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Synthesis and structure–activity relationships of tri-substituted thiazoles as RAGE antagonists for the treatment of Alzheimer's disease

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ABSTRACT

A series of thiazole derivatives were designed, and prepared to develop RAGE antagonist for the treatment of Alzheimer's disease (AD). SAR studies were performed to optimize inhibitory activity on Aβ-RAGE binding. SAR studies showed that introducing an amino group at part A was essential for inhibitory activity on Aβ-RAGE binding. Compounds selected from Aβ-RAGE binding screening displayed inhibitory activity on Aβ transport across BBB. They also showed inhibitory activity against Aβ-induced NF-κB activation. These results indicated that our derivatives had a potential as therapeutic agent for the treatment of AD.

Alzheimer's disease (AD) is a neurodegenerative disorder and is the most common cause of dementia. The pathological hallmarks of AD include extracellular amyloid plaques that are consist of aggregated Aβ, cleaved peptides from amyloid precursor protein (APP).[1] Although pathogenic process of AD is not yet clear, the beginning of AD is due to abnormal accumulation of Aβ in the brains causing neurodegeneration and finally the clinical symptoms of dementia.[2] Therefore, Aβ is considered to play a central role in the pathogenesis of AD. In addition to accumulation of Aβ, evidences for the involvement of inflammatory responses in the pathogenesis of AD have been documented. A number of inflammatory mediators with Aβ-associated NF-κB activation have been reported to be up-regulated in AD brain.[3]

Aβ peptides were initially thought to derive solely from neurons and glia.[4] It is now believed that Aβ originates from the entire tissues and circulating Aβ in plasma can contribute to accumulation of neurotoxic Aβ in the brain through transport of plasma Aβ.[5] The accumulation of Aβ in AD brain was related with the balance between production and clearance of Aβ. Although there has been a lot of reports regarding regulation of Aβ level in the brain for the treatment strategy of AD, Aβ-lowering strategy by receptors that mediate Aβ transport across blood–brain barrier (BBB) recently begin to receive attention.[6]

Aβ transport across BBB is mediated by the low-density lipoprotein receptor-related protein 1 (LRP-1) and the receptor for advanced glycation end-products (RAGE). LRP-1 and RAGE play contrary roles in Aβ transport across BBB; efflux and influx of Aβ. RAGE is a multiligand cell surface receptor which is normally expressed in endothelium, microglia and neurons. RAGE was originally identified as a receptor for the advanced glycation end products (AGEs) and amphoterin. Along with the involvement of RAGE in disease and age-related manner, it was reported that expression of RAGE was up-regulated in AD brain tissues and RAGE acted as a receptor/transporter for Aβ from blood into brain.[7] Also, RAGE in the surface of microglia and astrocyte activated NF-κB signaling pathway and triggered oxidative stress and inflammatory response, causing cellular perturbation.[7b,8] These studies suggested that RAGE was a key target in the inflammatory and neurotoxic cascade.

Our goal is to discover a potent antagonist that prevents Aβ transport across the BBB and decreases inflammatory response in the brain. Initially, compounds known for RAGE antagonist were considered as our strategy for molecular design of RAGE antagonist (Fig. 1). Our newly designed molecule was divided into four parts; parts A (aminoalkyl phenyl), part B (heterocyclic core), part C (alkoxyphenyl), and part D (aliphatic chain). BBB permeation of compounds was required for anti-inflammatory action of compound in brain. Therefore, more lipophilic thiazole ring than benzimidazole was selected for structural modification of heterocyclic

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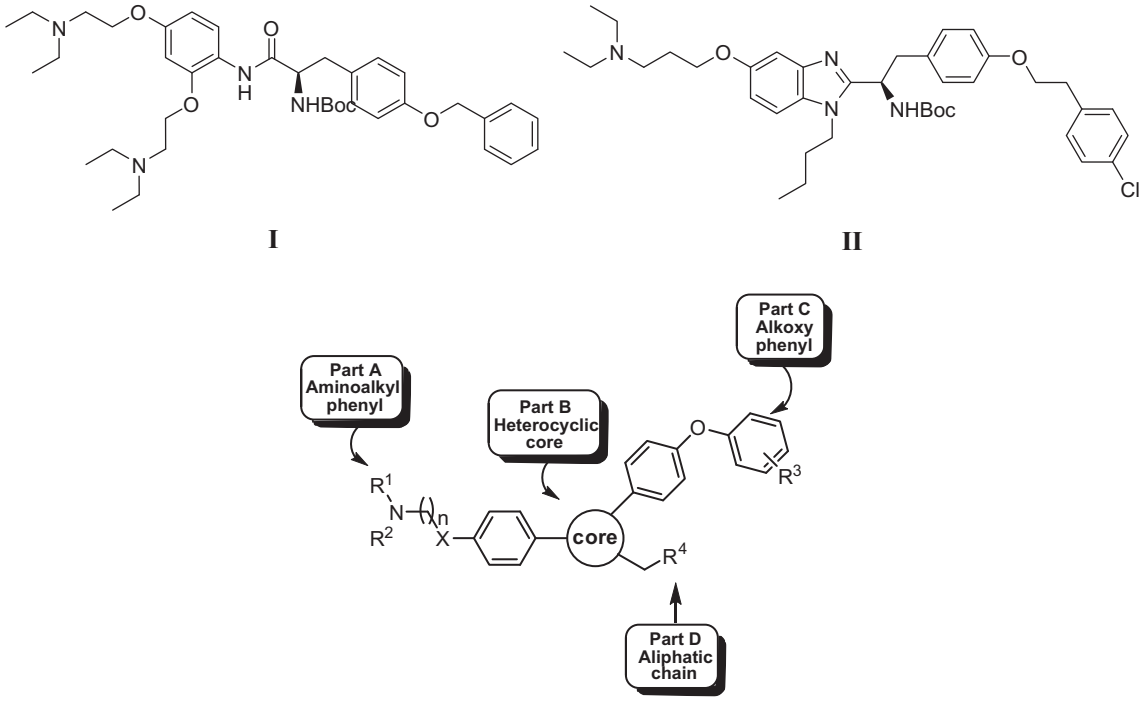
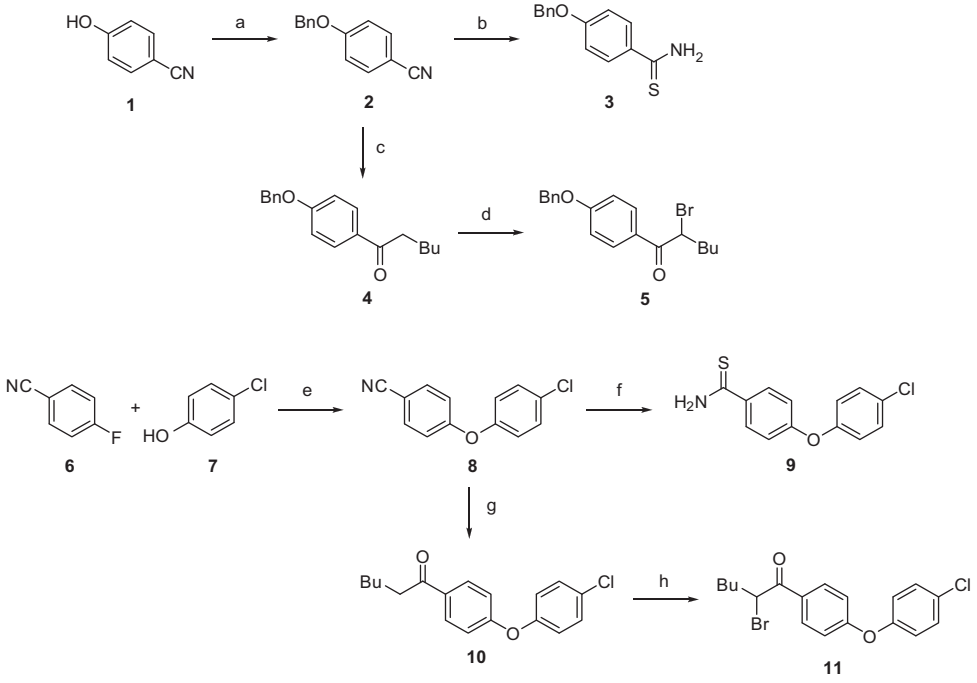


Figure 1. Compounds known for RAGE antagonist and synthetic strategy for new compounds.[9]

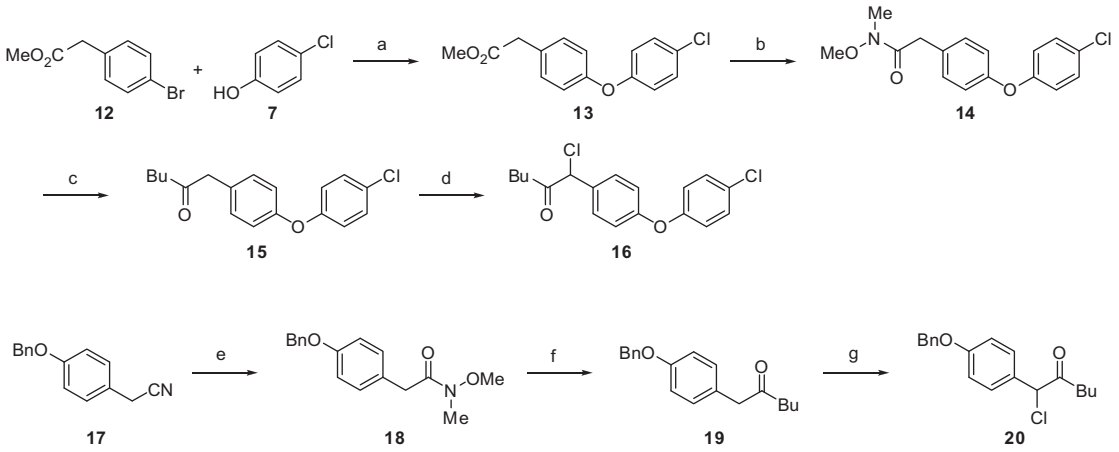
core at part B. In this study, we reported the synthesis of newly designed compounds and established SAR studies for RAGE binding.

General synthesis for thiazole core ring was achieved by cyclization reaction of thioamide and α-haloketone. Synthesis of intermediates thioamide and α-haloketone was shown in Scheme 1 and Scheme 2. Treatment of benzonitriles (**2** and **8**) with NaSH and $MgCl_2$ in DMF provided desired thioamides **3** and **9**.[10] Benzonitriles (**2** and **8**) were subject to reaction with pentylMgBr, followed by acidic hydrolysis to afford ketones **4** and **10**.[11] These ketones were then brominated with $CuBr_2$ to give α-bromoketones **5** and **11**.[12] Preparation of α-chloroketones **16** and **20** was shown in Scheme 2. Ullmann reaction of arylbromide **12** and phenol **7** provided diphenyl ether **13**. Ketones **15** and **19** were obtained from **14** and **18** by Weinreb ketone synthesis strategy. Regiospecific α-chloroketones **16** and **20** were synthesized by treating **15** and **19** with sulfuryl chloride, respectively.[13]

Thiazoles **21a**–**24a** which had positional diversity were obtained by cyclization reaction with intermediates thioamide and



Scheme 1. Reagents and conditions: (a) BnBr, K_2CO_3, acetone, reflux, 98%; (b) NaSH, $MgCl_2$, DMF, rt, 95%; (c) (i) pentylMgBr, THF, 65 °C; (ii) aqueous H_2SO_4, 86%; (d) $CuBr_2$, EtOAc, 70 °C, 61%; (e) K_2CO_3, DMF, reflux, 96%; (f) NaSH, $MgCl_2$, DMF, rt, 91%; (g) (i) pentylMgBr, THF, 65 °C; (ii) aqueous H_2SO_4, 53%; (h) $CuBr_2$, EtOAc, 70 °C, 97%.



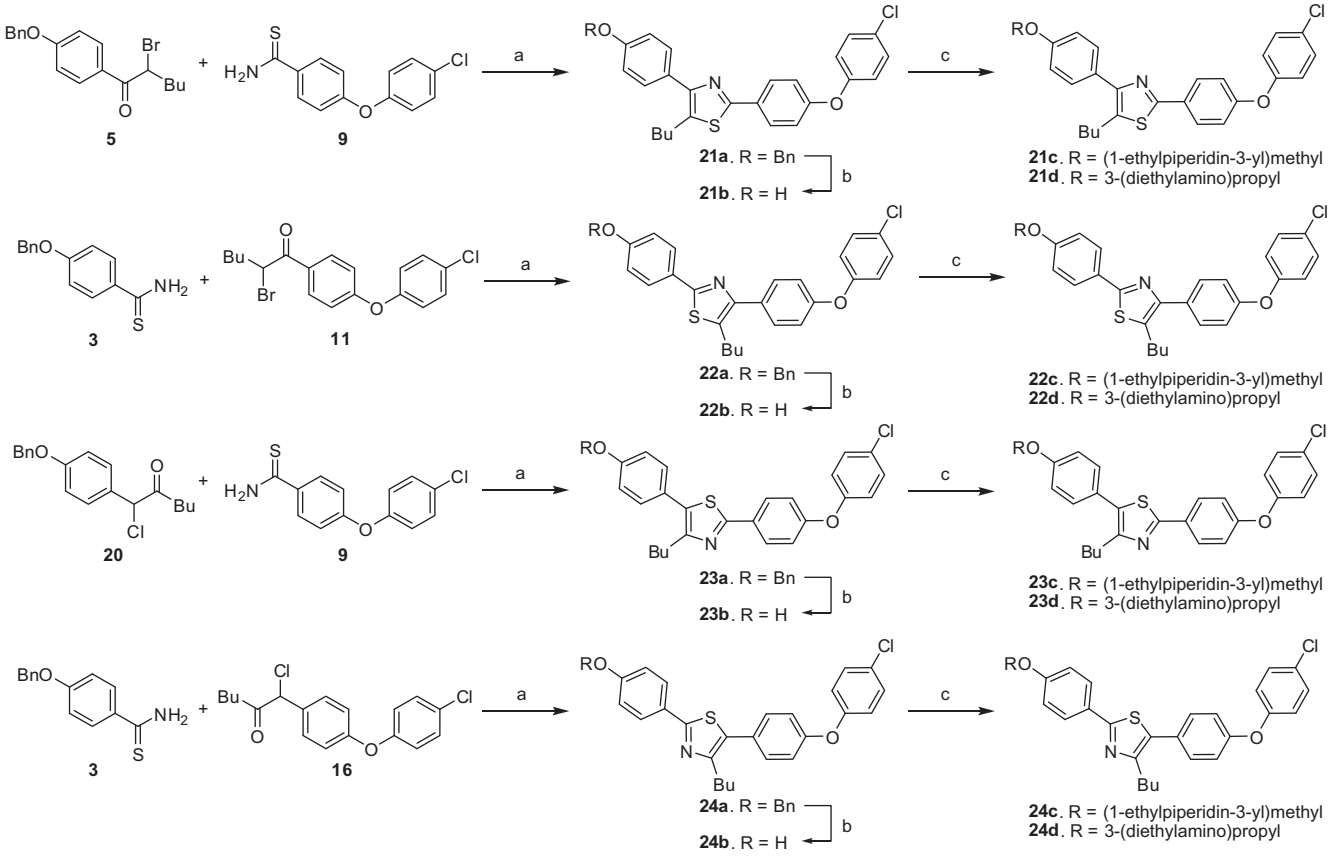
Scheme 2. Reagents and conditions: (a) Cu_2O, Cs_2CO_3, *N*,*N*-dimethylglycine, molecular sieve, DMF, 110 °C, 42%; (b) (i) 1 N NaOH, MeOH, reflux, then aqueous HCl, 95%; (ii) $SOCl_2$, reflux; (iii) *N*,*O*-dimethylhydroxylamine, NEt_3, CH_2Cl_2, 0 °C, 55%; (c) *n*-BuLi, THF, −78 °C, 52%; (d) SO_2Cl_2, CH_2Cl_2, −10 °C, 75%; (e) (i) 30% NaOH, reflux, then aqueous HCl, 88%; (ii) CDI, THF, then *N*,*O*-dimethylhydroxylamine, 32%; (f) *n*-BuLi, THF, −78 °C, 86%; (g) SO_2Cl_2, CH_2Cl_2, −10 °C, 42%.

α-haloketone in EtOH (Scheme 3). After debenzylation, Mitsunobu reaction with appropriate aminoalcohols provided desired compounds **21c**–**24d**.

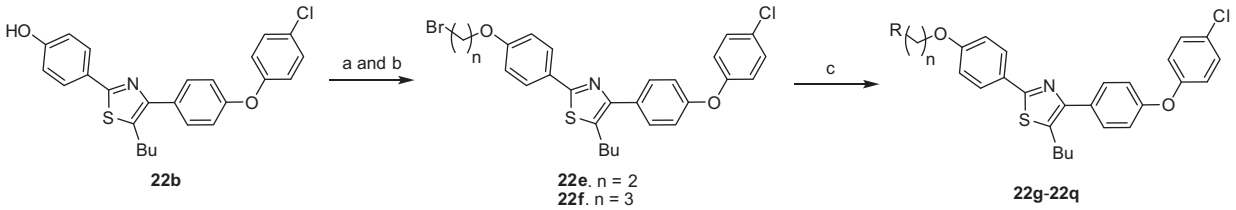
Alternatively, for modification of alkylamino group, **22b** reacted with dibromoalkane followed by the amination using the corresponding amines to afford compounds **22g**–**q** (Scheme 4).

Compound **27a** was prepared from **26b** by amide coupling reaction (Scheme 5). Compound **27b** which is reverse amide form of **27a** was prepared by condensation reaction with thiourea and **11**, followed by amidation, debenzylation, and Mitsunobu reaction (Scheme 6). Compound **27c** was obtained by cyclization reaction with **28** and **11** (Scheme 7).

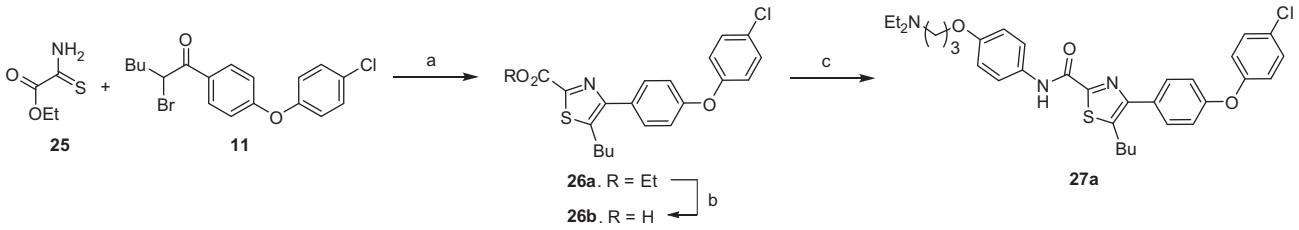
We introduced thiazole ring at heterocyclic core of molecules and attempted to investigate the effect on substitution site of thiazole ring. Their inhibitory activity on Aβ-RAGE binding was evaluated using in vitro enzyme-linked immunosorbent assay (ELISA).[14] The results of Aβ-RAGE binding study were summarized in Table 1 and 2. It showed that there was no difference in inhibitory activity between 2,4-phenyl-substituted (Table 1) and 2,5-phenyl-substituted (Table 2) derivatives. Also, the shifting for R^1 (part A) and R^2 (part B) had no effect on inhibitory activity. Inhibitory activity depended rather on amino group of part A. Compounds **21a**–**24b** that did not contain amino group showed no activity, whereas compounds **21c**–**24d** with amino group



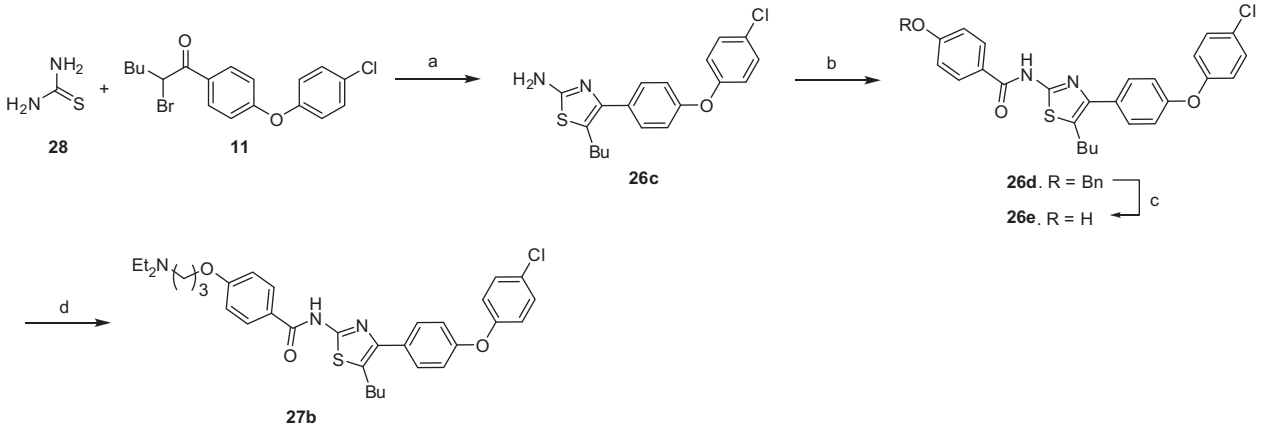
Scheme 3. Reagents and conditions: (a) EtOH, reflux, 38–79%; (b) BBr_3, CH_2Cl_2, −78 °C, 72–92%; (c) aminoalcohols, PPh_3, DIAD, THF, 0 °C → rt, 18–47%.



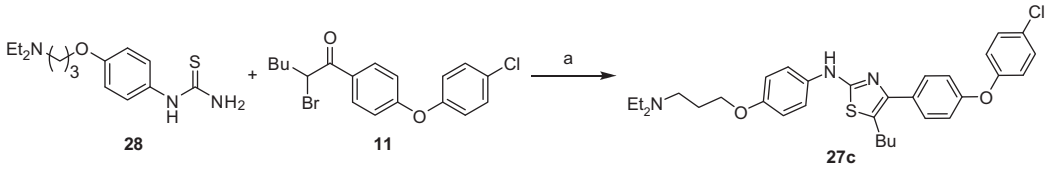
Scheme 4. Reagents and conditions: (a) dibromoethane, K_2CO_3, MeCN, reflux, 67%; (b) dibromopropane, K_2CO_3, MeCN, reflux, 80%; (c) amines, K_2CO_3, MeCN, reflux, 48–94%.



Scheme 5. Reagents and conditions: (a) EtOH, reflux, 44%; (b) 1 N NaOH, EtOH, reflux, then aqueous, HCl 84%; (c) (i) $SOCl_2$, reflux; (ii) 4-(3-diethylaminopropoxy)aniline, Et_3N, CH_2Cl_2, 0 °C, 21%.



Scheme 6. Reagents and conditions: (a) EtOH, reflux, 94%; (b) 4-benzyloxybenzoyl chloride, Et_3N, CH_2Cl_2, 0 °C, 50%; (c) BBr_3, CH_2Cl_2, −78 °C, 77%; (d) 3-diethylamino-1-propanol, PPh_3, DIAD, THF, 0 °C → rt, 25%.



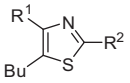
Scheme 7. Reagents and conditions: (a) EtOH, reflux, 40%.

ncreased inhibitory activity for Aβ-RAGE binding (% inhibition, 47.7–58.9% at 10 μM).

As thiazole ring of part B with positional diversity was not related to inhibition of Aβ-RAGE binding, we further continued to identify influence of part B in binding assay by modifying thiazole ring. Heterocyclic core at part B was converted into modified thiazole (compounds **27a**, **27b**, and **27c**). We inserted amide and amino groups between phenyl ring of part A and thiazole ring of part B, to change bond length between part A and part B, and log*P* value of compounds. Their inhibitory activity was kept when inserted amide and amine groups (Table 3). However, striking increase of activity was not observed (% inhibition, 48.1–60.9% at 10 μM), as compared with thiazole derivatives in Table 1 and Table 2 (% inhibition, 47.7–58.9% at 10 μM). The result confirmed that modification of heterocyclic core did not have a significant effect on increase of inhibitory activity.

Based on in vitro SAR study above, we selected compounds **22d**, which showed high binding activity (IC_{50} = 0.66 μM), to explore effect of aminoalkyl group at part A. With fixed diphenyl ether and butyl groups in part C and D, various amino groups with change of carbon chain length were substituted at part A. As shown in Table 4, most of compounds kept its inhibitory activity on Aβ-RAGE binding and exhibited good activity (IC_{50} = 0.91–3.18 μM). Although there was not remarkable difference in inhibitory activity

Table 1
In vitro inhibitory activity of 2,4-phenyl-substituted thiazole derivatives on Aβ-RAGE binding



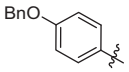
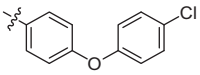
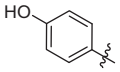
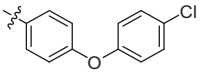
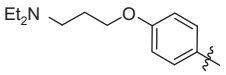
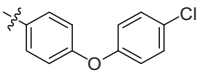
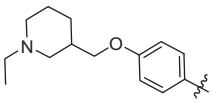
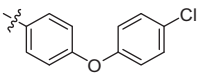
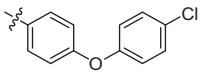
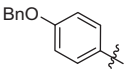
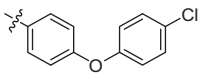
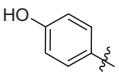
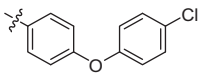
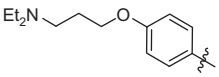
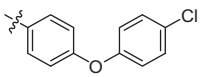
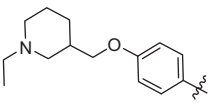
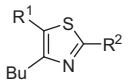
Compd.	R^1	R^2	% inhibition (10 μM)	IC_{50} (μM)
21a			2.4	–
21b			0.2	–
21c			57.0	1.12
21d			55.9	1.91
22a			–	–
22b			–	–
22c			58.9	1.72
22d			58.0	0.66

Table 2
In vitro inhibitory activity of 2,5-phenyl-substituted thiazole derivatives on Aβ-RAGE binding



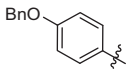
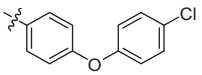
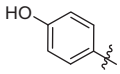
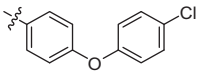
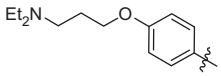
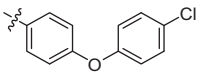
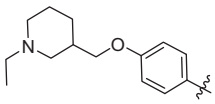
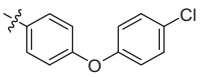
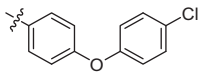
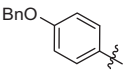
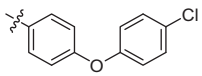
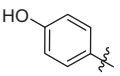
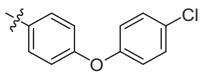
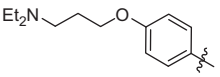
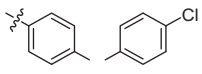
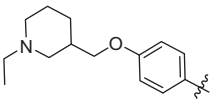
Compd.	R^1	R^2	% inhibition (10 μM)	IC_{50} (μM)
23a			–	–
23b			–	–
23c			57.1	2.01
23d			53.9	1.14
24a			–	–
24b			–	–
24c			49.6	–
24d			47.7	–

Table 3
In vitro inhibitory activity of modified thiazole derivatives on Aβ-RAGE binding

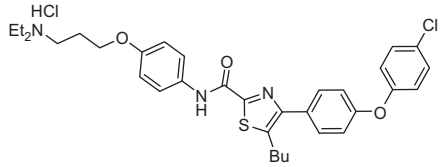
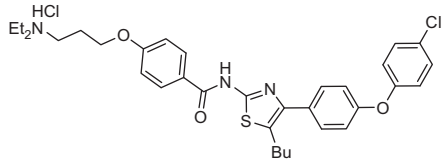
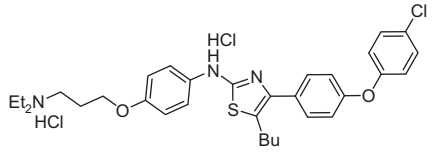
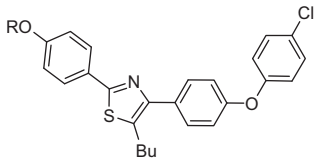
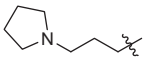
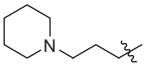
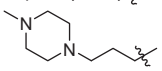
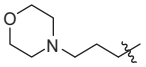
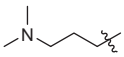
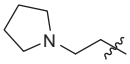
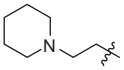
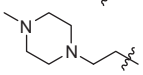
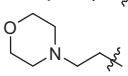
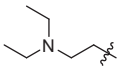
Compd.	Structure	% inhibition (10 μM)	IC_{50} (μM)
27a		48.1	–
27b		60.2	5.59
27c		60.9	0.55

Table 4
In vitro inhibitory activity of thiazole derivatives on Aβ-RAGE binding



Compd.	R	% inhibition (10 μM)	IC_{50} (μM)
22g		47.8	–
22h		59.2	1.84
22i		59.2	1.24
22j		40.6	–
22k		46.2	–
22l		64.2	0.91
22m		60.1	1.04
22n		54.7	1.21
22o		22.9	–
22p		51.8	3.18
22q		55.1	1.58

according to amine substituents, morpholine seemed to be not good substituent for inhibition of Aβ-RAGE binding (% inhibition, **22j** = 40.6%, **22o** = 22.9%). These results of binding assay suggested that amino group of part A was essential for inhibitory activity on Aβ-RAGE binding, and inhibitory activity depended more on amino group of part A than part B.

For study regarding RAGE antagonist, we further identified inhibitory activity on Aβ transport and Aβ-induced NF-κB activation using BBB and reporter gene assay, respectively (Table 5)[15,16] In BBB assay, RAGE-overexpressing bEND.3 cells with tight junction were used for artificial BBB system. The result of BBB assay was consistent with binding assay. Most of compounds with over 50% inhibitory activity in binding assay inhibited Aβ transport across BBB (% inhibition = 39.5–77.5% at 10 μM). Particularly, compound **22d** that was efficient for inhibition of Aβ-RAGE binding, with IC_{50} value of 0.66 μM, showed the highest activity in the BBB assay (77.5% at 10 μM). On the other hand, active compounds in binding assay were less active in luciferase reporter assay. Inhibitory activity of compounds on Aβ-induced NF-κB activation had relatively weak potency (% inhibition, 2.1–17.2% at 1 μM). It appeared that these series of compounds had partial antagonistic effect on Aβ-associated NF-κB activation.

Table 5
In vitro inhibitory activity of compounds on Aβ transport across BBB and NF-κB activation

Compd.	BBB permeation of Aβ % inhibition (10 μM)	Luciferase assay % inhibition (1 μM)
21c	71.7	–
21d	68.3	–
22c	73.3	–
22d	77.5	3.0
23c	63.5	10.3
23d	61.5	4.9
22h	64.3	11.7
22i	64.3	3.1
22l	39.5	2.5
22m	55.3	13.5
22n	68.6	17.2
22p	66.6	2.1
22q	NT	11.3

In summary, thiazole derivatives were designed, synthesized, and evaluated for RAGE antagonist. In SAR studies, alkylamino group of part A was essential for inhibitory activity of Aβ-RAGE binding, and compounds that can bind to RAGE inhibited transport of Aβ. In particular, we identified that compound **22n** inhibited not only transport of Aβ across BBB but also Aβ-associated NF-κB activation by blocking Aβ-RAGE binding. This study indicated that our synthetic compounds had a potential as promising agents for RAGE antagonist.

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14. *Aβ-RAGE binding assay*: 1 μg of purified biotinylated-human-RAGE, 1 μL of 10 μM Aβ solution and 10 μM of the compound in 100 μL of TBS-T with 2.5% BSA were incubated on a streptavidin-coated plate for 60 min at ambient temperature. After washing the plate with TBS-T, the horseradish-peroxidase conjugated 4G8 antibody (4G8-HRP, 1:1000 dilution) in 100 μL of TBS-T with 2.5% BSA was added into each well to detect the bound Aβ. The plate was incubated for 60 min at ambient temperature. After washing with TBS-T, the plate was developed with TMB substrate: the reaction was stopped with sulfuric acid. The absorbance was read on a Sunrise plate reader (TECHAN) at 450 nm.
15. *Measurement of BBB-permeated Aβ*: Collagen-coated transwell-Col inserts (Corning) were washed with Krebs-ringer bicarbonate buffer (Sigma). bEND.3 cells were grown for 3–4 days to confluence on inserts, and the resistance of inserts was measured using the Millicell ERS Voltohmmeter (Millipore, Billerica, MA). The transendothelial electrical resistance (TEER) of the inserts was calculated by subtracting the resistance of blank inserts from that of the inserts with bEND.3 cells and multiplying the subtracted values by the area of the insert. The TEER was used as an index of the barrier property of the bEND.3 monolayer. For the efficacy of RAGE antagonists, 5 μM of Aβ42-FITC (BACHEM) with indicated compound (10 μM) was treated in the inserts with bEND.3 cells. To measure transported Aβ42-FITC, the fluorescence level of the media from donor chamber was measured using SAPIRE (TECHAN) at 520 nm.
16. *Luciferase reporter gene assay*: For the measurement of RAGE-NF-κB signaling, the pNF-κB-Luciferase vector was transfected on CHO cell line and stable cell line was generated following manufacture's protocol (*Exp. Mol. Med.* **2006**, *38*, 445). CHO-NF-κB-Luciferase cells were plated at a density of 20,000 cells/well in 96-well plates in 100 μL of Opti-MEM (Invitrogen, Carlsbad, CA). After overnight incubation, compounds (1 μM) were added to the cultures for 18 h at 37 °C with or without 5 μM of Aβ42. For measure luciferase level, the cells were lysis with Passive lysis buffer (Promega). Cell lysates were transferred to new 96 well plate and mixed with Luciferase assay substrate (Promega). The values of relative light units (RLU) were measured using luminometer (Turner Designs, Sunnyvale, CA).