility of the hydrochloride salt of **3a** (16.7 mg/mL) has also been significantly improved as compared with curcumin (<0.1 mg/mL).

Alzheimer's disease (AD) is a neurodegenerative disease with a huge associated impact on individuals, families, and society. Though the disease has been found for more than 100 years, it is still incurable due to our incomplete understanding of its pathogenesis. Substantial evidence revealed that many biological and physiological steps are involved in the eventual pathological condition of AD, suggesting AD is a multifactorial disease.[1] Currently, the clinical available therapeutics shows only limited effectiveness in ameliorating the symptoms of AD and in improving cognitive ability. Thus, developing an effective agent to combat AD is therefore an immediate and important challenge nowadays.

Natural products are rich and unexplored sources of novel leading compounds with bioactive properties. Curcumin (**1**, Fig. 1) is a natural phenolic compound originally isolated from turmeric, a rhizome used in India for centuries as a spice and medicinal agent. Curcumin has a wide variety of bioactivities, including chemopreventive, antiinflammatory, antioxidant, antitumor as well as anti-AD properties.[2] There are evidences that the consumption of curcumin-containing natural compounds in various Asian populations is associated with a protective effect against AD.[3] The mechanism of the anti-AD property of curcumin is probably due to its ability of enhancing the brain clearance of amyloid-β (Aβ). Besides, it is also suggested that the antioxidant and the immune-stimulating properties of curcumin may be responsible for the anti-AD activity.[4] Despite curcumin showed many promising bioactivities, some drawbacks, especially the low bioavailability, the poor stability and water solubility, have been highlighted as the major problems in its therapeutic applications.[5,6]

Counteracting the shortcomings of curcumin mentioned above, various curcumin analogs/derivatives have been designed and synthesized in order to enhance the metabolic stability and the anti-AD activity, such as monofunctional curcumin derivatives,[7] curcumin derived pyrazoles and isoxazoles,[8] curcumin bioconjugates,[9] and so on. Before long, we reported a series of dimethylaminomethyl-substituted curcumin derivatives (e.g., **2a** and **2b**, Fig. 1) which showed potent antioxidant activity, good stability, and improved water-solubility, presenting good leading structures for further explorations.[10] Substantial evidence had revealed that substituent groups on the aromatic rings of curcumin had important influence on the activity of curcumin. For example, Xiao et al reported that the heptadiendione bridge chain and the phenolic hydroxy group are essential for the neuroprotective effect of curcumin,[11] and Cui et al found that bulky substituent on the aromatic ring is tolerate or even beneficial for the Aβ binding ability.[12] Based on these findings as well as our previous work, we have designed and synthesized another series of curcumin derivatives which possess the following structural characteristics: (i) the heptadiendione bridge chain and one phenolic hydroxy group are retained so that the newly synthesized derivatives could keep the neuroprotective effect of curcumin; (ii) a bulky dimethylaminomethyl group was introduced to the *ortho* position of the phenolic

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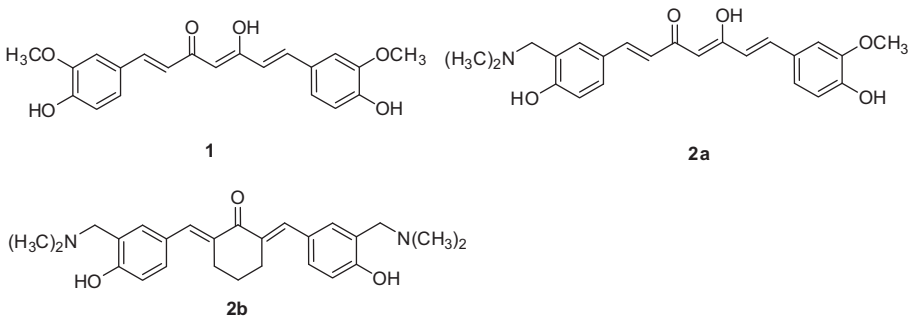
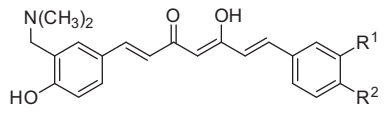


Figure 1. Structures of curcumin (**1**) and its derivatives reported (**2a** and **2b**).

hydroxy group. As our former study revealed that the introduction of dimethylaminomethyl group could improve the stability and the antioxidant activity of the derivatives. (iii) On another aromatic ring different substituent groups, including electron-withdrawing groups (e.g., Cl and F) and electron-donating groups (e.g., CH_3O), were employed with the hope to investigate the structure–activity relationship (SAR) of the substituents. The structures of the derivatives are shown in Figure 2.

The synthesis of the asymmetric curcumin derivatives was performed according to a literature method.[10,13] Generally, *p*-hydroxybenzaldehyde, dimethylamine and formaldehyde were applied to perform Mannich reaction to give intermediate **4** in a moderate yield. Thereafter compound **4** was reacted with acetylacetone to produce **5** via a condensation reaction. In this step, when **4** was directly treated with acetylacetone on a basic condition, the unwanted Knoevenagel reaction occurred and consequently reduced the yield of the desired product. Thus boron oxide was firstly applied to build a boron complex with acetylacetone. After the addition of compound **4** and a base, the condensation of the acetylacetone–boron complex with the aldehyde proceeded and an additional elimination occurred; eventual heating with dilute acid cleaved the boron complex to give compound **5**. Finally, intermediate **5** was reacted with corresponding benzaldehydes to give **3a**–**3h**, respectively (Scheme 1). For the synthesis of the hydrochloride salt of **2a** and **3a**, the solution of **2a** and **3a** in dichloromethane was treated with HCl gas and then the salt deposit was collected by filtration.

The antioxidant effect is believed to be responsible for many biological activities of curcumin, such as neuroprotective activity. In order to investigate whether the target compounds retain the antioxidant activity of curcumin or not, the potency of the target compounds to eliminate 2,2-diphenyl-1-picrylhydrazyl free radicals (DPPH) as well as galvinoxyl radicals was determined in vitro using a literature method.[14] The results are presented in Table 1. All of the tested compounds showed a moderate to strong free radical scavenging activity (FRSA). Like the positive control curcumin, the FRSA of the target compounds towards DPPH radicals is generally higher than that towards galvinoxyl radicals.

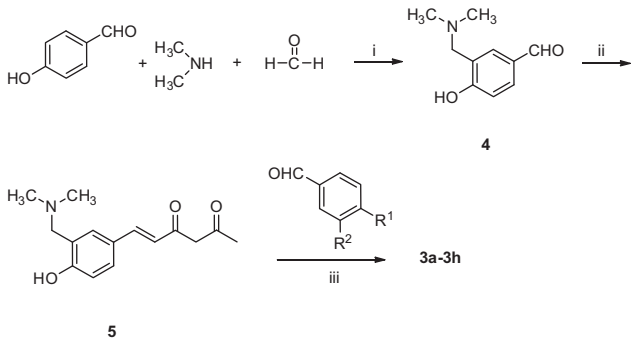


3a, R^1=$CH_2N(CH_3)_2$, R^2=OH; **3e**, R^1=H, R^2=F;
3b, R^1=H, R^2=OH; **3f**, R^1=OH, R^2=H;
3c, R^1=H, R^2=OCH_3; **3g**, R^1=H, R^2=CH_3;
3d, R^1=OCH_3, R^2=OCH_3; **3h**, R^1=H, R^2=Cl

Figure 2. Structures of the target compounds.

Three target compounds (**3a**, **3b** and **3f**) have IC_{50} values less than 5 μM, much lower than that of curcumin (IC_{50} 26.5 μM) while the other compounds showed a moderate FRSA with IC_{50} values around 20 μM. As for galvinoxyl radicals, a similar behavior was observed. Compounds **3a**, **3b** and **3f** showed a potent FRSA with IC_{50} values at single digit micro molar level while the others showed a relative low activity with IC_{50} values at doulble digit micro molar level.

The phenolic hydroxy groups clearly play an important role in the antioxidant activity. All the target compounds, which have at least one phenolic hydroxy group, showed positive antioxidant effect. Particularly, compounds **3a**, **3b** and **3f** which have two phenolic hydroxy groups showed higher FRSA than the others. Except the phenolic hydroxy group the other substituent group(s) seem to have little influence on the activity. For instance, the activity of **3e** which possess two electron-donating OCH_3 groups is similar



Scheme 1. Synthetic procedure of the target compounds. Reagents and conditions: (i) CH_3OH, 50 °C, overnight; (ii) 2,4-pentanedione, B_2O_3, EtOAc, 40 °C, 4 h; (iii) B_2O_3, EtOAc, 40 °C, 4 h.

Table 1
In vitro free radical scavenging activity (IC_{50} values) of the target compounds towards DPPH and galvinoxyl radicals

Compound	IC_{50} (μM) ± S.E.M.[a]	
	DPPH	Galvinoxyl radicals
Curcumin	26.5 ± 5.5[b]	>100[b]
3a	1.6 ± 0.7	4.9 ± 0.5
3b	1.5 ± 0.7	7.6 ± 1.4
3c	15.1 ± 6.1	11.9 ± 3.1
3d	29.9 ± 6.6	27.4 ± 2.6
3e	24.1 ± 8.0	22.3 ± 8.0
3f	4.0 ± 0.8	6.9 ± 1.6
3g	18.7 ± 4.3	22.0 ± 4.8
3h	11.1 ± 4.8	41.1 ± 8.8

[a] Data are the means of at least three determinations.
[b] Values are cited from Ref. 10.

to that of **3f** which possess an electron-withdrawing F substituent. The FRSA of the target compounds towards DPPH radicals is generally better than that towards galvinoxyl radicals. This is probably because they are two different types of radicals: DPPH is a kind of typical nitrogen radicals while galvinoxyl radicals belong to reactive oxygen species. From a chemical point of view, DPPH radicals are more reactive than galvinoxyl radicals, and are more easily captured by phenolic hydroxy groups. In comparison with curcumin, the FRSA of the target compounds is significantly improved. This is probably due to the introduction of the dimethylaminomethyl substituent which is an electron donating group and may subsequently enhance the free radical-capturing ability of the phenolic hydroxy group.

Aβ, especially Aβ42, and its aggregates have been considered as the key etiological factors triggering a neurotoxic cascade and finally causing neurodegeneration in AD brains.[1] In vivo monomeric Aβ42 peptides come from the amyloid precursor protein (APP) cleaved by β- and γ-secretase. The monomeric Aβ42 peptides, spontaneously or bioactivated by various factors, prone to aggregate, forming more toxic oligomeric intermediates and plaque-associated amyloid fibrils. Thus, the inhibition of Aβ-aggregation has been presented as a promising approach for the development of anti-AD agents. In order to investigate the inhibitory effect of the derivatives on Aβ self-aggregation, compound **3a** (5, 50, 100 μM) was selected to perform Thioflavin T (ThT) assay. Curcumin was used as positive control. The results are presented in Figure 3. At concentration of 5 μM, neither curcumin nor **3a** showed significant inhibitory effect on the self-aggregation of Aβ42. As the concentration increased, the inhibition rate of curcumin and **3a** was enhanced. At concentration of 50 μM, the inhibition rate of curcumin and **3a** reached 24% and 28%, respectively. When the concentration was raised to 100 μM, the inhibition rate of curcumin and **3a** was also increased to 29% and 32%, respectively, which is indicative of a dosage-dependent manner of the inhibition. Clearly, in addition to the antioxidant activity compound **3a** also possesses positive anti-Aβ42 self-aggregation activity, which may alleviate the Aβ-induced toxicity and eventually benefit the treatment of AD.

The poor stability of curcumin is a major problem limiting its clinical application. An investigation showed that 50% of curcumin has decomposed after 8 h culture in cell culture medium containing 0.1% fetal bovine serum (FBS) or in human blood.[6] In vivo curcumin can be rapidly metabolized into curcumin glucuronides and sulfates conjugating at the phenolic hydroxy groups. Thus, the introduction of dimethylaminomethyl groups, which have a large steric hindrance, to the *ortho* position of the hydroxy groups may slow down the formation of the glucuronides and sulfates, and prolong the half time of the target compounds. To investigate the stability of the target compounds in a physiological media, the absorption variation of curcumin and **3a** was measured in phosphate-buffered solutions (pH = 7.4) in the presence or absence of 0.1% FBS under daylight condition using a previous reported method (Fig. 4).[6] It was found that curcumin degraded very rapidly. In the absence of 0.1% FBS more than 80% curcumin degraded within 120 min, which is consistent with the previous report.[6] In the presence of 0.1% FBS, curcumin was a little stable, but still 60% curcumin degraded during the measurement. The stability of **2a** was also poor, but better than curcumin. About half of **2a** were decomposed within 120 min under both measurement conditions. In contrast, the analogue **3a** was much more stable. The absorption of **3a** only slight decreased during the measurement, no matter 0.1% FBS was present or absent, indicating a good stability of **3a**. Analyzing the structures of **2a** and **3a**, it can be found that the structural difference between these two compounds is that the methoxy group of **2a** was replaced with the dimethylaminomethyl group of **3a**. The improvement of the stability of **3a**, as compared with **2a**, indicated that the introduction of the dimethylaminomethyl group can indeed prevent the degradation of the compound.

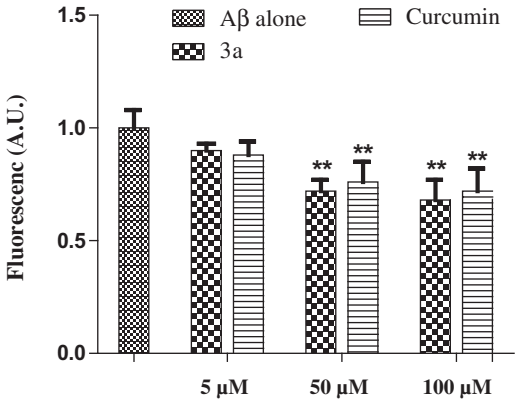


Figure 3. Inhibition of amyloid formation by compound **3a** and curcumin monitored by ThT fluorescence. Each result is the mean value of 3 measurements ($n = 3$) ± SEM. Statistical analysis was performed by the one-way ANOVA followed by Tukey's multiple comparison test. $^{**}p < 0.01$ versus Aβ alone.

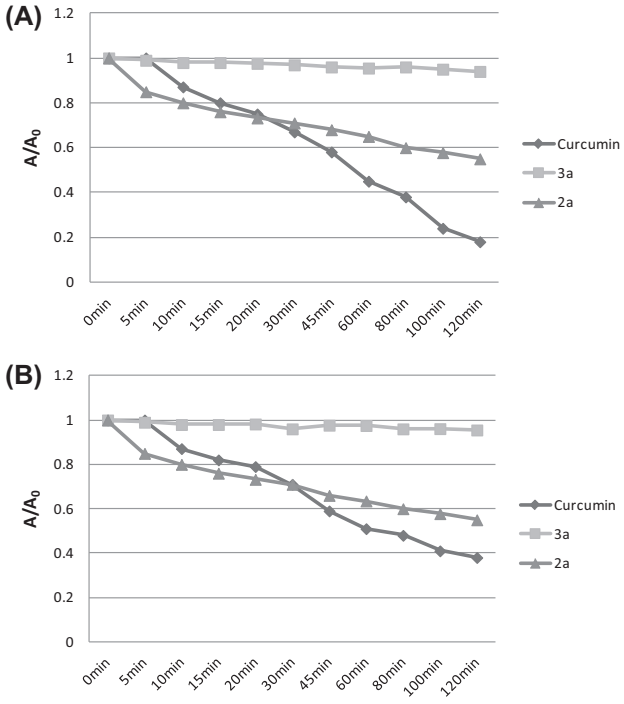


Figure 4. Stability measured by visible absorption in the presence (**A**) or absence (**B**) of 0.1% FBS. A_0 means absorption of the solution measured at 410 nm at 0 min; A means absorption of the solution measured at 410 nm. The data of 2a and curcumin were cited from Ref. 10

Table 2
The log*P* values of the target compounds and curcumin

Compound	Log*P*	Compound	Log*P*
3a	3.70[a] (3.48 [b])	**3e**	4.32[a]
3b	3.84[a]	**3f**	3.84[a]
3c	4.06[a]	**3g**	4.70[a]
3d	3.92[a]	**3h**	4.83[a]
2a	3.72[a] (3.65[b])	Curcumin	3.85[a]

[a] Calculated by software Marvin sketch.
[b] Experimental values determined in the *n*-octanol–water system.

The log*P* value is an important criterion to evaluate the drug-likeness of substances, especially for the anti-AD agents which must possess the ability to penetrate the blood–brain-barrier (BBB). In order to preliminarily evaluate whether the synthesized compounds possess such ability, the log*P* value of each compound was calculated using software Marvin Sketch (Table 2). For confirming the accuracy of the calculated values given by the software, the experimental values of two representative compounds **3a** and **2a** were also determined in the octanol–water system. As expected, the obtained experimental values (3.48 for **3a** and 3.65 for **2a**) are similar to the values (3.70 and 3.72, respectively) calculated by the software, indicating the reliability of the software. The calculated log*P* values of the target compounds are around 4.00, ranging from 3.70 to 4.83, suggesting a good lipophilicity and a potential ability to penetrate the BBB. According to the Lipinski's Rule of Five which suggests the optimal log*P* value of drug candidate should be not higher than 5,[15] it can be expected that the synthesized compounds, at least in the terms of the oil-water partition coefficient, possess a good potential to be drug candidates.

The poor aqueous solubility of curcumin is another problem limiting its clinical application. As far as our synthesized compounds are concerned, the introduction of the basic aminegroup makes them possible to be converted into the hydrochloride salt forms which will be likely to benefit the aqueous solubility. Using UV absorption spectroscopy we measured the aqueous solubility of **3a**·**2HCl** and **2a**·**HCl**, respectively. As compared with curcumin whose aqueous solubility is very poor (<0.1 mg/mL), the aqueous solubility of the dimethylaminomethyl-substituted derivatives **3a**·**2HCl** (16.7 mg/mL) and **2a**·**HCl** (1.2 mg/mL) has been significantly improved. The hydrochloride salt of **3a** which has two dimethylaminomethyl substituent groups has the highest solubility, while the solubility of the hydrochloride salt of **2a** which has only one dimethylaminomethyl substituent group is relatively low, with a value of 1.2 mg/mL.

In summary, we have designed and synthesized a series of dimethylaminomethyl-substituted curcumin derivatives which showed potential anti-AD properties with high FRSA towards both DPPH and galvinoxyl radicals and potent inhibitory effect on the Aβ self-aggregation. Furthermore, the in vitro stability and the aqueous solubility of the target compounds have also been significantly improved as compared with curcumin. The calculated log*P* values suggested that the target compounds possess a good lipophilicity and may have the ability to penetrate the BBB. All together, the distinct antioxidant and Aβ self-aggregation inhibitory activity as well as the much improved physical properties make the novel curcumin derivatives as promising anti-AD drug candidates.

Acknowledgments

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Supplementary data

Supplementary data associated (detailed experimental procedures for the synthesis and the pharmacological investigations and the spectral datum of the target compounds) with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.011.

References and notes

1. Querfurth, H. W.; LaFerla, F. M. N. *Engl. J. Med.* **2010**, *362*, 329.
2. Fang, L.; Gou, S.; Fang, X.; Cheng, L.; Fleck, C. *Mini. Rev. Med. Chem.* **2013**, *13*, 870.
3. Chandra, V.; Pandav, R.; Dodge, H. H.; Johnston, J. M.; Belle, S. H.; DeKosky, S. T.; Ganguli, M. *Neurology* **2001**, *57*, 985.
4. Lim, G. P.; Chu, T.; Yang, F.; Beech, W.; Frautschy, S. A.; Cole, G. M. *J. Neurosci.* **2001**, *21*, 8370.
5. Anand, P.; Kunnumakkara, A. B.; Newman, R. A.; Aggarwal, B. B. *Mol. Pharm.* **2007**, *4*, 807.
6. Blasius, R.; Duvoix, A.; Morceau, F.; Schnekenburger, M.; Delhalle, S.; Henry, E.; Dicato, M.; Diederich, M. *Ann. N. Y. Acad. Sci.* **2004**, *1030*, 442.
7. Shi, W.; Dolai, S.; Rizk, S.; Hussain, A.; Tariq, H.; Averick, S.; L'Amoreaux, W.; El Idrissi, A.; Banerjee, P.; Raja, K. *Org. Lett.* **2007**, *9*, 5461.
8. Narlawar, R.; Pickhardt, M.; Leuchtenberger, S.; Baumann, K.; Krause, S.; Dyrks, T.; Weggen, S.; Mandelkow, E.; Schmidt, B. *ChemMedChem* **2008**, *3*, 165.
9. Harish, G.; Venkateshappa, C.; Mythri, R. B.; Dubey, S. K.; Mishra, K.; Singh, N.; Vali, S.; Bharath, M. M. *Bioorg. Med. Chem.* **2010**, *18*, 2631.
10. Fang, X.; Fang, L.; Gou, S.; Cheng, L. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1297.
11. Xiao, Z.; Lin, L.; Liu, Z.; Ji, F.; Shao, W.; Wang, M.; Liu, L.; Li, S.; Li, F.; Bu, X. *Brain Res.* **2010**, *1361*, 115.
12. Cui, M.; Ono, M.; Kimura, H.; Liu, B.; Saji, H. *J. Med. Chem.* **2011**, *54*, 2225.
13. Mazumder, A.; Neamati, N.; Sunder, S.; Schulz, J.; Pertz, H.; Eich, E.; Pommier, Y. *J. Med. Chem.* **1997**, *40*, 3057.
14. Feng, J.-Y.; Liu, Z.-Q. *J. Agric. Food Chem.* **2009**, *57*, 11041.
15. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.