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Synthesis and evaluation of curcumin derivatives toward an inhibitor of beta-site amyloid precursor protein cleaving enzyme 1

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ABSTRACT

To research a new non-peptidyl inhibitor of beta-site amyloid precursor protein cleaving enzyme 1, we focused on the curcumin framework, two phenolic groups combined with an sp_2 carbon spacer for low-molecular and high lipophilicity. The structure–activity relationship study of curcumin derivatives is described. Our results indicate that phenolic hydroxy groups and an alkenyl spacer are important structural factors for the inhibition of beta-site amyloid precursor protein cleaving enzyme 1 and, furthermore, non-competitive inhibition of enzyme activity is anticipated from an inhibitory kinetics experiment and docking simulation.

Alzheimer's disease (AD)[1] is caused by the aggregation of amyloid-beta (Aβ),[2] which is produced from amyloid precursor protein (APP), a transmembrane protein expressed in many tissues and organs, cleaved by the both β-secretase (β-site amyloid precursor protein cleaving enzyme 1, BACE1[3]) and γ-secretase in the brain. BACE1 has been recognized as a valuable target for the treatment of AD. Therefore, BACE1 inhibitors have potential to be developed as *anti*-dementia drugs. A variety of inhibitors against BACE1 have been reported in the literature in past decades, however it has not been permitted to give BACE1 inhibitors as AD therapeutic agents to date. Most peptidomimetic inhibitors with potent activity are promisingly based on the cleaving site of the APP sequence,[4–7] but these inhibitors tend to be *P*-glycoprotein substrates and have restricted brain penetration. Although non-peptidyl BACE1 inhibitors rationally designed from fragment-based screening techniques have also been reported by several groups,[8,9] the cytotoxicity of the candidate compounds is frequently a serious problem. In contrast, natural products are promising bioactive libraries from which *anti*-AD agents from microorganisms or food plants have been isolated,[10] although they tend to offer low inhibitory activity and/or an unknown mechanism of action for the target enzyme in exchange for oral bioavailability and brain penetration.

Curcumins[11] are components of turmeric, which is consumed as a curry spice and is especially used in traditional Indian medicine to treat biliary disorders, anorexia, coughs, etc. around South Asia. The main ingredient of curcumins is curcumin 1 (**1**) [1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-1,6-diene-3,5-dione], so-called curcumin, and other compounds exist as curcumin 2 (**2**) [desmethoxycurcumin] and curcumin 3 (**3**) [bis-desmethoxycurcumin]. These curcumins form stable enols and are responsible for the yellow color. A variety of extensive investigations in past decades have indicated that curcumin 1 (**1**) is an antioxidant and *anti*-inflammatory and has promising *anti*-Alzheimer's disease activity.[12–14]

In the course of a research program on BACE1 inhibitors,[15] we focused on the curcumin[16,17] framework as non-peptidyl compounds with low-molecular weight and high lipophilicity. Two crucial structural features of curcumins have been associated with BACE1 inhibitors: phenolic rings and an alkenyl spacer to join the two rings.[18,19]

In the present study, the inhibitory activities of natural curcumins 1, 2 and 3 (**1**), (**2**) and (**3**) were evaluated against recombinant β-site amyloid precursor protein cleaving enzyme 1 (rBACE1)[15] and subsequently, the effects of phenolic hydroxy groups, double bonds and ketone groups were examined. Furthermore, a structure–activity

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relationship study of the aromatic substituents of curcumin derivatives and determination of the inhibition mechanism were attempted. Therefore, 37 curcumin derivatives were prepared from curcumin 1 (**1**) or benzaldehyde derivatives and subsequently screened for their effect on *anti*-BACE1 activity at a concentration of 1.3 or 0.67 mM. Then IC_{50} values of the selected inhibitors were determined and interactions between the inhibitors and rBACE1 was estimated (Fig. 1).

To investigate the importance of phenolic hydroxy groups, double bonds and keto carbonyl groups, we prepared a variety of curcumin derivatives from curcumin 1 (**1**). First of all, phenolic hydroxy groups of curcumin 1 (**1**) were protected using several protecting groups because of the insolubility of the curcumins in organic solvents. Although the selected protecting groups were TBS, MEM, MOM ethers, chemical yields of **4a**–**4f** remained moderate or low because of insolubility in reaction solvents. In addition, treatment of curcumin 1 (**1**) with methyl iodide and potassium carbonate in acetone afforded α,α-dimethyl-di-*O*-methylcurcumin (**4g**) in 47% yield. Attempts to deprotect phenolic methyl ether of **4g** under several conditions were unsuccessful to give complexed mixtures. A three step sequence with acetylation of phenolic hydroxyl groups/methylation of α,α-carbon on the spacer/deacetylation afforded **4h** in a trace amount (Table 1).

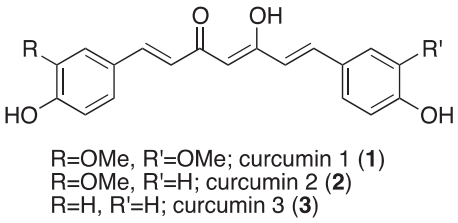


Figure 1. Structure of curcumins.

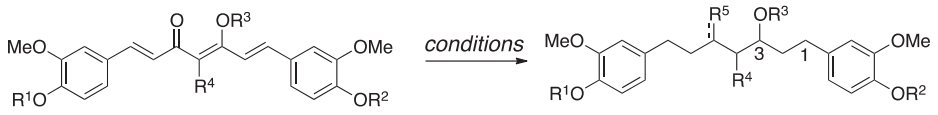
As depicted in Table 2, curcumin derivatives (**5a**)–(**9b**) were prepared by hydrogenolysis of double bonds and reduction of ketones. As the above-mentioned results, using the reductive reactions of curcumin derivatives it was difficult to afford one or more products in satisfied yield. Although reactions at 0 °C or −20 °C were attempted, insolubility of the substrates prevented use of the reactants. In contrast, conjugated alkenyl groups of the curcumin framework often gave the over-reduced products. In entry 1, hydrogenation of curcumin 1 (**1**) with Pd/C in MeOH/AcOEt at H_2 atmosphere afforded the expected compound (**5a**) and ketone-reduced compounds (**5b**) and (**5c**) in 69%, 10% and 8% yields, respectively. On the other hand, treatment of **4c** with $NaBH_4$ in MeOH/AcOEt gave **9a** in 30% yield with the recovered material (**4c**) in ca. 50% yield (entry 5). Stereochemistry of hydroxyl groups of these products was not still determined in this case (Table 2).

Table 1
Protected curcumin derivatives (**4a**)–(**4h**)

Entry	Conditions	Product	R^1	R^2	R^3	R^4	Yield (%)
1	TBSCl, imidazole, DMF	**4a**	TBS	H	H	H	45
		4b	TBS	TBS	H	H	33
2	MEMCl, iPr_2NEt, CH_2Cl_2	**4c**	MEM	MEM	H	H	49
		4d	MEM	H	H	H	32
		4e	MEM	MEM	MEM	H	5
3	MOMCl, iPr_2NEt, CH_2Cl_2	**4f**	MOM	MOM	H	H	30
4	MeI, K_2CO_3, acetone	**4g**[a]	Me	Me	H	Me_2	47
5	(a) Ac_2O, py.; (b) MeI, K_2CO_3; (c) 1M NaOH	**4h**[a]	H	H	H	Me_2	1

[a] **4g** and **4h** were isolated with keto form.

Table 2
Protected curcumin derivatives (**5a**)–(**9b**)



Entry	Substrate	Conditions	Product	R^1	R^2	R^3	R^4	R^5		Yield (%)
1	**1**	H_2/Pd-C	**5a**	H	H	H	H	O	Δ^3	69
		MeOH/AcOEt	**5b**	H	H	H	H	O		10
			5c	H	H	H	H	OH, H		8
2	**4b**	H_2/Pd-C	**6a**	TBS	TBS	H	H	O	Δ^3	23
		MeOH/AcOEt								
3	**4c**	H_2/Pd-C	**7a**	MEM	MEM	H	H	O	Δ^3	45
		MeOH/AcOEt	**7b**	MEM	MEM	H	H	O		25
4	**4g**	H_2/Pd-C	**8a**[a]	Me	Me	—	Me_2	O		28
		MeOH/AcOEt	**8b**	Me	Me	H	Me_2	O		7
			8c	Me	Me	H	Me_2	OH, H		3
5	**4c**	$NaBH_4$	**9a**	MEM	MEM	H	H	OH, H	$\Delta^{1,3}$	30
		MeOH/AcOEt	**9b**	MEM	MEM	H	H	O	Δ^3	3

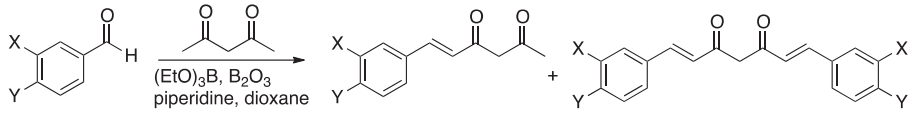
[a] Chemical structure of **8a** was shown diketone derivative.

For the structure–activity relationship study of aromatic substituents of curcumin derivatives, we attempted to synthesize curcumin derivatives (**22**)–(**36**) by an aldol reaction. As depicted in Table 3, diketone building blocks (**16**)–(**21**) were constructed using Pabon's protocols,[20,21] although the conventional conditions of the aldol reaction afforded 3-substituted-2,4-diketone derivatives mainly. Treatment of benzaldehyde (**10**) and 2,4-pentanedione with $(EtO)_3B/B_2O_3$/piperidine in dioxane gave phenyl diketone (**16**) and curcumin 1 (**1**) in 22% and 8% yields, respectively. The spectral data (^1H NMR, MS) of synthetic curcumin 1 (**1**) were identified with those of commercially available curcumin 1 (**1**). Unfortunately, it is difficult for us to increase the chemical yields using these several conditions as efficiently as the preparation of **16** and/or **1**. Similarly, the aldol reaction using the hydroxy or methoxy substituted benzaldehyde (**11**)–(**15**) with 2,4-pentanedione gave the corresponding diketone detivatives (**17**)–(**21**) and symmetrical curcumin derivatives (**22**)–(**25**) and curcumin 3 (**3**) in low yields. As a consequence, the combination of several phenolic aldehydes with 2,4-pentanedione could be directly converted to the symmetric curcumin frameworks by this methodology (Table 3).

Subsequently, we attempted to prepare the asymmetrical curcumin analogues using diketone (**16**) and (**21**) and substituted benzaldehydes under the above-mentioned condition. Diketone (**21**) was subjected to a second aldol reaction with benzaldehyde (**11**) to give curcumin derivative (**26**) in 7% yield with abundant starting material after purification. In spite of the poor yield, the Pabon's protocol was employed to prepare asymmetrical curcumin derivatives because other conditions to perform the aldol reaction did not isolate the desired products. As a result, asymmetrical curcumin derivatives (**26**)–(**35**)[22] were prepared for the evaluation of BACE1 inhibitors (Table 4).

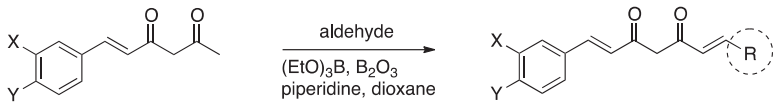
The inhibition of rBACE1 activity was determined by the previous procedure using a synthetic dodecapeptide with the BACE1 cleavage sequence of the Swedish type as a substrate.[23] Commercially available natural curcumins 1, 2 and 3 (**1**), (**2**) and (**3**) and 37 synthetic curcumin analogues from several structural subclasses were investigated, including hydroxy group-protected curcumin (**4a**)–(**4h**), curcumin-reduced derivatives (**5a**)–(**9b**), and symmetrical and asymmetrical curcumin analogues obtained by the Pabon–aldol reaction (**22**)–(**36**). The inhibition potency of curcumin derivatives was screened for rBACE1 inhibition at 1.3 or 0.67 mM

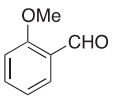
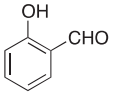
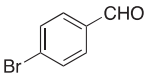
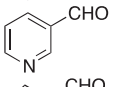
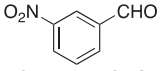
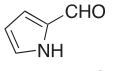
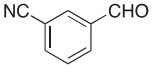
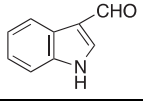
Table 3
Synthesis of symmetric curcumin derivatives by Pabon's reaction of benzaldehyde (**10**)–(**15**) with 2,4-pentanedione



Entry	Benz-aldehyde	X	Y	Phenyl diketone	Yield (%)	Curcumin derivative	Yield (%)
1	**10**	OMe	OH	**16**	22	**1**	8
2	**11**	H	H	**17**	17	**22**	50
3	**12**	OMe	OMe	**18**	24	**23**	4
4	**13**	H	OH	**19**	20	**3**	10
5	**14**	OH	H	**20**	17	**24**	12
6	**15**	OH	OH	**21**	35	**25**	38

Table 4
Synthesis of curcumin derivatives (**26**)–(**35**) by Pabon's reaction



Entry	Diketone	Aldehyde	Product	Yield (%)	Entry	Diketone	Aldehyde	Product	Yield (%)
1	**21**	**11**	**26**	7	6	**21**		**31**	7
2	**21**	**13**	**27**	10	7	**21**		**32**	6
3	**16**		**28**	21	8	**21**		**33**	3
4	**21**		**29**	2	9	**21**		**34**	4
5	**21**		**30**	2	10	**21**		**35**	15

concentration and subsequently, the IC_{50} values were determined only for the selected compounds. As depicted in Table 5, many compounds showed relatively less potent inhibitory effects of rBACE1 activity by the comparison with naturally curcumin 2 (**2**). It is suggested that phenolic hydroxyl groups and the planer structure with an sp_2 carbon spacer are essential and, additionally, the water solubility of compounds is important for this assay. It is noted that symmetrical curcumins 1 and 3 (**1**) and (**3**) showing no inhibitory activities were particularly interesting (Table 5).

Next, the structure–activity relationship study of phenolic analogues (**22**)–(**35**) with an sp_2 carbon spacer against rBACE1 was attempted at 0.67 mM concentration of the inhibitors shown in Table 6. Curcumin derivatives (**24**), (**25**), (**26**), (**27**), (**31**) and (**32**) with the attachment of multiple hydroxy groups exhibited potent activity. Nitrogen-containing compounds (**33**), (**34**) and (**35**) also showed inhibitory activity against rBACE1. In contrast, symmetrical biphenyl-(**22**) and tetramethoxy-(**23**) and *p*-bromo phenyl derivative (**28**) were inactive; therefore, non-substituted phenyl groups and methoxy and *p*-halogenated groups had a tendency to decrease inhibitory activities markedly. The results for these analogues suggest that regio-specific phenolic hydroxy groups interact site-specifically with the pharmacophore or the periphery of BACE1 using the hydrogen bond network (Table 6).

Additionally, the inhibitory activities of six selected curcumin analogues were evaluated based on IC_{50} values. The IC_{50} value, melting point and C log *P* of selected inhibitors are summarized in Table 7. The naturally occurring curcumin 2 (**2**) control exhibited an IC_{50} value of 1.6 mM. Symmetrical tetrahydroxy curcumin analogue (**25**) and dihydroxy-nitro curcumin analogue (**29**) showed IC_{50} values of 2.2 and 1.8 mM, respectively. Surprisingly *ortho*-substituted trihydroxy curcumin derivative (**32**) showed IC_{50} calue of 250 μM. Indole-type compounds (**35**) inhibited increase rBACE1 with an IC_{50} value of 450 μM. These results are partly proportional to the melting points and accordingly, water solubility also seems to be an important factor to access the target enzyme. However, there is no correlation between the IC_{50} value and *C* log *P* in this case (Table 7).

Table 5
rBACE1 inhibitory activity of curcumin and its analogues (**1**)–(**9b**)

Compound	Inhibition[a]	Compound	Inhibition[a]
1	NI	**5a**	7% (1.3 mM)
2	35% (0.67 mM)	**5b**	7% (1.3 mM)
3	9% (0.67 mM)	**5c**	NI
4a	22% (1.3 mM)	**6a**	NI
4b	NI	**7a**	17% (1.3 mM)
4c	19% (1.3 mM)	**7b**	15% (1.3 mM)
4d	17% (1.3 mM)	**8a**	5% (0.67 mM)
4e	36% (1.3 mM)	**8b**	1% (0.67 mM)
4f	7% (0.67 mM)	**8c**	9% (0.67 mM)
4g	4% (0.67 mM)	**9a**	NI
4h	6% (0.67 mM)	**9b**	17% (1.3 mM)

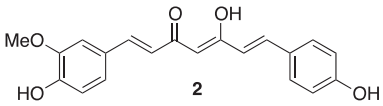
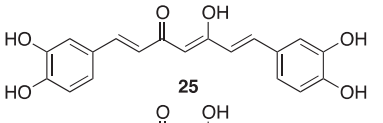
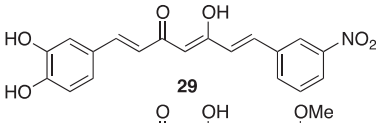
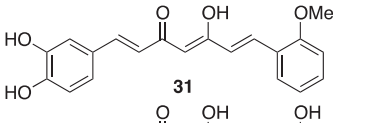
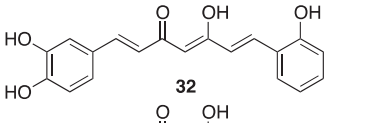
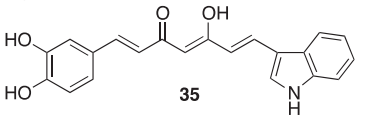
[a] NI: no inhibition.

Table 6
rBACE1 inhibitory activity of synthesized curcumin analogues (**22**)–(**35**)

Compound	Inhibition[a] (%)	Compound	Inhibition[a] (%)
22	1	**29**	18
23	0	**30**	21
24	33	**31**	47
25	25	**32**	100
26	26	**33**	21
27	18	**34**	30
28	5	**35**	71

[a] Inhibitory activity was measured by 0.67 mM.

Table 7
IC_{50} values against rBACE1

Compound	IC_{50} (μM)	mp (°C)	C Log *P*
 **2**	1600	ND[a]	3.676
 **25**	2200	225–227	2.047
 **29**	1800	186–189	0.019
 **31**	1400	156–158	3.230
 **32**	250	111–115	2.644
 **35**	450	165–168	3.301

[a] ND: no data.

To estimate the inhibitory mechanism, an inhibitory kinetics experiment with **32** was performed by constructing a Lineweaver–Burk plot (Fig. 2). The cleavage rate of different amounts of substrate [*S*] by rBACE1 in the absence or presence of **32** (0, 200 and 250 μM) was monitored during the initial 10–15 min reaction period using HPLC. The enzymatic reaction rate was obtained by monitoring the decrease in the area corresponding to the substrate, and the resulting 1/*v* was plotted against 1/*S*. The plots resulted in three straight lines with the close *x*-axis intercept reflecting non-competitive inhibition toward the protease (Fig. 2).

The binding mode of **32** to BACE1 was predicted by docking simulations using MOE from Ryoka Systems Inc. A cylinder-shaped space near the P3 pocket fitted with the bis-phenyl group of **32**.

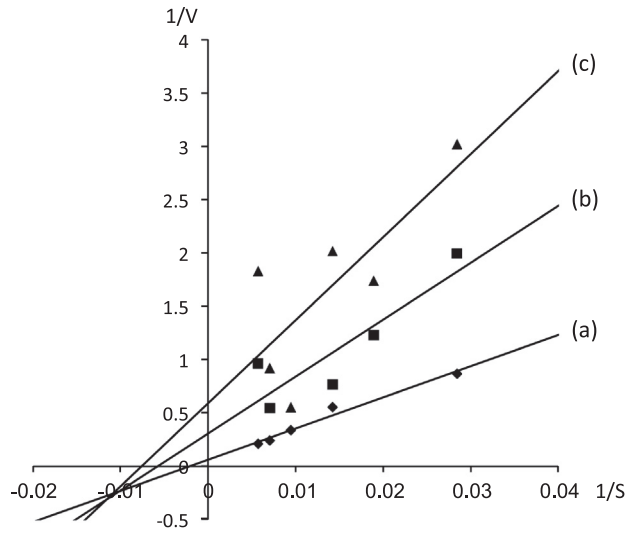


Figure 2. (a) Incubated without inhibitor; (b) incubated with 200 μM of **32**; (c) incubation of 250 μM of **32**.

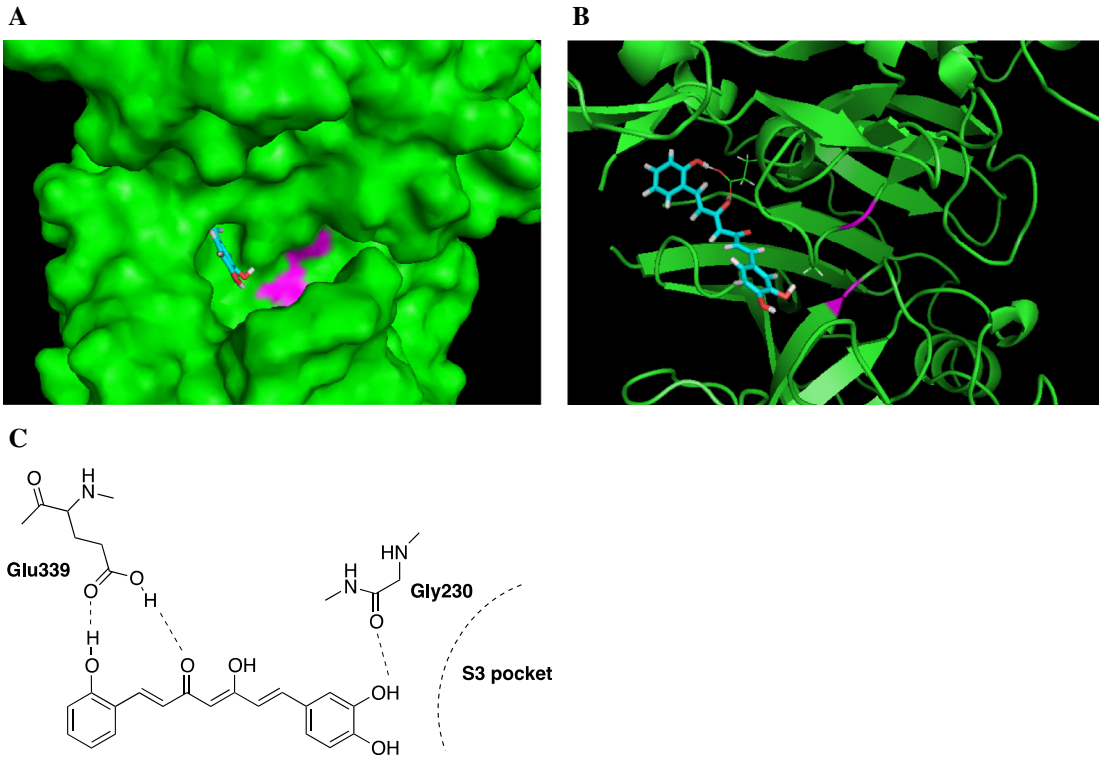


Figure 3. Docking simulation of inhibitor (**32**) bound to BACE1 (PDB code 2ZHT) using MOE from Ryoka Systems Inc. Molecular graphics image showed using PyMol from Schrödinger; oxygen (red) and carbon (blue) of **32**; Asp32 and Asp228 (purple) of BACE1. (A) Surface mode. (B) Cartoon mode to eliminate the side chains of BACE1. (C) Model of interaction.

Two polar phenolic hydroxy groups and a ketone of **32** were involved in hydrogen bonding interactions (Gly230 and Glu339). Inhibitor **32** did not directly interact the active site of two aspartic acids (Asp32, Asp228) of BACE1, but binding of the substrate of the P3 pocket partially interfered with the mode of action. Therefore, non-competitive inhibition of enzyme activity is expected. The cylinder-shaped space near the P3 pocket might be unique and a new potential target for *anti*-BACE1 agents (Fig. 3).

The present study indicated that free hydroxy groups in the place of bis-phenols were preferable for the activity but were not potently effective compared with those of curcumin 2 (**2**). In addition, both ketones and double bonds in the spacer were essential motifs and the corresponding reductive compounds showed no inhibitory activities because of the high degree of flexibility. Therefore, a planer sp_2 carbon unit was important to maintain the inhibitory activity. We propose that both the rigid structure of the spacer and phenolic hydroxy groups act in a cooperative manner on BACE1 with the support of a specific conformation. In particular, the *o*-phenol motif showed a good result from the point of maintaining both planer structures for inhibitory activity and water solubility. It is suggested that replacement of phenols with indole and pyrrole is also an effective method and is expected to occur in the interaction with hydrogen bonds for BACE1. Thus, in order to examine the potential of these compounds as novel BACE1 inhibitors of Alzheimer's disease, it seems worthwhile to design and study more simple molecules incorporating key elements of curcumin 2 (**2**). Taking into account our hypothesis regarding the essential structure, a structure-activity relationship study is now underway.

Acknowledgments

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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.2013.11.039.

References and notes

1. Selkoe, D. J. *Neuron* **1991**, *6*, 487.
2. Sinha, S.; Lieberburg, I. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11049.
3. Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. *Science* **1999**, *286*, 735.
4. Cheng, Y.; Judd, T. C.; Barberger, M. D.; Brown, J.; Chen, K.; Fremeau, R., Jr.; Hickman, D.; Hitchcock, S. A.; Jodan, B.; Li, V.; Lopez, P.; Louie, S. W.; Luo, Y.; Michelsen, K.; Nixey, T.; Powers, T. S.; Rattan, C.; Sickmier, E. A.; St. Jean, D. J., Jr.; Wahl, R. C.; Wen, P. H.; Wood, S. *J. Med. Chem.* **2011**, *54*, 5836.
5. Charrier, N.; Clarke, B.; Cutler, L.; Demont, E.; Dingwall, C.; Dunsdon, R.; Hawkins, J.; Howes, C.; Hubbard, J.; Hussain, I.; Maile, G.; Matico, R.; Mosley, J.; Naylor, A.; O'Brien, A.; Redshaw, S.; Rowland, P.; Soleil, V.; Smith, K. J.; Sweitzer, S.; Theobald, P.; Vesey, D.; Walter, D. S.; Wayne, G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3674.
6. Tagad, H. D.; Hamada, Y.; Nguyen, J. T.; Hidaka, K.; Hamada, T.; Sohma, Y.; Kimura, T.; Kiso, Y. *Bioorg. Med. Chem.* **2011**, *19*, 5238.
7. Tagad, H. D.; Hamada, Y.; Nguyen, J. T.; Hamada, T.; Abdel-Rahman, H.; Yamani, A.; Nagamine, A.; Ikari, H.; Igawa, N.; Hidaka, K.; Sohma, Y.; Kimura, T.; Kiso, Y. *Bioorg. Med. Chem.* **2010**, *18*, 3175.
8. Hilpert, H.; Guba, W.; Woltering, T. J.; Wostl, W.; Pinard, E.; Mauser, H.; Mayweg, A. V.; Rogers-Evans, M.; Humm, R.; Krummenacher, D.; Muser, T.; Schnider, C.; Jacobsen, H.; Ozmen, L.; Bergadano, A.; Banner, D. W.; Hochstrasser, R.; Kuglstratter, A.; Davis-Pierson, P.; Fischer, H.; Polara, A.; Narquizian, R. *J. Med. Chem.* **2013**, *56*, 3980.
9. Xu, Y.-Z.; Yuan, S.; Bowers, S.; Hom, R. K.; Chan, W.; Sham, H. L.; Zhu, Y. L.; Beroza, P.; Pan, H.; Brecht, E.; Yao, N.; Lougheed, J.; Yan, J.; Tam, D.; Ren, Z.; Ruslim, L.; Bova, M. P.; Artis, D. R. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3075.
10. Cai, Z.; Yan, L. J. *J. Biochem. Pharmacol. Res.* **2013**, *1*, 84.
11. Ahmed, T.; Gilani, A. H. *Phytother. Res.* **2013**. http://dx.doi.org/10.1002/ptr.5030.
12. Shieh, J.-M.; Chen, Y.-C.; Lin, Y.-C.; Lin, J.-N.; Chen, W.-C.; Chen, Y.-Y.; Ho, C.-T.; Way, T.-D. *J. Agric. Food Chem.* **2013**, *61*, 6366.

13. Guo, J.; Li, W.; Shi, H.; Xie, X.; Li, L.; Tang, H.; Wu, M.; Kong, Y.; Yang, L.; Gao, J.; Liu, P.; Wei, W.; Xie, X. *Mol. Cell Biochem.* **2013**, *382*, 1103–1111.
14. Lin, L.; Lin, Y.; Li, P.-K.; Fuchs, J.; Shibata, H.; Iwabuchi, Y.; Lin, J. *Br. J. Cancer* **2011**, *105*, 212.
15. Kakizawa, T.; Sanjoh, A.; Kobayashi, A.; Hattori, Y.; Teruya, K.; Akaji, K. *Bioorg. Med. Chem.* **2011**, *19*, 2785.
16. Yang, F.; Lim, G. P.; Begum, A. N.; Ubeda, O. J.; Simmons, M. R.; Ambegaokar, S. S.; Chen, P. P.; Kayed, R.; Glabe, C. G.; Frautschy, S. A.; Cole, G. M. *J. Biol. Chem.* **2005**, *280*, 5892.
17. Shi, W.; Dolai, S.; Rizk, S.; Hussain, A.; Tariq, H.; Averick, S.; L'Amoreaux, W.; Idrissi, A. E.; Banerjee, P.; Raja, K. *Org. Lett.* **2007**, *9*, 5461.
18. Murray, C.; Callaghan, O.; Chessari, G.; Cleasby, A.; Congreve, M.; Frederickson, M.; Hartshorn, M.; McMenamin, R.; Patel, S.; Wallis, N. *J. Med. Chem.* **2007**, *50*, 1116.
19. Congreve, M.; Aharony, D.; Albert, J.; Callaghan, O.; Campbell, J.; Carr, R.; Chessari, G.; Cowan, S.; Edwards, P.; Frederickson, M.; McMenamin, R.; Murray, C.; Patel, S. *J. Med. Chem.* **2007**, *50*, 1124.
20. Pabon, H. J. J. *Recl. Trav. Chim. Pays-Bas* **1964**, *83*, 379.
21. Wisut, W.; Nimmannit, U.; Wacharasindhu, S.; Rojsitthisak, P. *Molecules* **2011**, *16*, 1888.
22. *General procedure for the preparation of the curcumin framework*: To a solution of **21** (250 mg, 1.14 mmol) in 1,4-dioxane was added triethyl borate (250 mg, 3.6 mmol) at room temperature. After stirring for 30 min at 85 °C, 3,4-dihydroxybenzaldehyde (**15**) (250 mg, 1.81 mmol) and boron trioxide (580 μl, 3.41 mmol) were added to the reaction mixture at room temperature. After stirring for 5 h at room temperature, the mixture was added piperidine (350 μl, 3.54 mmol). After the stirring for 1 h at 100 °C, 1 M HCl was added to the reaction mixture and extracted with AcOEt. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by chromatography on silica gel (hexane/AcOEt) to give **25** (73.6 mg, 0.216 mmol, 19%) as a brown powder. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.91 (s, 1H), 6.49 (d, 2H, J = 16.0 Hz), 6.76 (d, 2H, J = 8.4 Hz), 6.95 (dd, 2H, J = 8.0, 1.6 Hz), 7.05 (d, 2H, J = 16.0 Hz), 7.46 (d, 2H, J = 16.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 113.9, 115.2, 120.6, 121.6, 127.2, 140.9, 145.5, 148.1, 183.4; IR (film) ν_{max} cm^{-1}: 3485, 3400, 3390, 3100, 1601, 1514, 1442, 1354, 1286, 1265, 1205, 1115, 976, 822, 739; ESI–HRMS $[M+Na]^+$ calcd for $C_{19}H_{16}O_6Na$ 363.0845, found: 363.0869.
23. *Measurement of inhibitory activity*: Enzyme assays were carried out using recombinant BACE1 at an enzyme concentration of 120 nM. The reaction mixture (40 mM AcONa buffer, pH 4.0, containing 10% grecerol, 10 mM DTT and 4 M NaCl) was analyzed on a Cosmosil 5C18-AR-II column (4.6 × 150 mm), employing a linear gradient of MeCN (10–40%, 30 min) in aq 0.1% TFA. Each IC_{50} value was obtained from a sigmoidal dose–response curve obtained from the decrease of the substrate in the reaction mixture. Each experiment was repeated three times.