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Structure activity relationship study of curcumin analogues toward the amyloid-beta aggregation inhibitor

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ABSTRACT

Inhibition of the amyloid β aggregation process could possibly prevent the onset of Alzheimer's disease. In this article, we report a structure–activity relationship study of curcumin analogues for anti amyloid β aggregation activity. Compound **7**, the ideal amyloid β aggregation inhibitor in vitro among synthesized curcumin analogues, has not only potent anti amyloid β aggregation effects, but also water solubility more than 160 times that of curcumin. In addition, new approaches to improve water solubility of curcumin-type compounds are proposed.

In 2013, Ministry of Health, Labor and Welfare (MHLW) has reported that the number of dementia patients has reached 4 million in Japan. It is an extremely serious problem because this number corresponds to 15% of the elderly population in an aging society. Estimated numbers of dementia patients globally are about 25 million by 2025,[1] so this problem is a growing medical need on a global scale. Most dementia has been classified as a type of Alzheimer's disease (AD). Unfortunately, no fundamental therapeutic medicine has been approved for commercial use.[2] In previous studies, the cause of AD pathogenesis was a toxic peptide[3] called amyloid-beta (Aβ), which is generated by the metabolism of amyloid precursor protein and cleaved by the both β and γ-secretase from within the brain.[4,5] Aβ possesses high aggregability and consequently its aggregate is very toxic.[6] Therefore, it is widely believed that a drug that can dissociate Aβ aggregate can become a fundamental treatment of AD.[7,8] However, ready-made drugs for AD focus on symptomatic treatment such as acetylcholine esterase (AChE) inhibitors and *N*-methyl-D-aspartic acid (NMDA) receptor antagonists. Development of Aβ aggregation inhibitors is not proceeding, even though the area is being intensively studied. Because drugs must be administered to AD patients very early and long-term, fundamental treatment is an important mission.

To research an Aβ aggregation inhibitor, we focused on curcumin (CUR) (**1**) contained in turmeric, which has been used as a food and medicine in Southeast Asia. CUR (**1**) shows a variety of biological activities including anti-inflammatory, -tumor, and -oxidant etc.[9–11] Especially, its anti Aβ aggregation ability has received attention in recent years.[12–14] In vivo, CUR (**1**) clearly reduced the amount of Aβ in the brain with intraperitoneal administration in mice.[15] However, a clinical trial of CUR (**1**) for AD patients in 2012 was disappointing because it hardly reduced the level of Aβ in the brain.[16] It is suggested that the poor water solubility of CUR (**1**) has a negative effect on drug absorption. To improve solubility is imperative for CUR (**1**) to produce a good clinical effect. Dolai et al. reported improved water solubility for a curcumin-type compound with PEG-mediated injection of a hydrophilic group.[17] However, this strategy accompanied with an increase in molecular weight is a disadvantage as a drug. In addition, no structure activity relationship study of CUR (**1**) for inhibition of Aβ aggregation has been reported in detail.

In this Letter, we report the optimization of curcumin analogues which have more potent inhibitory activity of Aβ aggregation than CUR (**1**). It is essential to understand the structural property of CUR (**1**) to identify the pharmacophore of curcumin framework for potent inhibitory activity against Aβ aggregation.

CUR (**1**) has three structural properties, which are olefin, β-diketone and two phenol rings. Additionally, it adopts a planar structure owing to extended π-conjugation constructed by olefin and β-diketone.[18] At first we prepared two analogues, bismethoxycurcumin (BMC) (**2**) and tetrahydrocurcumin (THC) (**3**) (Fig. 1).

BMC (**2**) were constructed using Pabon's protocols,[19] although the conventional conditions of the aldol reaction afforded mainly

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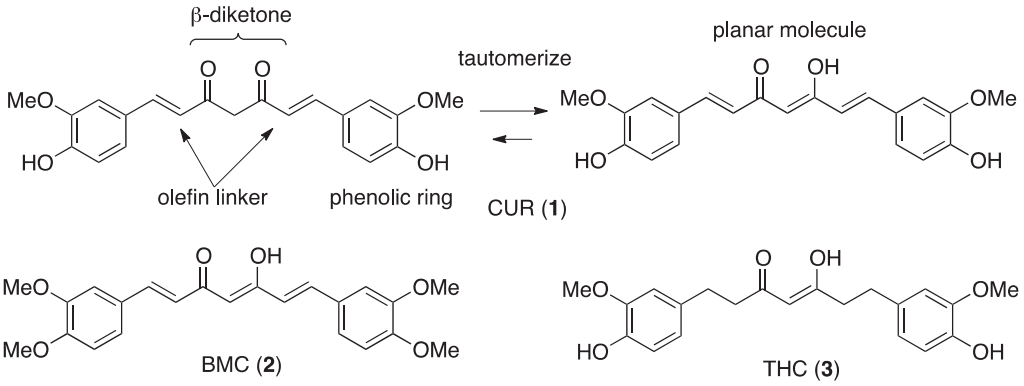
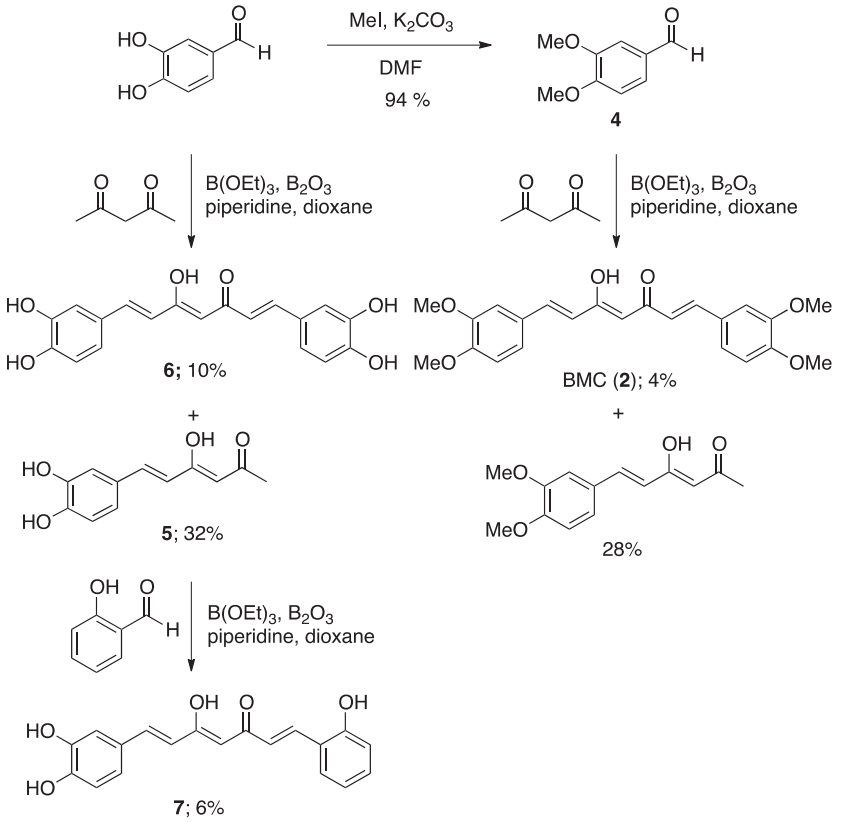


Figure 1. Structures of CUR (**1**), BMC (**2**) and THC (**3**).

3-substituted-2,4-diketone derivatives. Treatment of 3,4-dimethoxybenzaldehyde (**4**) derived from 3,4-dihydroxybenzaldehyde by methylation, and 2,4-pentanedione with $(EtO)_3B/B_2O_3$/piperidine in dioxane gave BMC (**2**) in 4% yield. Because 3-benzyl- 2,4-pentanedione is given as a by-product, the separation process of the mixture with similar physical properties by chromatography was needed to afford pure BMC (**2**) (Scheme 1). In contrast, hydrogenation of CUR (**1**) with Pd/C in MeOH/AcOEt at hydrogen atmosphere afforded THC (**3**) in 69% yield.[20] Aβ aggregation of inhibitory activity was estimated by the ThT method,[21] which has been widely used to estimate the amount of amyloid aggregate.[22–25] As shown in Figure 2A, 25 μM fresh amyloidogenic Aβ(1-42) (Peptide Institute) was incubated at 37 °C and the fluorescence of ThT followed a sigmoidal-like curve with a point of inflection. After an initiation lag for 2 h, quantum of Aβ(1-42) aggregation increased to be saturated between 4 h and 6 h. Although the final equilibrium level was achieved at this time, the quantum of Aβ(1-42) aggregation for 20 h was defined as 100%. When the mixture of 25 μM Aβ(1-42) and 10 μM CUR (**1**) was incubated at 37 °C, a similar sigmoidal-like curve could be drawn and the final equilibrium level was ~20%. CUR (**1**) played a role as an Aβ aggregation inhibitor at a ratio of 80% and this result was similar to Yang's report.[15] As for incubation of Aβ(1-42) with BMC (**2**) or THC (**3**), the Aβ aggregation inhibitory effect was hardly observed. These results suggested that there was an essential motif of the CUR (**1**) structure as an Aβ aggregation inhibitor. Figure 2B shows the ratio of the Aβ(1-42) aggregation for 20 h. Treatment of Aβ(1-42) with CUR (**1**) at 20 h was defined as about 20% aggregate as a control and accordingly following assay was evaluated (Fig. 2).

We considered that phenolic hydroxy groups of CUR (**1**) had important functionalities for the inhibition of Aβ aggregation. Thus, preparation of curcumin analogues focused on phenolic functionality and an evaluation were attempted. As depicted in Table 1, we designed and synthesized catechol or pyrogallol type curcumin



Scheme 1. Preparation of curcumin analogues **2**, **6** and **7**.

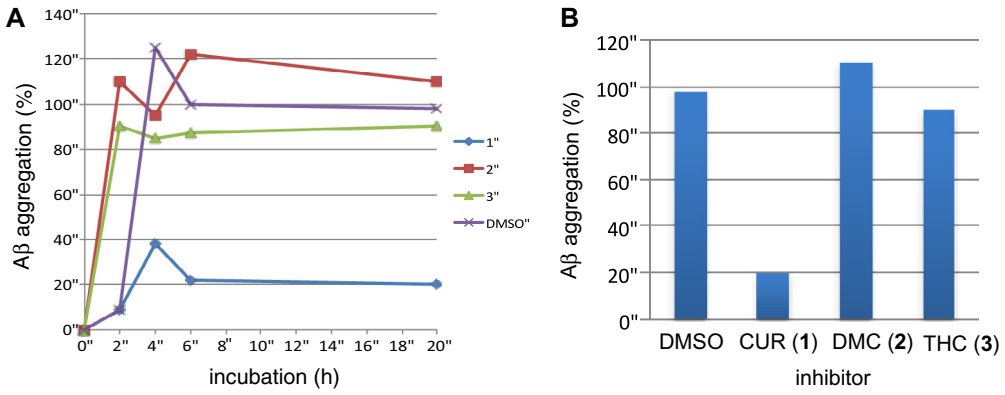
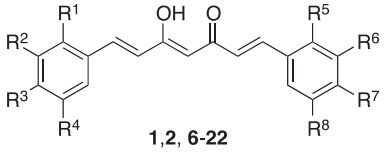


Figure 2. Aggregation kinetics of three compounds (**1**–**3**) of Aβ(1-42). (A) Time course of the fluorescence after the initiation of fAβ(1–42). (B) Quantity of Aβ Aggregation (%) after 20 h incubation at 25 μM.

Table 1
Structure of CUR (**1**) and its synthesized curcumin analogues (**2**, **6**–**22**) and their inhibitory rates against Aβ(1-42) aggregation after an 20 h incubation at 25 μM



1,2, 6-22

Entry	Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	Inhibition (%)
1	CUR (**1**)	H	OMe	OH	H	H	OMe	OH	H	80
2	BMC (**2**)	H	OMe	OMe	H	H	OMe	OMe	H	0
3	**6**	H	OH	OH	H	H	OH	OH	H	99
4	**7**	H	OH	OH	H	OH	H	H	H	100
5	**8**	H	OH	OH	H	OMe	H	H	H	100
6	**9**	H	OH	OH	H	H	OH	H	H	99
7	**10**	H	OH	OH	H	H	OMe	H	H	95
8	**11**	H	OH	OH	H	H	H	OH	H	89
9	**12**	H	OH	OH	H	H	H	OMe	H	95
10	**13**	H	OH	OH	H	H	OMe	OMe	OMe	95
11	**14**	H	OH	OH	H	H	OH	OH	OH	100
12	**15**	H	OH	OH	H	H	OH	OMe	OH	100
13	**16**	H	OH	OH	OH	H	OH	OH	OH	46
14	**17**	H	OH	OH	OH	OH	H	H	H	73
15	**18**	H	OH	OH	H	H	NO_2	H	H	97
16	**19**	H	OH	OH	H	H	CN	H	H	96
17	**20**	OH	H	H	H	OH	H	H	H	80
18	**21**	H	OH	H	H	H	OH	H	H	79
19	**22**	H	H	OH	H	H	H	OH	H	75

analogues. The designed curcumin analogues (**5**–**22**) were applied to Pabon's protocol to construct a curcumin structure (Scheme 1, Table 1 and supporting information). Curcumin analogues (**6**–**17**) that vary in the number, position or type of substituents were estimated to clarify their effects or tolerances as inhibitors. Curcumin analogues (**18**, **19**) were two bare heterocycles. As depicted in Scheme 1, the aldol reaction using the 3,4-dihydroxybenzaldehyde with 2,4-pentanedione gave the corresponding desmethyl curcumin (**6**) and phenyl diketone (**5**) in 10% and 32% yields, respectively. Reiteration of Pabon's aldol reaction between **5** and 2-hydroxybenzaldehyde was performed to afford an *o*-hydroxy curcumin derivative (**7**) in 6% yield. In spite of the poor yield, Pabon's protocol was employed to prepare asymmetrical curcumin derivatives because other conditions to perform the aldol reaction did not afford the desired products. As a result, a variety of curcumin derivatives (**6**–**22**)[20] could be directly prepared by this methodology for the evaluation of anti Aβ aggregation (Table 1).

The Aβ aggregation inhibitory activities of synthesized curcumin analogues (**6**–**22**) were estimated by the ThT method shown in Table 1. Inhibition rates (%) of curcumin derivatives were shown by the incubation of fAβ(1-42) for 20 h. Catechol type analogues (**6**–**15**, **18**, **19**) prevented Aβ fibril formation clearly (entry 3–12, 15, 16). Figure 3A shows the time course of the Aβ(1-42) aggregation rates in the presence of catechol type derivatives (**7**, **8**, **9**, **11** and **12**), which have an excellent inhibitory potency. Increase of the fluorescence descended from ThT was hardly observed for the assays with **7**, **8** and **9**. 10 μM of **7**, **8** and **9** inhibited 25 μM of Aβ aggregation after 20 h incubation as shown in Figure 3B. In contrast, inhibitory activity of a truncated analogue (**5**) (inhibitory rate = 75%) and pyrogallol type analogues (**16**, **17**) (inhibitory rate = 46% for **16**, 73% for **17**) were weaker than that of CUR (**1**) (entry 13, 14). Interestingly, asymmetrical catechol-pyrogallol hybrid compounds (**13**–**15**) maintained potent inhibitory activities (entry 10–12). These results suggested that the catechol unit was an important motif for Aβ aggregation inhibitory activities and that pyrogallol and/or phenolic units also played a role of another factors. To estimate the effect of the catechol motif, we designed symmetrical curcumin analogues (**20**–**22**) with two phenolic hydroxy groups. Symmetrical curcumin analogues; *o*-phenol type (**20**), *m*-phenol type (**21**) and *p*-hydroxy type (**22**); were

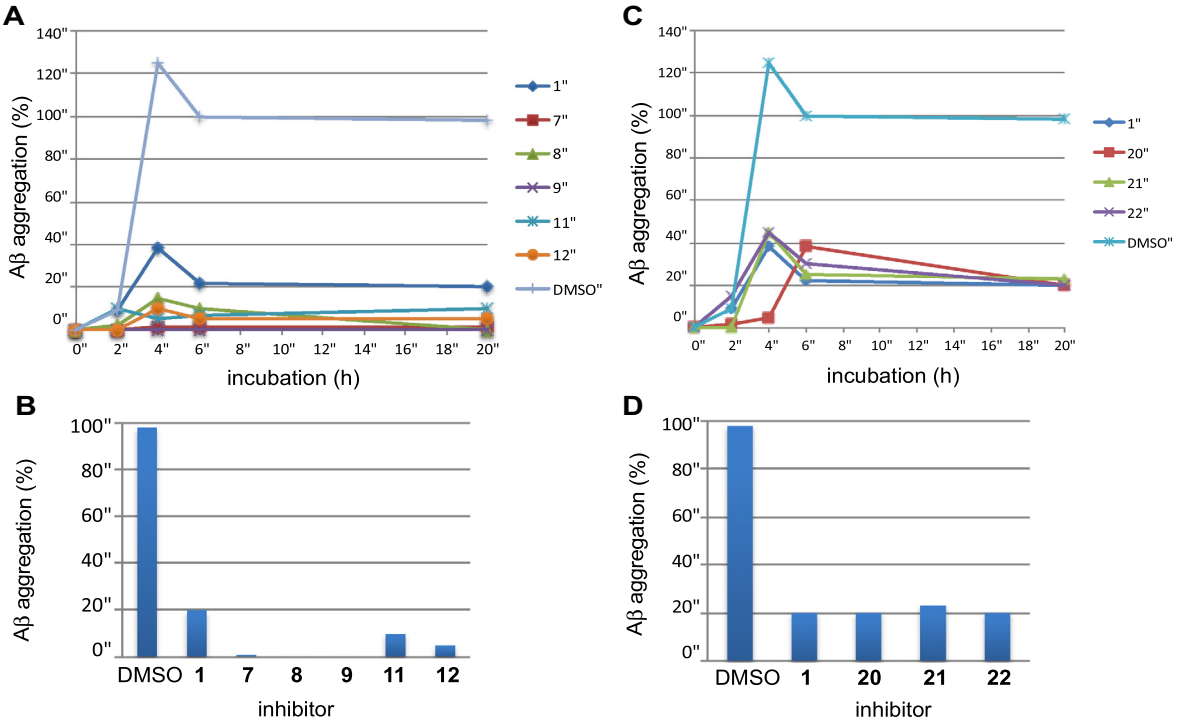


Figure 3. Aggregation kinetics of curcumin analogues of Aβ(1-42). (A) Time course of fluorescence after the initiation of fAβ(1-42) for catechol type derivatives (**7**, **8**, **9**, **11** and **12**). (B) Quantum of Aβ Aggregation (%) after 20 h incubation at 25 μM for **7**, **8**, **9**, **11** and **12**. (C) Time course of the fluorescence after the initiation of fAβ(1-42) for symmetrical derivatives (**20**, **21** and **22**), (D) Quantum of Aβ Aggregation (%) after 20 h incubation at 25 μM for **20**, **21** and **22**.

synthesized to clarify whether positional relationships of phenolic hydroxy groups have implications for Aβ aggregate inhibitory activities (entry 17–19). Interestingly, the Aβ aggregate inhibitory activities of three symmetrical curcumin analogues (**20**–**22**) were similar to that of CUR (**1**) (Fig. 3D). As shown in Figure 3C, each time course of fluorescence was similar despite the different positions of phenolic hydroxy groups on the curcumin frameworks. There was a high correlation between inhibitory activity and the number of phenolic hydroxy groups, and consequently the catechol motif potently inhibited Aβ aggregation.

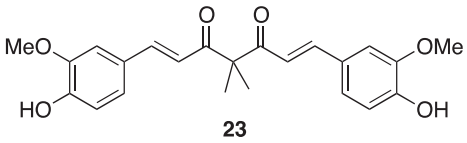
Since CUR (**1**) itself has poor water solubility and consequently low bioavailability, there is a major limitation for using CUR (**1**) as a drug. Therefore, curcumin type inhibitors were required to improve both Aβ aggregate inhibitory activities and water solubility. For these reasons, water solubility and physical properties of eleven selected compounds were depicted in Table 2. An ideal compound, which combines water solubility and membrane permeability, is essential for central nervous system (CNS) drugs.[26] A UV–vis experiment and HPLC analysis were conducted to evaluate several compounds compared to CUR (**1**). The same molar quantities of both CUR (**1**) and curcumin derivatives were vortexed in water, and the resulting solution was centrifuged to remove undissolved material. The supernatant liquid was concentrated and the resultant material was dissolved in MeOH for analysis by RP-HPLC. From the profile of HPLC, the water solubility is shown in Table 2. It should be noted that the solubility study outlined above measured just using water without other solvents for predissolution. Water solubility of catechol type derivatives with methoxyphenyl groups (**8**, **10** and **12**) was extremely poor compared to CUR (**1**). On the other hand, catechol type derivatives with free phenol groups (**6**, **9** and **10**), catechol-pyrogallol hybrid compound (**15**) and pyrogallol type derivative (**16**) increased water solubility. Especially, the water solubility of *o*-phenol types (**7** and **17**) and a catechol-pyrogallol hybrid compound (**14**) was 160∼260 times better than that of CUR (**1**). Although the correlations between water solubility and melting points or tPSA and Clog*P* are difficult to explain, we could assume that their low water solubilities and high melting points are due to tight crystal packing resulting from high planarity of molecules.[27]

Table 2
Physiological properties of water solubility, Mp, tPSA and Clog*P* of CUR (**1**) and selected curcumin analogues (**6**–**12**, **14**–**17**)

Entry	Compound	Solubility[a]	Mp (°C)[b]	tPSA (A)[c]	Clog*P*[c]
1	CUR (**1**)	0.19	180–183	96	2.939
2	**6**	2.4	225–227	118	2.047
3	**7**	31	115–138	98	2.644
4	**8**	0.12	155–160	87	3.230
5	**9**	6.2	173–178	98	2.644
6	**10**	0.11	151–155	87	3.230
7	**11**	0.66	218–222	98	2.644
8	**12**	0.009	173–177	87	3.230
9	**14**	50	215 dec.	138	1.380
10	**15**	5.4	129–142 dec.	127	1.630
11	**16**	6.2	250 dec.	159	0.713
12	**17**	47	198 dec.	118	1.977

[a] Water solubilities were measured by the Dolai protocol[17] to show the values with μM.
[b] Melting points (Mps) were measured by an AS ONE ATM-02.
[c] tPSA and Clog*P* were calculated by ChemBio3D Ultra 12.0 (PerkinElmer).

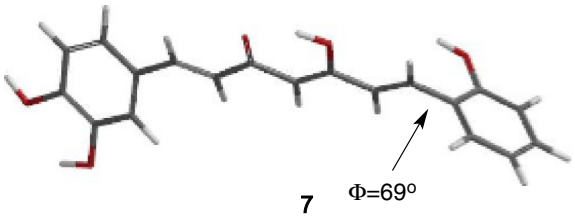
To demonstrate this hypothesis, α,α-dimethylcurcumin (αDMC) (**23**), with much improved water solubility, was designed because of its lack of planarity to disrupt a π-conjugated structure. αDMC (**23**) was prepared from CUR (**1**) in three steps (see supporting information). Physical properties and Aβ aggregate inhibitory activity of αDMC (**23**) are also shown to compare with those of CUR (**1**) (Fig. 4). Surprisingly, αDMC (**23**) had 63-fold better solubility than CUR (**1**), despite an increased hydrophobicity (vs CUR (**1**), Clog*P* = 2.25) by two methyl groups. By a drastic decrease in its melting point (73 °C), the water solubility of αDMC (**23**) was increased to diminish the molecular interaction. This idea is expected to offer a new approach that improves water solubility



23

solubility = 12 μM, mp = 73°C, ClogP = 2.87
inhibition = 30% for 25 μM of Aβ(1-42)

Figure 4. Structure of αDMC (**23**) and its physical properties.



7

Figure 5. Stable conformer of **7** calculated by SPARTAN.

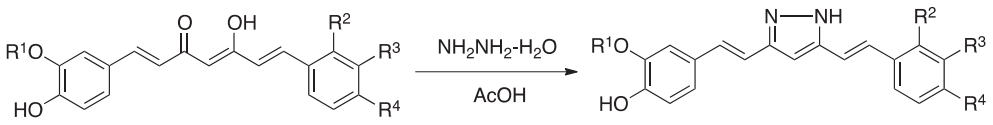
of curcumin type compounds. Unfortunately, Aβ aggregate inhibitory activity showed a value of 30% indicating the enol structure of the α,α-position and/or the molecular planarity of the curcumin framework are essential factors in producing interaction between curcumin derivatives and Aβ peptide (see Fig. 5).

In contrast, an *o*-phenol type derivative (**7**), which showed 31 μM for water solubility, was an attractive compound. This value of water solubility of **7** is excellent compared with those of another isomers such as *m*-phenol type derivative (**9**) (6.2 μM) and *p*-phenol type derivative (**11**) (0.66 μM). In order to interpret these results, we assume an interaction of hydrogen bonds between the *o*-phenolic hydroxyl group of **7** and water. Thus, water solubility between trihydroxy analogues (**7**, **9** and **11**) and the corresponding methoxy compounds (**8**, **10** and **12**) were compared based on the assumption and the difference in water solubility between **7** and **8** (260-fold) was extremely higher than those of the other two pairs (**9** vs **10**, 56-fold and **11** vs **12**, 73-fold). To further investigate the interaction with an *o*-phenol type derivative (**7**) and water, pyrazole–curcumin analogues (**24**–**27**) were prepared and their physical properties were evaluated because they would preserve a number of hydrogen bonds and planarity structure such as original analogues (Table 3). The water solubility of CUR (**1**) and its analogues (**6**,**9**) increased to convert β-diketone to a pyrazole ring except for **7** to show the values of **24**, **25** and **27**. However, **26** derived from **7** hardly increased water solubility. Therefore, the β-diketone moiety of the curcumin structure is important to construct the shape of the curcumin planarity framework, as well as act as an Aβ aggregation inhibitor. Fujita reported that strained molecules with increased dihedral angles had high water solubility.[28] The molecular force field for selected curcumin analogues was calculated by SPARTAN. Stable conformers were indicated and the dihedral angles of the *o*-phenol ring and neighbor olefin of **7** and **26** were 69° and 68°, respectively. Owing to the smaller dihedral angles of selected compounds (41°∼48°), our results are supported by Fujita's theory. Partial polarity of **9** and **11** were also dissimilar clearly. These results attract us to *o*-phenol type derivative (**7**) and pyrogallol type derivative (**14**). In order to have both potent Aβ aggregation inhibitory activity and water solubility (Tables 1 and 2), they are of reasonable prospect as an Aβ aggregation inhibitor. However, it was a significant disadvantage that a pyrogallol type derivative (**14**) was accompanied by a decrease in molecular stability in water and estimated membrane permeability (tPSA = 138 A). For this reason, we recommend an *o*-phenol type derivative (**7**) that is an deal compound candidate Aβ aggregation inhibitor.

Although a variety of Aβ aggregation inhibitors have been reported to date,[7] it is not clear the interaction between Aβ and its inhibitors. The phenolic groups of CUR (**1**) can interact with the aromatic residues in amyloid fibrils disrupting peptide π–π stacking while the hydroxyl groups and β-diketone unit can act as β-sheet breakers via competitive hydrogen bonding. However, this mechanism is still incompletely elucidated. It is causally related to the handling difficulty of Aβ which is non-crystalline, has poor water solubility and self-cohesive properties. We found a pharmacophore that included the catechol motif to inhibit Aβ aggregation by structure–activity relationship studies of curcumin analogues and additionally revealed tolerant molecular modification of curcumin analogues with a catechol motif. We expect that the molecular design of suitably modified analogues such as bioprobes to investigate Alzheimer's disease will become available. We found two new approaches for improved water solubility of curcumin analogues; (a) reduce molecular planarity and (b) use a β-diketone. The *o*-phenolic hydroxyl group in curcumin analogues played an important role in a tight connection with a ketone group by participation of water to stabilize the torsion of the *o*-phenol ring and neighboring olefin.

In conclusion, we found an effective pharmacophore for anti Aβ aggregation inhibitory activity by a structure activity relationship study of curcumin analogues. Consequently, an attractive inhibitor (**7**) that improved the low water solubility of CUR (**1**) was produced. Moreover, useful knowledge gathered in the present study will help in new approaches to improve water solubility of curcumin-type compounds. Application of water soluble curcumin analogues for in vivo use is expected.

Table 3
Synthesis and physical properties of pyrazole–curcumin analogues (**24**–**27**)



Entry	Substrate	R^1	R^2	R^3	R^4	Product	Yield (%)	Solubility[a]	Mp (°C)	Inhibition[b]
1	1	Me	H	OMe	OH	24	76	1.2	210–213	51
2	6	H	H	OH	OH	25	38	98	220–223	90
3	7	H	OH	H	H	26	38	16	230–236	47
4	9	H	H	OH	H	27	37	10	232–236	ND

[a] Water solubilities were measured by the Fujita's protocol to show the values with μM.
[b] Aggregation inhibition values of Aβ(1-42) after a 20 h incubation at 25 μM were indicated.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.076.

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