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Small molecules that protect against β-amyloid-induced cytotoxicity by inhibiting aggregation of β-amyloid

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ABSTRACT

Aggregated β-amyloid (Aβ) plays crucial roles in Alzheimer's disease (AD) pathogenesis, therefore blockade of Aβ aggregation is considered as a potential therapeutic target. We designed and synthesized small molecules to reduce Aβ-induced cytotoxicity by inhibiting Aβ aggregation. The small molecules were screened via ThT, MTT, and cell-based cytotoxicity assay (Aβ burden assay). Selected compounds **1c**, **1d**, **1e**, and **1f** were then investigated by evaluating their effects on cognitive impairment of acute AD mice model. Learning and memory dysfunction by injection of Aβ(1–42) was recovered by administration of these molecules. Especially, **1d** showed the best recovery activity in Y-maze task, object recognition task, and passive avoidance task with dose dependent manner. These results suggest that **1d** has high potential as a therapeutic agent for AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and its clinical symptoms are characterized by dysfunction in cognition and memory. One of the major pathologic hallmarks of AD is accumulation of extracellular β-amyloid (Aβ) peptide. A number of studies have provided strong evidences that aggregation of Aβ induces neurodegeneration, neurotoxicity, and oxidative stress in AD brains.[1]

Aβ is derived from the sequential proteolytic processing of amyloid precursor protein (APP) by β-secretase and γ-secretase.[2] The cleavage of APP at different positions by γ-secretase produces two predominant Aβ peptide residues, Aβ(1–40) and Aβ(1–42). The most abundant variant in vascular Aβ is Aβ(1–40), but Aβ(1–42), the longer form, is more fibrillogenic and neurotoxic than Aβ(1–40). Although production of Aβ is a normal process, the over-production of Aβ or an increased proportion of Aβ(1–42) appears to cause early onset of AD.[3]

Since the progressive accumulation of Aβ aggregates is widely believed to be fundamental to the initial development of neurodegenerative pathology, many therapeutic approaches in AD are focused on reducing neurotoxicity by decreasing the concentration of cerebral Aβ. Various approaches to decrease Aβ include inhibiting β, γ-secretases, increasing Aβ clearance, or blocking Aβ aggregation with peptides, antibodies, and small molecules.[4]

There are numbers of small molecules reported to have anti-neurodegenerative activities. Styryl benzene (SB) derivatives have excellent binding affinities to β-sheet rich Aβ fibrils (Fig. 1). Also, SB type molecules are known to bind to oligomeric Aβ which is relatively smaller and more soluble fibrils, leading to prohibit the formation of larger and insoluble fibrils.[5] Ferulic acid (FA), a well-known anti-oxidant, inhibited fibril formation of Aβ(1–40) and Aβ(1–42) and neutralized Aβ(1–42)-induced toxicity.[6] It was also demonstrated that long-term administration of FA exhibited anti-dementia activity in vivo.[7] Resveratrol, a polyphenol compound, exhibited a wide range of biological and pharmacological activities including anti-oxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic, and anti-angiogenic effects.[8] Resveratrol inhibited Aβ fibril formation and exerted neuroprotective effect against cell death induced by Aβ.[9] Curcumin has potent anti-oxidant and anti-inflammatory activities and can suppress oxidative damage, inflammation, and amyloid accumulation. It directly binds to small Aβ species, blocking aggregation and fibril formation in vitro and in vivo.[10]

The recent studies suggest that fibrils are formed through oligomeric Aβ assembly intermediates, which are more toxic than fibrils.[11] In the light of these findings, a need for inhibitors of pathogenic oligomeric assembly formation has been recognized. We previously introduced a small molecule, KMS4005, with decent

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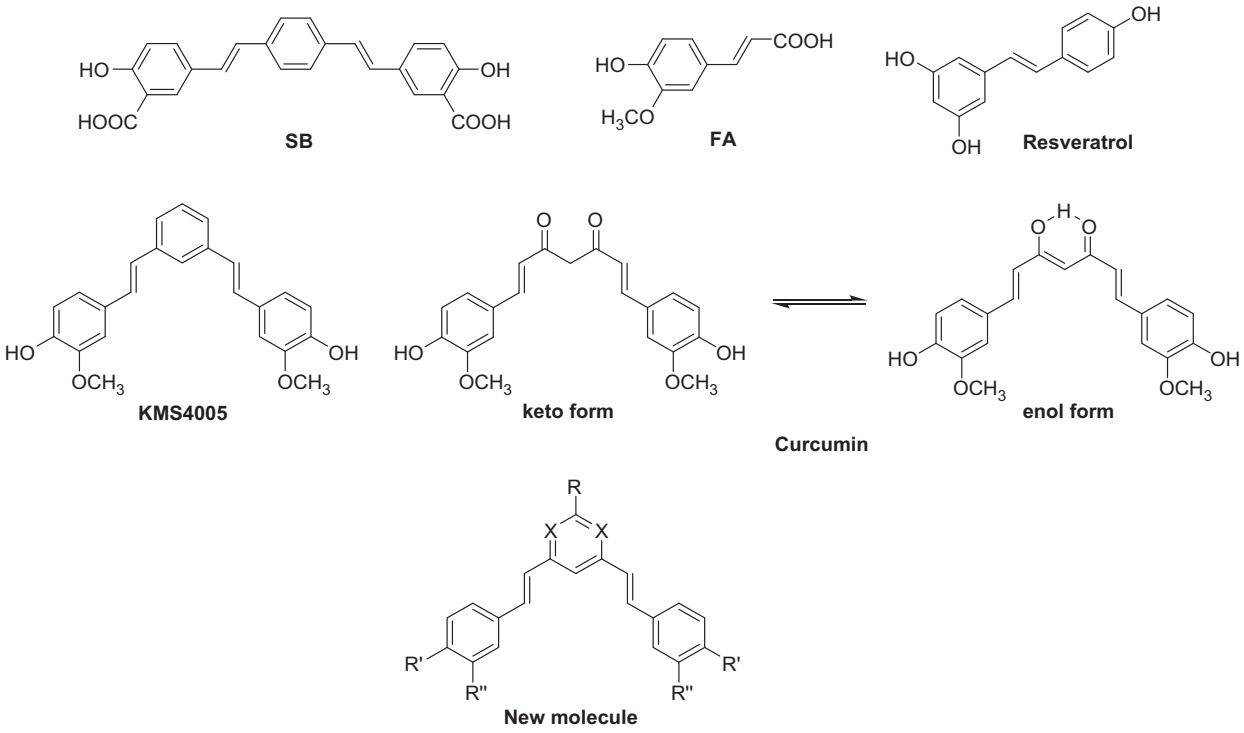


Figure 1. Structures of styryl benzene (SB), ferulic acid (FA), resveratrol, KMS4005, curcumin, and new molecule.

binding affinity and specificity to the non-fibrous and monomer-like Aβ.[12] By extension of these results, we here designed and synthesized new molecules which are derived from previously reported KMS4005. A central benzene ring of KMS4005 into pyrimidine ring was modified and substituents on central ring were added. According to our earlier study, hydroxyl groups on outer rings may play an important role in its anti-amyloidogenic activity and is considered as necessary pharmacophore to have protective effect on Aβ-induced cytotoxicity. Therefore, we added naturally occurring substituents such as hydroxy and methoxy, and lipophilic substituents such as dimethylamino group on outer ring.

Several clinical studies proved that curcumin has a chemotherapeutic activity in AD and is non-toxic even at very high doses.[13] In spite of its pharmacological efficacy on AD and safety, curcumin has not yet been approved as a therapeutic agent due to its poor bioavailability, high rate of metabolism, and instability under neutral-basic conditions.[14] Newly designed molecules have central pyrimidine or benzene ring which is equivalent to the hexagonal shape of enol form of curcumin.

In this study, we synthesized new small molecules as described above (Fig. 1). Their activities to prevent fibril formation and alleviate Aβ-induced cytotoxicity were investigated using fluorescence assay in vitro and cell-based assay. We utilized acute AD mouse model to assess the recovery abilities of titled compounds against Aβ-induced behavioral abnormality.

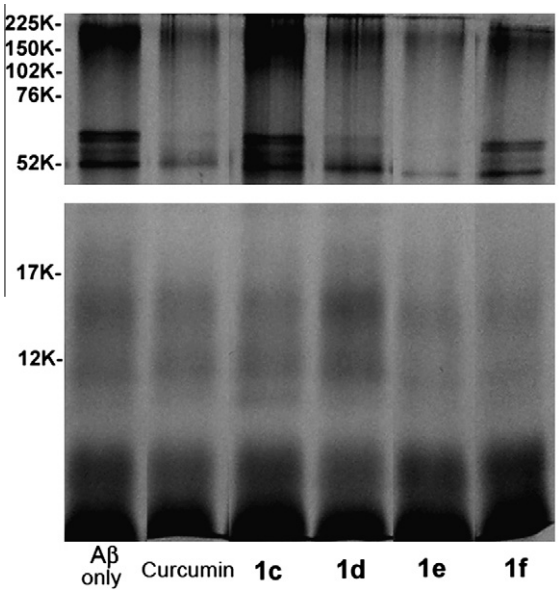
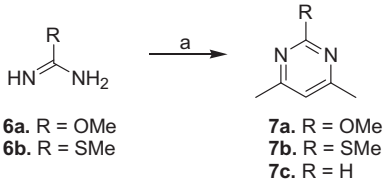


Figure 2. Effect of compounds on Aβ oligomerization. Monomerized Aβ(1–42) (25 μM) was incubated for 2 h at 37 °C in the presence of curcumin, **1c**, **1d**, **1e**, and **1f** (2 μM). After cross-linking of peptide using PICUP technique, the mixture was applied on SDS–PAGE gel.

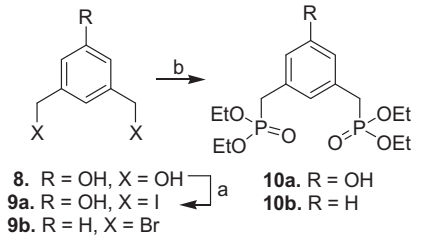
2. Results and discussion

2.1. Chemistry

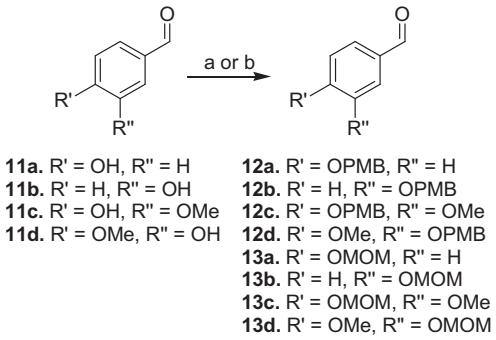
Compounds **1a**–**5f** are shown in Fig. 2 and were prepared according to Schemes 1–5. Cyclization of *O*-methylisourea **6a** and *S*-methylisothiourea **6b** with acetylacetone in aqueous K_2CO_3 solution afforded 2-methoxypyrimidines **7a** and 2-thiomethylpyrimidine **7b**, respectively (Scheme 1). Halogenation of benzyl alcohol **8** followed by Arbuzov reaction gave diethyl phosphonates **10**



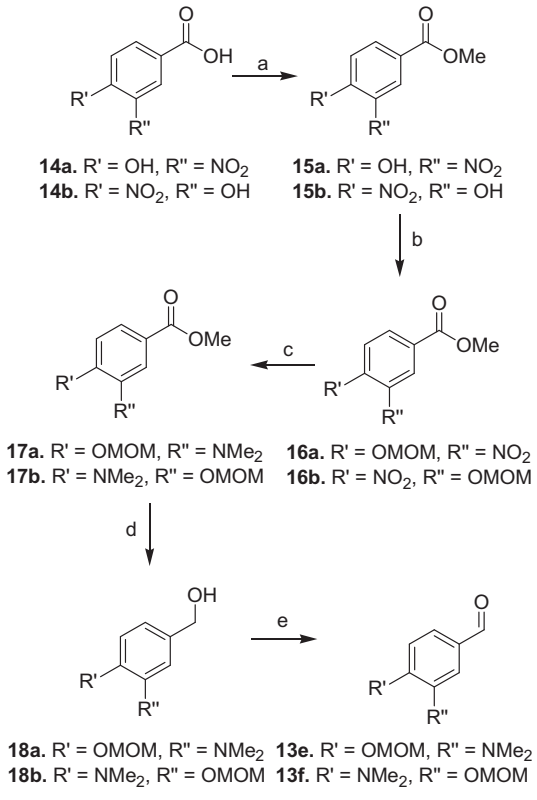
Scheme 1. Reagents and conditions: (a) acetylacetone, aqueous K_2CO_3, reflux.



Scheme 2. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, KI, 1,4-dioxane, rt; (b) $P(OEt)_3$, toluene, reflux.



Scheme 3. Reagents and conditions: (a) PMB-Cl, K_2CO_3, DMF, 60 °C; (b) MOM-Cl, Et_3N, THF, 0 °C.



Scheme 4. Reagents and conditions: (a) concd H_2SO_4, MeOH, reflux; (b) MOM-Cl, Et_3N, THF, 0 °C; (c) H_2, 10% Pd/C, formaldehyde, MeOH; (d) $LiAlH_4$, THF, 0 °C; (e) MnO_2, CH_2Cl_2.

(Scheme 2). The protection of commercial hydroxybenzaldehydes **11** with *p*-methoxybenzyl (PMB) or methoxymethyl (MOM) groups afforded O-protected benzaldehydes **12a**–**12d** and **13a**–**13d**, respectively (Scheme 3). Benzaldehydes **13e** and **13f** possessing dimethylamino group were prepared from nitrobenzoic acid **14a** and **14b**, respectively (Scheme 4). Condensation of **7** with **12**, **13e**, and **13f** in aqueous NaOH solution, and Horner-Emmons reaction of **10** with **13** in the presence of *t*-BuOK as a base afforded protected molecules **1a′**–**5f′** (Scheme 5). Removal of PMB and MOM groups afforded the target compounds **1a**–**5f**.

2.2. Anti-amyloidogenic activity and cytotoxicity

To determine anti-amyloidogenic activity and cytotoxicity of the compounds, a fluorescence intensity assay using thioflavin T (ThT) and MTT assay were performed.[15] Most of compounds showed inhibitory effects on Aβ fibril formation (Table 1). It appeared that the central pyrimidine and benzene rings were responsible for the inhibition of Aβ fibril formation, but R substituents of the central ring did not have a meaningful activity. Generally, compounds **1** and **5** except compounds **b** and **g** showed a higher inhibitory activity than others. The inhibitory activity depended rather strongly on R′ and R″ substituents of outer rings. Non-substituted compounds **2g**–**5g** at outer ring inhibited only 20% of fibril formation (**1g**: 47.4%, **2g**: 18.8%, **3g**: 11.6%, **4g**: 19.8%, **5g**: 19.8%). Introduction of e-donating groups at R′ and R″ had a tendency to lower fibril formation. In proportion to the strength of e-donating group at outer ring substituents (H < OMe < NMe_2), inhibitory activity on Aβ fibril formation was increased (a < c < e, b < d < f). Although e-donating group on both R′ and R″ was critical for inhibition of Aβ fibril formation, e-donating group on R′ played a more important role than that on R″ (a > b, c ≈ d, e < f). Summing up in vitro data, compounds **f** had the best inhibitory activity compared to others, due to relatively high binding affinity to Aβ with *N*,*N*-dialkylamino group.[16] According to the cell cytotoxicity data obtained in treating compounds to HT22 cell line, **1a**–**2f** except **2b** and **2c** had no cytotoxicity. Among them, **1a**–**1f** except **1b** and **2f** were non-toxic, and also effectively blocked fibrillogenesis in vitro.

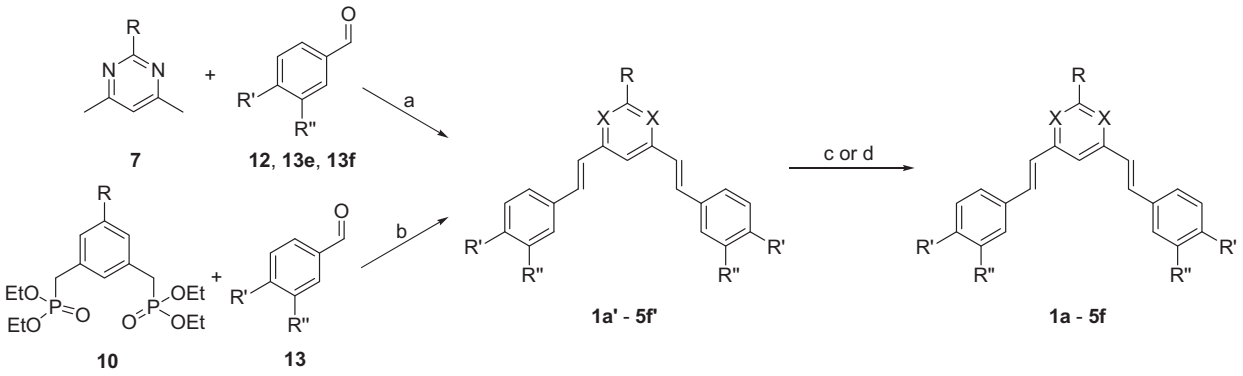
IC_{50} values of compounds with over 80% inhibition activity at 10 μM were shown in Table 2, and they had IC_{50} values in the range of 0.8–3.7 μM. Compounds **2f** and **3f** blocked fibril formation of Aβ most effectively (IC_{50} = 0.8 and 1.1 μM, respectively).

2.3. Protective effect against Aβ-induced cytotoxicity

For the further investigation of activity, we selected compounds with % inhibition over 80% and cytotoxicity under 10%, and examined a protective effect of these compounds on Aβ-induced cytotoxicity with HT22 cell line. Aβ(25–35) was used to induce Aβ cytotoxicity. The truncated fragment of Aβ is often used as a component for amyloid-induced neurotoxicity assays for drug screening. Aβ(25–35) is known to generate toxicity similar to Aβ(1–42), and causes neuronal cell death and oxidative stress in cell cultures.[17] As shown in Table 3, Aβ(25–35)-induced cytotoxicity decreased the cell viability by 44%. Compounds **1c**–**1f** and **2f** had a protective effect on Aβ-induced cytotoxicity (67–103%). In particular, Compound **1c**, which was structurally similar to the enol form of curcumin, had no cytotoxicity, and alleviated Aβ-induced cytotoxicity (103%). Compound **2f** that blocked fibril formation of Aβ in ThT assay most effectively (IC_{50} = 0.8) showed a little protective effect (67%) against Aβ compared with compounds **1c**–**1f**.

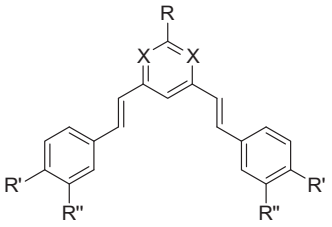
2.4. Inhibitory activity on formation of oligomeric species

We previously reported that KMS4005 (**4c**) exhibited specific interaction with non-fibrous and monomeric Aβ and contributed to the inhibitory effect on the pathogenic oligomer or fibril formation.[12] Non-effect of central ring on Aβ aggregation inhibition and structural analogousness with **4c** implies that specific interaction



Scheme 5. Reagents and conditions: (a) Bu_4NHSO_4, 5 N NaOH, reflux; (b) *t*-BuOK, THF, 0 °C; (c) EtOH, 1 N HCl, reflux; (d) TFA, CH_2Cl_2, rt.

Table 1
Inhibitory activity on Aβ fibril formation and cytotoxicity of compounds: thioflavin T (ThT)[a] and MTT assay[b]



Compd	X	R	R′	R″	% Inhibition of fibril	Viability (%)	Compd	X	R	R′	R″	% Inhibition of fibril	Viability (%)
1a	N	H	OH	H	64.3 ± 7.0	104.2 ± 7.1	**3d**	N	SMe	OMe	OH	75.2 ± 11.1	78.8 ± 9.3
1b	N	H	H	OH	14.1 ± 19.7	142.5 ± 5.6	**3e**	N	SMe	OH	NMe_2	78.6 ± 10.3	77.3 ± 15.1
1c	N	H	OH	OMe	79.3 ± 5.4	215.6 ± 3.5	**3f**	N	SMe	NMe_2	OH	88.8 ± 4.2	85.3 ± 8.1
1d	N	H	OMe	OH	80.9 ± 0.6	135.6 ± 6.1	**4a**	CH	H	OH	H	58.4 ± 6.2	102.1 ± 3.9
1e	N	H	OH	NMe_2	83.5 ± 2.6	146.2 ± 26.6	**4b**	CH	H	H	OH	−28.7 ± 8.2	47.0 ± 6.7
1f	N	H	NMe_2	OH	93.7 ± 1.8	132.8 ± 41.1	**4c**[c]	CH	H	OH	OMe	71.5 ± 1.1	74.8 ± 1.2
2a	N	OMe	OH	H	67.4 ± 7.3	101.2 ± 31.8	**4d**	CH	H	OMe	OH	67.9 ± 1.1	69.5 ± 4.2
2b	N	OMe	H	OH	−28.7 ± 11.6	45.9 ± 4.2	**4e**	CH	H	OH	NMe_2	63.9 ± 10.1	95.7 ± 8.3
2c	N	OMe	OH	OMe	68.0 ± 11.6	75.2 ± 12.5	**4f**	CH	H	NMe_2	OH	56.0 ± 8.3	100.8 ± 7.6
2d	N	OMe	OMe	OH	55.0 ± 9.2	98.4 ± 2.7	**5a**	CH	OH	OH	H	80.7 ± 1.1	37.4 ± 4.7
2e	N	OMe	OH	NMe_2	74.1 ± 7.3	96.6 ± 13.7	**5b**	CH	OH	H	OH	7.1 ± 12.4	59.2 ± 15.4
2f	N	OMe	NMe_2	OH	90.4 ± 3.7	101.4 ± 2.5	**5c**	CH	OH	OH	OMe	79.0 ± 2.4	80.9 ± 13.1
3a	N	SMe	OH	H	67.4 ± 7.4	65.3 ± 6.0	**5d**	CH	OH	OMe	OH	84.5 ± 3.8	73.5 ± 14.1
3b	N	SMe	H	OH	−53.3 ± 16.3	43.7 ± 6.6	**5e**	CH	OH	OH	NMe_2	74.2 ± 5.8	66.0 ± 12.1
3c	N	SMe	OH	OMe	67.6 ± 12.5	79.0 ± 7.9	**5f**	CH	OH	NMe_2	OH	83.3 ± 0.7	64.4 ± 4.2

[a] Aβ (25 μM) and compounds (10 μM) were incubated at rt for 1 h, before adding ThT solution.
[b] Cytotoxicity of compounds (10 μM) in HT22 cell.
[c] KMS4005.

Table 2
IC_{50} of compounds with over 80% inhibition at 10 μM concentration in ThT assay

Entry	Compd	IC_{50} (μM)
1	**1c**	3.2
2	**1d**	2.1
3	**1e**	3.7
4	**1f**	3.4
5	**2f**	0.8
6	**3f**	1.1
7	**5a**	1.6
8	**5d**	1.6
9	**5f**	3.3

Table 3
Protective effect of compounds on Aβ-induced cytotoxicity: Aβ burden assay[a]

Entry	Compd	Protective effect (%)
1	**1c**	103.0 ± 13.0
2	**1d**	91.8 ± 8.8
3	**1e**	85.3 ± 0.1
4	**1f**	76.6 ± 12.1
5	**2e**	59.0 ± 5.7
6	**2f**	67.4 ± 5.1
	Aβ(25–35)	56.0 ± 0.6

[a] Aβ(25–35) (25 μM) and compounds (10 μM) were incubated for 18 h.

of our new compounds with monomeric Aβ might induce an equilibrium shift in favor of monomers, consequently inhibiting formation of pathogenic oligomers and fibrils.

To confirm the inhibitory activity of compounds **1c**, **1d**, **1e**, and **1f** against oligomers, we further characterized the aggregation state in the presence and absence of compounds using SDS–PAGE

(sodium dodecyl sulfate polyacrylamide gel electrophoresis) and PICUP (photo-induced cross-linking of unmodified proteins) technique (Fig. 2). For this study, compounds were prepared at final concentration of 2 μM, based on their IC_{50} values (2.1–3.7 μM). In the absence of compounds, Aβ was spontaneously aggregated into high molecular weight oligomers (HMW oligomers, bands ranging from 150 to 225 kDa and discrete bands above 52 kDa) and the low molecular weight oligomers (LMW oligomers, bands below 17 kDa corresponding to dimmers, trimers, and tetramers). In the presence of compounds, inhibitory effect on the formation of HMW oligomers was observed. Compounds **1d**–**1f** prevented formation of the HMW oligomers strongly. However, compound **1c** showed weak inhibitory effect on the formation of oligomers than compounds **1d**–**1f**. It shows that there is a good relationship in ThT assay and SDS–PAGE results of compounds **1d**–**1f**.

2.5. Efficacy in acute AD animal model

Several behavioral tests were developed for learning and memory in mice model. To examine the efficacy of compounds for the animal model, behavioral tests were performed to test whether they could recover the Aβ-induced learning and memory impairment in ICR mice. Intracerebroventricular (ICV) injection of Aβ is well known to cause memory deficits.[7,18] Therefore, we used this method to evaluate efficacy of compounds for in vivo mouse model.

The experimental schedules are summarized in Fig. 3. All samples were administered via oral routes (100 mg/kg) or food pellets. After the ICV injection of Aβ(1–42), the compounds were administered for 2 days (for Y-maze and the object recognition), 3 days (for passive avoidance), or 7 days (for watermaze) to the mice, then they were subjected to the Y-maze on day 2, the object cognition task on days 2–3, the passive avoidance task on days 3–4, and the watermaze task on days 3–7.

Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task.[19] Spontaneous alternation behavior was considered to reflect spatial working memory. As shown in Fig. 5a, mice injected with Aβ(1–42) had impaired memory (62%), and recovered memory with statistical significance in the compound-treated groups (67–72%). Treating group with **1d** displayed the highest effect (72%) with a decrease of alternation behavior.

Non-spatial and long-term memory recovery activity was evaluated by time that spends to explore the object in the object recognition task.[20] Memory was assessed by measuring mice's ability to discriminate the familiarity of objects previously encountered. Acute AD mice showed reduced preference for the new object by about 17% (preference index = 0.53) compared to the control (preference index = 0.64). However, they recovered by 7–13% (preference index = 0.59–0.61, but no statistical significance) when administered with the compounds, **1c**, **1d**, and **1f** (Fig. 4b). Compound **1e** showed no effect in the object recognition task. Treating group with **1c** showed similar activity to control group (preference index = 0.61) for the new object task.

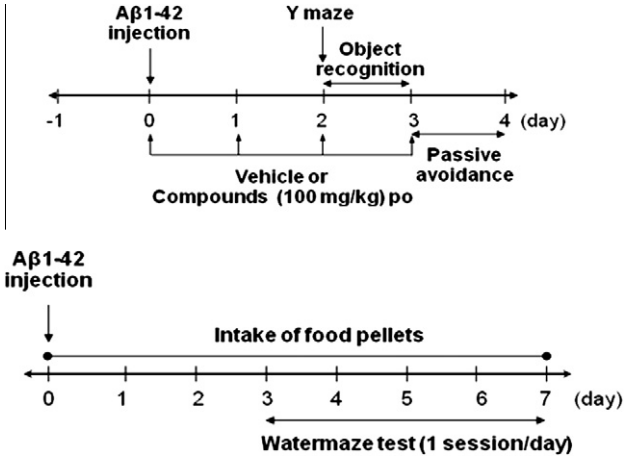


Figure 3. Experimental schedules of acute AD model.

In passive avoidance task,[21] long-term memory was assessed by measuring a natural tendency (step-through latency) of mice to enter darkened space. Aβ(1–42) injected mice showed reduced step-through latency by 53% (90 s) compared to control (190 s), but they recovered latency (118–144 s, but no statistical significance) when administered with the compounds (Fig. 4c). A group of treatment with **1d** also showed the highest effect (144 s) for step-through latency.

Results of the watermaze task designed to test spatial learning ability are depicted in Fig. 4d.[22] As seen in Fig. 4d, the escape latency of mice injected with Aβ(1–42) was longer than that of control group. When administered with compounds **1c**, **1d**, **1e**, and **1f**, the latencies of compound-treated groups were shortened than those of mice injected with Aβ(1–42) in the fourth and fifth training sessions. In the watermaze study, impairment of spatial learning abilities caused by Aβ(1–42) was restored by administration of compounds.

Taken together, the results obtained in acute AD mouse model study demonstrated that the four compounds **1c**–**f** enhanced learning and memory ability impaired by injection of Aβ(1–42) (see Fig. 4a–d). Especially, acute AD mice administrated with **1d** showed the highest improvement compared to others (**1d**, 72% vs the rest, 67–71% in Fig. 4a; **1d**, 93% vs the rest, 83–96% in Fig. 4b; **1d**, 76% vs the rest, 62–73% in Fig. 4c). From results of ThT assay, SDS–PAGE, and Aβ burden assay, compound **1d** inhibited Aβ aggregation (% inhibition of fibril formation: **1d**, 19% in Table 1) and alleviated Aβ-induced cytotoxicity (protective effect: **1d**, 91% in Table 3). It suggests that the improvement effect of **1d** on learning and memory in vivo is due to alleviation of cytotoxicity by inhibiting Aβ aggregation. As shown in Fig. 5, **1d** also showed learning and memory improvement effects in a dose-dependent manner over 50–200 mg/kg po, suggesting that these improvement effects came from administration of **1d**. Administration of **1d** could prevent cognitive deficits at the lower dose than curcumin (500 mg/kg curcumin chow) and ferulic acid (daily 14–19 mg/kg for 2–4 weeks).[7,23]

3. Conclusion

Aβ aggregates play crucial roles in AD pathogenesis and blockade of Aβ aggregation is a promising target. It is reported that soluble oligomeric intermediates are more toxic than fully formed mature amyloid fibrils and are recognized as a key pathogen in AD.[3,24] To reduce Aβ aggregation and alleviate toxicity, a number of small molecules and peptides that specifically focused on soluble and toxic oligomers were reported.[4e,25,26] We synthesized new compounds and evaluated their inhibitory ability and protective effect on Aβ aggregates. We showed that compounds, especially **1c**, **1d**, **1e**, and **1f**, can inhibit Aβ aggregation, reduce Aβ-induced cytotoxicity in vitro, and attenuate the Aβ-induced learning and memory impairment in acute AD mouse model. Among them, **1d** is most effective, and a promising drug candidate to inhibit fibril formation of Aβ for treatment and prevention of AD.

4. Experimental section

4.1. General methods

All chemicals were obtained from commercial supplies and used without further purification. Solvents were distilled (THF

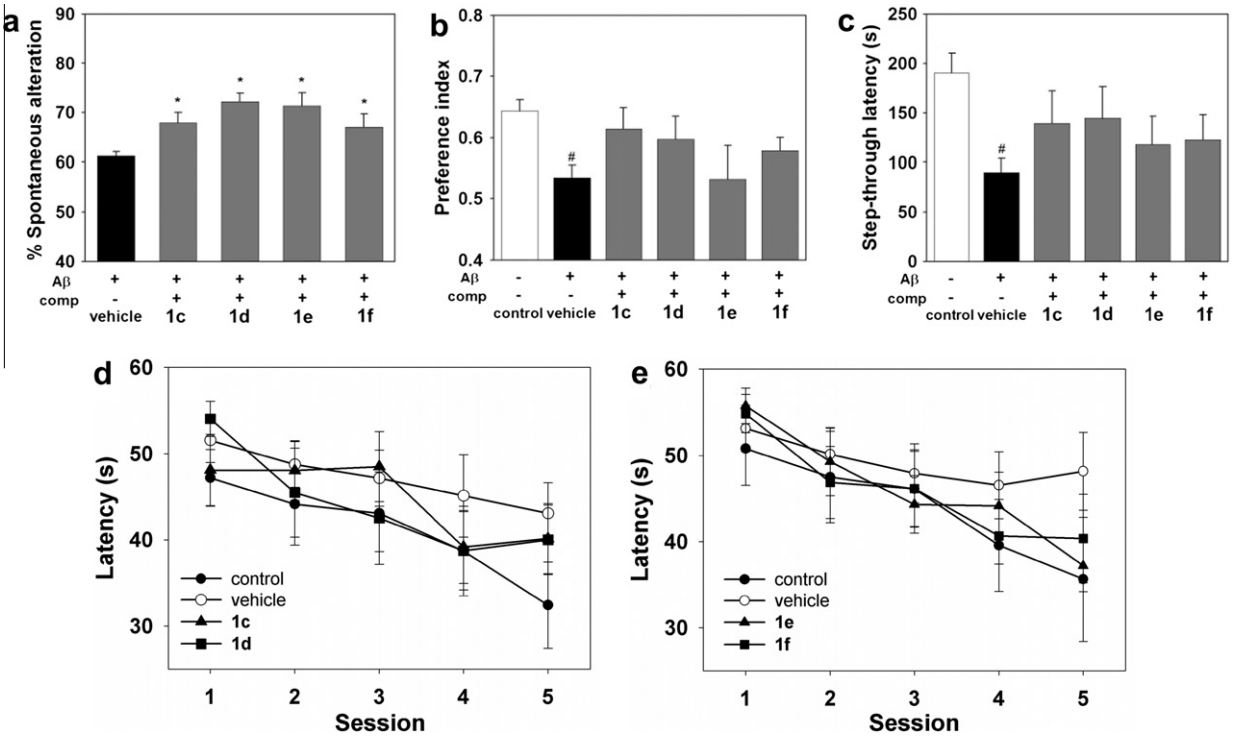


Figure 4. Recovery effect of **1c**, **1d**, **1e,** and **1f** on the Aβ(1–42)-induced impairment in learning and memory ability of acute AD mice. After the injection of aggregated Aβ(1–42) (10 nM), mice (n = 10) was administered with the compounds of **1c**, **1d**, **1e**, and **1f** (100 mg/kg po). The data are expressed as mean ± SEM. (a) Y-maze task. (b) Object recognition task. (c) Passive avoidance task. (d) Watermaze task. $^{*}t$-test, P <0.05, $^{\#}t$-test, P <0.05.

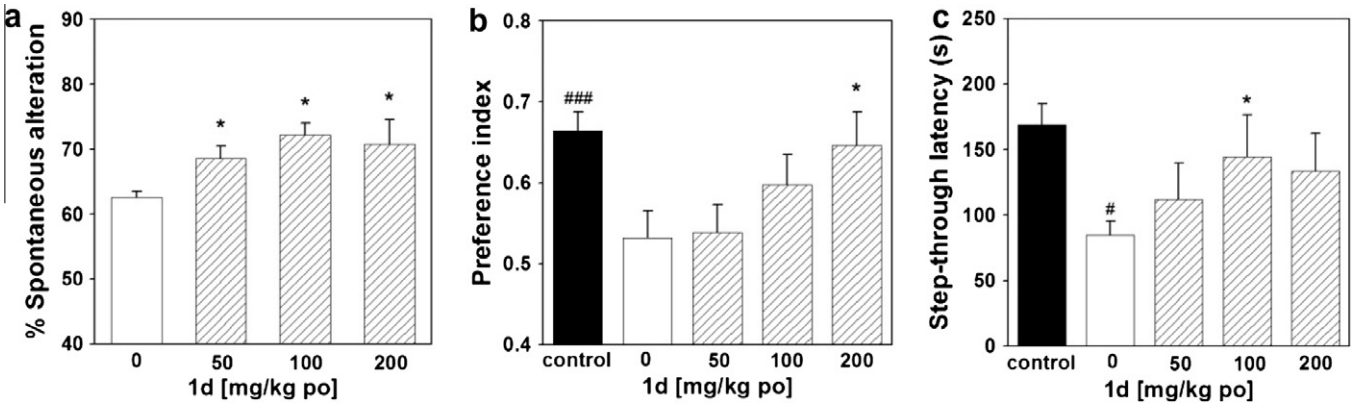


Figure 5. Dose dependence of **1d**. After the injection of aggregated Aβ(1–42) (10 nM), mice (n = 10) was administered with the various doses of **1d**. The data are expressed as mean ± SEM. (a) Y-maze task. (b) Object recognition task. (c) Passive avoidance task. $^{*}t$-test, P <0.05, $^{\#}t$-test, P <0.05.

from Na/Benzophenone; CH_2Cl_2 from CaH_2). Flash column chromatography was performed with Silica Gel 60 (40–63 μm, 230–400 mesh, Merck). Thin layer chromatography (TLC) was performed Silica Gel 60 F_{254} precoated plates (0.25 mm thickness, Merck, Darmstadt). All ^{1}H and ^{13}C NMR spectra were recorded on a Brucker Avance 400 spectrometer. Chemical shifts were reported in part per million relative to internal tetramethylsilane (TMS). All ^{13}C NMR spectra were recorded with complete proton decoupling.

4.2. Synthesis procedures and spectral data

4.2.1. General procedure A

Preparation of **1a′**–**3f′** and **1g**–**5g**. A mixture of pyrimidines (2.00 mmol) and benzaldehydes (4.00 mmol) in 5 N NaOH solution (20 mL) containing tetrabutylammonium hydrogen sulfate (0.10 g, 0.29 mmol) was refluxed. After cooling, the precipitates were filtered off. The products were purified by recrystallization from EtOAc. Otherwise, the mixture was extracted with CH_2Cl_2. Then, the organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography to give the products.

4.2.2. General procedure B

Preparation of **4a′**–**5f′**. To a stirred solution of 1,3-bis(diethylphosphonomethyl)benzene or 3,5-bis(diethylphosphonomethyl)phenol (2.00 mmol) in dry THF (50 mL) was added benzaldehydes (4.00 mmol). Then, potassium *tert*-butoxide was added at 0 °C and the mixture was stirred for 2 h at the same temperature. The excess H_2O was added into the mixture. If the products precipitated, it was collected by filtration. Otherwise, the

mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography to give the products.

4.2.3. (*E*,*E*)-4,6-Bis[4′-(4″-methoxybenzyloxy)styryl]pyrimidine (1a′)

Compound **1a′** was prepared from 4,6-dimethylpyrimidine and **12a** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **1a′** (0.93 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.85 (d, J = 15.9 Hz, 2H), 7.56 (d, J = 8.7 Hz, 4H), 7.37 (d, J = 8.7 Hz, 4H), 7.22 (d, J = 0.9 Hz, 1H), 7.00 (d, J = 8.7 Hz, 4H), 6.94 (d, J = 15.9 Hz, 2H), 6.93 (d, J = 8.7 Hz, 4H), 5.04 (s, 4H), 3.83 (s, 6H).

4.2.4. (*E*,*E*)-4,6-Bis[3′-(4″-methoxybenzyloxy)styryl]pyrimidine (1b′)

Compound **1b′** was prepared from 4,6-dimethylpyrimidine and **12b** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **1b′** (0.84 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (d, J = 0.7 Hz, 1H), 7.89 (d, J = 16.0 Hz, 2H), 7.66 (d, J = 0.8 Hz, 1H), 7.41–7.27 (m, 12H), 7.01 (dd, J = 1.3, 7.9 Hz, 2H), 6.94 (d, J = 8.6 Hz, 4H), 5.07 (s, 4H), 3.74 (s, 6H).

4.2.5. (*E*,*E*)-4,6-Bis[3′-methoxy-4′-(4″-methoxybenzyloxy)styryl]pyrimidine (1c′)

Compound **1c′** was prepared from 4,6-dimethylpyrimidine and **12c** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **1c′** (1.0 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (d, J = 0.8 Hz, 1H), 7.81 (d, J = 15.9 Hz, 2H), 7.37 (d, J = 8.6 Hz, 5H), 7.16 (d, J = 1.7 Hz, 2H), 7.11 (dd, J = 8.3, 1.7 Hz, 2H), 6.93 (d, J = 15.9 Hz, 2H), 6.91 (d, J = 8.3 Hz, 2H), 6.91 (d, J = 8.6 Hz, 4H), 5.12 (s, 4H), 3.93 (s, 6H), 3.80 (s, 6H).

4.2.6. (*E*,*E*)-4,6-Bis[4′-methoxy-3′-(4″-methoxybenzyloxy)styryl]pyrimidine (1d′)

Compound **1d′** was prepared from 4,6-dimethylpyrimidine and **12d** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **1d′** (1.0 g, 81%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 7.78 (d, J = 15.9 Hz, 2H), 7.40 (d, J = 8.5 Hz, 4H), 7.22 (s, 1H), 7.19 (s, 2H), 7.18 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.5 Hz, 4H), 6.91 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 15.9 Hz, 2H), 5.12 (s, 4H), 3.91 (s, 6H), 3.81 (s, 6H).

4.2.7. (*E*,*E*)-4,6-Bis(4′-methoxymethoxy-3′-*N*,*N*-dimethylaminostyryl)pyrimidine (1e′)

Compound **1e′** was prepared from 4,6-dimethylpyrimidine and **13e** according to general procedure A. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 2:1) to give **1e′** (0.51 g, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (d, J = 1.0 Hz, 1H), 7.87 (d, J = 15.9 Hz, 2H), 7.67 (d, J= 1.0 Hz, 1H), 7.24 (dd, J = 8.9, 1.9 Hz, 2H), 7.23 (d, J = 1.9 Hz, 2H), 7.11 (d, J = 15.9 Hz, 2H), 7.05 (d, J = 8.9 Hz, 2H), 5.24 (s, 4H), 3.42 (s, 6H), 2.77 (s, 12H).

4.2.8. (*E*,*E*)-4,6-Bis(3′-methoxymethoxy-4′-*N*,*N*-dimethylaminostyryl)pyrimidine (1f′)

Compound **1f′** was prepared from 4,6-dimethylpyrimidine and **13f** according to general procedure A. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1.5:1) to give **1f′** (0.42 g, 43%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (s, 1H), 7.82 (d, J = 15.9 Hz, 2H), 7.66 (s, 1H), 7.35 (d, J = 1.8 Hz, 2H), 7.26 (dd, J = 8.3, 1.8 Hz, 2H), 7.03 (d, J = 15.9 Hz, 2H), 6.90 (d, J = 8.3 Hz, 2H), 5.26 (s, 4H), 3.45 (s, 6H), 2.80 (s, 12H).

4.2.9. 2-Methoxy-(*E*,*E*)-4,6-bis[4′-(4″-methoxybenzyloxy)styryl]pyrimidine (2a′)

Compound **2a′** was prepared from **7a** and **12a** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **2a′** (0.57 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 15.9 Hz, 2H), 7.65 (d, J = 8.7 Hz, 4H), 7.38 (d, J = 8.6 Hz, 4H), 7.23 (s, 1H), 7.07 (d, J = 15.9 Hz, 2H), 7.05 (d, J = 8.7 Hz, 4H), 6.94 (d, J = 8.6 Hz, 4H), 5.06 (s, 4H), 3.97 (s, 3H), 3.75 (s, 6H).

4.2.10. 2-Methoxy-(*E*,*E*)-4,6-bis[3′-(4″-methoxybenzyloxy)styryl]pyrimidine (2b′)

Compound **2b′** was prepared from **7a** and **12b** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **2b′** (0.53 g, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, J = 15.9 Hz, 2H), 7.38 (d, J = 8.6 Hz, 4H), 7.31 (dd, J = 8.0, 8.0 Hz, 2H), 7.21–7.20 (m, 4H), 7.02–6.91 (m, 9H), 5.03 (s, 4H), 4.11 (s, 3H), 3.82 (s, 6H).

4.2.11. 2-Methoxy-(*E*,*E*)-4,6-bis[3′-methoxy-4′-(4″-methoxybenzyloxy)styryl]pyrimidine (2c′)

Compound **2c′** was prepared from **7a** and **12c** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **2c′** (0.73 g. 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (d, J = 15.9 Hz, 2H), 7.37 (d, J = 8.6 Hz, 4H), 7.35 (d, J = 1.8 Hz, 2H), 7.21 (dd, J = 8.4, 1.8 Hz, 2H), 7.12 (d, J = 15.9 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.6 Hz, 4H), 5.04 (s, 4H), 3.98 (s, 3H), 3.83 (s, 6H), 3.75 (s, 6H).

4.2.12. 2-Methoxy-(*E*,*E*)-4,6-bis[4′-methoxy-3′-(4″-methoxybenzyloxy)styryl]pyrimidine (2d′)

Compound **2d′** was prepared from **7a** and **12d** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **2d′** (0.71 g, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (d, J = 15.9 Hz, 2H), 7.46 (d, J = 1.6 Hz, 2H), 7.41 (d, J = 8.6 Hz, 4H), 7.25 (s, 1H), 7.24 (dd, J = 8.4, 1.6 Hz, 2H), 7.12 (d, J = 15.9 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 8.6 Hz, 4H), 5.08 (s, 4H), 3.98 (s, 3H), 3.79 (s, 6H), 3.75 (s, 6H).

4.2.13. 2-Methoxy-(*E*,*E*)-4,6-bis(4′-methoxymethoxy-3′-*N*,*N*-dimethylaminostyryl)pyrimidine (2e′)

Compound **2e′** was prepared from **7a** and **13e** according to general procedure A. The crude product was purified by flash column chromatography (MeOH/CH_2Cl_2 = 1:50) to give **2e′** (0.58 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 15.8 Hz, 2H), 7.20–7.13 (m, 6H), 6.93 (s, 1H), 6.91 (d, J = 15.9 Hz, 2H), 5.30 (s, 4H), 4.12 (s, 3H), 3.54 (s, 6H), 2.86 (s, 12H).

4.2.14. 2-Methoxy-(*E*,*E*)-4,6-bis(3′-methoxymethoxy-4′-*N*,*N*-dimethylaminostyryl)pyrimidine (2f′)

Compound **2f′** was prepared from **7a** and **13f** according to general procedure A. The crude product was purified by flash column chromatography (MeOH/CH_2Cl_2 = 1:50) to give **2f′** (0.53 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 15.8 Hz, 2H), 7.39 (s, 2H), 7.21 (d, J = 8.1 Hz, 2H), 6.92 (d, J = 8.1 Hz, 2H), 6.91 (s, 1H), 6.89 (d, J = 15.8 Hz, 2H), 5.29 (s, 4H), 4.10 (s, 3H), 3.56 (s, 6H), 2.86 (s, 12H).

4.2.15. 2-Methylthio-(*E*,*E*)-4,6-bis[4′-(4″-methoxybenzyloxy)styryl]pyrimidine (3a′)

Compound **3a′** was prepared from **7b** and **12a** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **3a′** (0.84 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (d, J = 15.9 Hz, 2H), 7.65 (d, J = 8.7 Hz, 4H), 7.37 (d, J = 8.6 Hz, 4H), 7.27 (s, 1H), 7.05 (d, J = 8.7 Hz, 4H), 7.05 (d, J = 15.9 Hz, 2H), 6.94 (d, J = 8.6 Hz, 4H), 5.06 (s, 4H), 3.74 (s, 6H), 2.58 (s, 3H).

4.2.16. 2-Methylthio-(*E*,*E*)-4,6-bis[3′-(4″-methoxybenzyloxy) styryl]pyrimidine (3b′)

Compound **3b′** was prepared from **7b** and **12b** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **3b′** (0.79 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 15.9 Hz, 2H), 7.38 (d, J = 8.6 Hz, 4H), 7.31 (dd, J = 8.1, 8.1 Hz, 2H), 7.21–7.19 (m, 4H), 6.99 (d, J = 15.9 Hz, 2H), 6.99–6.95 (m, 3H), 6.93 (d, J = 8.6 Hz, 4H), 5.04 (s, 4H), 3.83 (s, 6H), 2.68 (s, 3H).

4.2.17. 2-Methylthio-(*E*,*E*)-4,6-bis[3′-methoxy-4′-(4″-methoxybenzyloxy)styryl]pyrimidine (3c′)

Compound **3c′** was prepared from **7b** and **12c** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **3c′** (1.20 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 15.9 Hz, 2H),7.37 (d, J = 7.4 Hz, 5H), 7.14 (s, 2H), 7.09 (d, J = 8.2 Hz, 2H), 6.92–6.84 (m, 8H), 5.12 (s, 4H), 3.93 (s, 6H), 3.81 (s, 6H), 2.67 (s, 3H).

4.2.18. 2-Methylthio-(*E*,*E*)-4,6-bis[4′-methoxy-3′-(4″-methoxybenzyloxy)styryl]pyrimidine (3d′)

Compound **3d′** was prepared from **7b** and **12d** according to general procedure A. The crude product was purified by crystallization from EtOAc to give **3d′** (1.24 g, 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (d, J = 15. 9 Hz, 2H), 7.47 (d, J = 1.6 Hz, 2H), 7.40 (d, J = 8.6 Hz, 4H), 7.29 (s, 1H), 7.25 (dd, J = 8.3, 1.6 Hz, 2H), 7.10 (d, J = 15.9 Hz, 2H), 7.01 (d, J = 8.3 Hz, 2H), 6.95 (d, J = 8.6 Hz, 4H), 5.07 (s, 4H), 3.78 (s, 6H), 3.74 (s, 6H), 2.60 (s, 3H).

4.2.19. 2-Methythio-(*E*,*E*)-4,6-bis(4′-methoxymethoxy-3′-*N*,*N*-dimethylaminostyryl)pyrimidine (3e′)

Compound **3e′** was prepared from **7b** and **13e** according to general procedure A. The crude product was purified by flash column chromatography (THF/*n*-hexane = 1:1) to give **3e′** (0.47 g, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 15.9 Hz, 2H), 7.20–7.13 (m, 6H), 6.97 (s, 1H), 6.89 (d, J = 15.9 Hz, 2H), 3.54 (s, 6H), 2.86 (s, 12H), 2.69 (s, 3H).

4.2.20. 2-Methylthio-(*E*,*E*)-4,6-bis(3′-methoxymethoxy-4′-*N*,*N*-dimethylaminostyryl)pyrimidine (3f′)

Compound **3f′** was prepared from **7b** and **13f** according to general procedure A. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:2) to give **3f′** (0.54 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 15.9 Hz, 2H), 7.39 (d, J = 1.6 Hz, 2H), 7.21 (dd, J = 8.3, 1.6 Hz, 2H), 6.95–6.86 (m, 4H), 5.30 (s, 4H), 3.56 (s, 6H), 2.89 (s, 12H), 2.68 (s, 3H).

4.2.21. (*E*,*E*)-3,5-Bis(4′-methoxymethoxystyryl)benzene (4a′)

Compound **4a′** was prepared from **10a** and **13a** according to general procedure B. The crude product was purified by crystallization from CH_2Cl_2/*n*-hexane (1:8) solution to give **4a′** (0.50 g, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (s, 1H), 7.54 (d, J = 8.6 Hz, 4H), 7.43–7.31 (m, 3H), 7.26 (d, J = 16.4 Hz, 2H), 7.11 (d, J = 16.4 Hz, 2H), 7.03 (d, J = 8.6 Hz, 4H), 5.20 (s, 4H), 3.37 (s, 6H).

4.2.22. (*E*,*E*)-3,5-Bis(3′-methoxymethoxystyryl)benzene (4b′)

Compound **4b′** was prepared from **10a** and **13b** according to general procedure B. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:5) to give **4b′** (0.70 g, 88%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (s, 1H), 7.50–7.23 (m, 13H), 6.93 (dd, J = 7.7, 1.3 Hz, 2H), 5.23 (s, 4H), 3.39 (s, 6H).

4.2.23. (*E*,*E*)-3,5-Bis(3′-methoxy-4′-methoxmethoxystyryl) benzene (4c′)

Compound **4c′** was prepared from **10a** and **13c**according to the general procedure B to give **4c′** (0.77 g, 84%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.44–7.33 (m, 3H), 7.28 (s, 2H), 7.26 (d, J = 16.4 Hz, 2H), 7.17 (d, J = 16.4 Hz, 2H), 7.09 (dd, J = 8.3, 1.5 Hz, 2H), 7.05 (d, J = 8.3 Hz, 2H), 5.15 (s, 4H), 3.84 (s, 6H), 3.38 (s, 6H).

4.2.24. (*E*,*E*)-3,5-Bis(4′-methoxy-3′-methoxmethoxystyryl) benzene (4d′)

Compound **4d′** was prepared from **10a** and **13d** according to the general procedure B to give**4d′** (0.69 g, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (s, 1H), 7.34–7.32 (m, 5H), 7.23 (d, J = 16.4 Hz, 2H), 7.21 (dd, J = 8.5, 1.9 Hz, 2H), 7.07 (d, J = 16.4 Hz, 2H), 7.01 (d, J= 8.5 Hz, 2H), 5.20 (s, 4H), 3.78 (s, 6H), 3.41 (s, 6H).

4.2.25. (*E*,*E*)-3,5-Bis(4′-methoxymethoxy-3′-*N*,*N*-dimethylaminostyryl)benzene (4e′)

Compound **4e′** was prepared from **10a** and **13e** according to general procedure B. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:1) to give **4e′** (0.68 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.43–7.32 (m, 3H), 7.24 (d, J = 16.4 Hz, 2H), 7.14 (d, J = 1.8 Hz, 2H), 7.11 (dd, J = 8.2, 1.8 Hz, 2H), 7.10 (d, J = 16.4 Hz, 2H), 7.00 (d, J = 8.2 Hz, 2H), 5.19 (s, 4H), 3.41 (s, 6H), 2.75 (s, 12H).

4.2.26. (*E*,*E*)-3,5-Bis(3′-methoxymethoxy-4′-*N*,*N*-dimethylaminostyryl)benzene (4f′)

Compound **4f′** was prepared from **10a** and **13f** according to general procedure B (0.88 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (s, 1H), 7.41–7.29 (m, 3H), 7.26 (d, J = 1.7 Hz, 2H), 7.22 (d, J = 16.3 Hz, 2H), 7.16 (dd, J = 8.2, 1.7 Hz, 2H), 7.04 (d, J = 16.3 Hz, 2H), 6.88 (d, J = 8.2 Hz, 2H), 5.24 (s, 4H), 3.44 (s, 6H), 2.74 (s, 12H).

4.2.27. (*E*,*E*)-3,5-Bis(4′-methoxymethoxystyryl)phenol (5a′)

Compound **5a′** was prepared from **10b** and **13a** according to general procedure B. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:2) to give **5a′** (0.47 g, 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.45 (s, 1H), 7.53 (d, J = 8.7 Hz, 4H), 7.23 (s, 1H), 7.15 (d, J = 16.3 Hz, 2H), 7.02 (d, J = 8.7 Hz, 4H), 7.02 (d, J = 16.3 Hz, 2H), 6.83 (d, J = 1.1 Hz, 2H), 5.19 (s, 4H).

4.2.28. (*E*,*E*)-3,5-Bis(3′-methoxymethoxystyryl)phenol (5b′)

Compound **5b′** was prepared from **10b** and **13b** according to general procedure B. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:2) to give **5b′** (0.45 g, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (br s, 1H), 7.32–7.22 (m, 7H), 7.17 (d, J = 1.5 Hz, 4H), 6.93–6.91 (m, 2H), 6.88 (d, J = 1.1 Hz, 2H), 5.22 (s, 4H), 3.38 (s, 6H).

4.2.29. (*E*,*E*)-3,5-Bis(3′-methoxy-4-methoxymethoxystyryl) phenol (5c′)

Compound **5c′** was prepared from **10b** and **13c** according to general procedure B. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:1) to give **5c′** (0.61 g, 64%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26 (d, J = 1.4 Hz, 3H), 7.14 (d, J = 16.3 Hz, 2H), 7.08 (dd, J = 8.4, 1.7 Hz, 2H), 7.07 (d, J = 16.3 Hz, 2H), 7.04 (d, J = 8.4 Hz, 2H), 6.83 (d, J = 1.1 Hz, 2H), 5.14 (s, 4H), 3.83 (s, 6H), 3.38 (s, 6H).

4.2.30. (*E*,*E*)-3,5-Bis(4′-methoxy-3′-methoxymethoxystyryl) phenol (5d′)

Compound **5d′** was prepared from **10b** and **13d** according to general procedure B. The crude product was purified by crystallization from EtOAc/*n*-hexane (1:3) to give **5d′** (0.49 g, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 7.32 (d, J = 1.8 Hz, 2H), 7.25 (s, 1H), 7.19 (dd, J = 8.4, 1.8 Hz, 2H), 7.12 (d, J = 16.3 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 16.3 Hz, 2H), 6.81 (d, J = 0.8 Hz, 2H), 5.19 (s, 4H), 3.78 (s, 6H), 3.41 (s, 6H).

4.2.31. (*E*,*E*)-3,5-Bis(4′-methoxymethoxy-3′-*N*,*N*-dimethylaminostyryl)phenol (5e′)

Compound **5e′** was prepared from **10b** and **13e** according to general procedure B. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:2) to give **5e′** (0.44 g, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.44 (s, 1H), 7.28 (s, 1H), 7.16–7.00 (m, 10H), 6.83 (d, J = 0.9 Hz, 2H), 5.20 (s, 4H), 3.42 (s, 6H), 2.76 (s, 12H).

4.2.32. (*E*,*E*)-3,5-Bis(3′-methoxymethoxy-4′-*N*,*N*-dimethylaminostyryl)phenol (5f′)

Compound **5f′** was prepared from **10b** and **13f** according to general procedure B. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:2) to give **5f′** (0.69 g, 69%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 7.24 (s, 1H), 7.24 (d, J = 1.8 Hz, 2H), 7.14 (dd, J = 8.3, 1.8 Hz, 2H), 7.12 (d, J = 16.3 Hz, 2H), 6.94 (d, J = 16.3 Hz, 2H), 6.87 (d, J = 8.3 Hz, 2H), 6.79 (d, J = 1.0 Hz, 2H), 5.24 (s, 4H), 3.43 (s, 6H), 2.74 (s, 12H).

4.2.33. General procedure C

Removal of *p*-methoxybenzyl and methoxymethyl protecting groups in **1a′**–**5f′** for the preparation of **1a**–**5f**. A solution of **1a′**–**5f′** in EtOH/1 N HCl (3:1) was refluxed. When the reaction was completed, the reaction mixture was allowed to cool to room temperature and excess H_2O was added. (Compounds containing dimethylamino group were neutralized with $NaHCO_3$ before adding H_2O) If the product precipitated, it was collected by filtration. Otherwise, the reaction mixture was extracted with EtOAc. The organic layer was washed with brine and concentrated in vacuo. The residue was purified by recrystallization or flash column chromatography to give the products.

4.2.34. General procedure D

Removal of *p*-methoxybenzyl and methoxymethyl protecting groups in **1a′**–**5f′** for preparation of **1a**–**5f**. To a stirred solution of **1a′**–**5f′** in CH_2Cl_2 was added trifluoroacetic acid (TFA) and the mixture was stirred at room temperature for 1–2 h. When the reaction was complete, CH_2Cl_2 and trifluoroacetic acid was evaporated. The residue was purified by recrystallization or flash column chromatography to give the products.

4.2.35. (*E*,*E*)-4,6-Bis-(4′-hydroxystyryl)pyrimidine (1a)

Compound **1a′** (1.00 g, 1.80 mmol) was deprotected in EtOH/1 N HCl (80 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **1a** (0.55 g, 96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (br s, 2H), 8.99 (s, 1H), 7.90 (d, J = 16.0 Hz, 2H), 7.67 (s, 1H), 7.58 (d, J = 8.6 Hz, 4H), 7.06 (d, J = 16.0 Hz, 2H), 6.84 (d, J = 8.6 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1, 160.6, 154.2, 141.3, 130.7, 126.4, 119.9, 116.5, 115.3. HRMS: calcd for $C_{20}H_{16}N_2O_2 + H^+$, 317.1290; found (ESI, $[M+H]^+$), 317.1289. Mp 292 °C.

4.2.36. (*E*,*E*)-4,6-Bis-(3′-hydroxystyryl)pyrimidine (1b)

Compound **1b′** (0.90 g, 1.60 mmol) was deprotected in EtOH/1 N HCl (48 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **1b** (0.39 g, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (br s, 2H), 9.04 (d, J = 0.7 Hz, 1H), 7.84 (d, J = 16.0 Hz, 2H), 7.74 (d, J = 0.8 Hz, 1H), 7.25–7.13 (m, 6H), 7.07 (d, J = 1.7 Hz, 2H), 6.79 (dd, J = 7.9, 1.7 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.7, 158.3, 158.2, 137.7, 137.1, 130.4, 125.9, 119.2, 117.2, 116.8, 114.5. HRMS: calcd for $C_{20}H_{16}N_2O_2 + H^+$, 317.1290; found (ESI, $[M+H]^+$), 317.1289. Mp 218 °C.

4.2.37. (*E*,*E*)-4,6-Bis-(4′-hydroxy-3′-methoxystyryl)pyrimidine (1c)

Compound **1c′** (1.06 g, 1.70 mmol) was deprotected in EtOH/1 N HCl (48 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **1c** (0.46 g, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (br s, 2H), 8.95 (s, 1H), 7.83 (d, J = 16.0 Hz, 2H), 7.57 (s, 1H), 7.32 (d, J = 1.6 Hz, 2H), 7.13 (dd, J = 8.1, 1.6 Hz, 2H), 7.09 (d, J = 16.0 Hz, 2H), 6.82 (d, J = 8.1 Hz, 2H), 3.84 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 157.9, 149.1, 148.4, 138.0, 127.4, 122.8, 122.7, 116.1, 115.6, 111.3, 56.1. HRMS: calcd for $C_{22}H_{20}N_2O_4 + H^+$, 377.1501; found (ESI, $[M+H]^+$), 377.1496. Mp 242 °C.

4.2.38. (*E*,*E*)-4,6-Bis-(3′-hydroxy-4′-methoxystyryl)pyrimidine (1d)

Compound **1d′** (1.06 g, 1.70 mmol) was deprotected in EtOH/1 N HCl (48 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **1d** (0.60 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (s, 2H), 8.95 (d, J = 1.0 Hz, 1H), 7.78 (d, J = 15.9 Hz, 2H), 7.62 (d, J = 1.0 Hz, 1H), 7.13 (d, J = 1.9 Hz, 2H), 7.11 (dd, J = 8.3, 1.9 Hz, 2H), 6.98 (d, J = 15.9 Hz, 2H), 6.97 (d, J = 8.3 Hz, 2H), 3.80 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 158.6, 149.7, 147.2, 137.0, 128.9, 123.9, 120.8, 116.1, 114.1, 112.5, 56.0. HRMS: calcd for $C_{22}H_{20}N_2O_4 + H^+$, 377.1501; found (ESI, $[M+H]^+$), 377.1503. Mp 202 °C.

4.2.39. (*E*,*E*)-4,6-Bis-(4′-hydroxy-3′-*N*,*N*-dimethylaminostyryl) pyrimidine (1e)

Compound **1e′** (0.51 g, 1.00 mmol) was deprotected in EtOH/1 N HCl (48 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **1e** (0.31 g, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.62 (br s, 2H), 8.93 (s, 1H), 7.81 (d, J = 15.9 Hz, 2H), 7.59 (s, 1H), 7.19 (d, J = 1.8 Hz, 2H), 7.16 (dd, J = 8.1, 1.8 Hz, 2H), 7.01 (d, J = 15.9 Hz, 2H), 6.81 (d, J = 8.1 Hz, 2H), 2.73 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.0, 158.6, 152.1, 141.7, 137.4, 127.4, 123.1, 123.0, 117.7, 116.3, 115.6, 43.0. HRMS: calcd for $C_{24}H_{26}N_4O_2 + H^+$, 403.2134; found (ESI, $[M+H]^+$), 403.2133. Mp 129 °C.

4.2.40. (*E*,*E*)-4,6-Bis-(3′-hydroxy-4′-*N*,*N*-dimethylaminostyryl) pyrimidine (1f)

Compound **1f′** (0.43 g, 0.87 mmol) was deprotected in EtOH/1 N HCl (48 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **1f** (0.28 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (br s, 2H), 8.93 (s, 1H), 7.76 (d, J = 15.9 Hz, 2H), 7.63 (s, 1H), 7.07 (s, 2H), 7.06 (d, J = 8.0 Hz, 2H), 6.91 (d, J = 15.9 Hz, 2H), 6.83 (d, J = 8.0 Hz, 2H), 2.75 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 158.6, 149.9, 143.0, 137.0, 129.1, 123.3, 120.7, 118.1, 115.8, 114.2, 42.6. HRMS: calcd for $C_{24}H_{26}N_4O_2 + H^+$, 403.2134; found (ESI, $[M+H]^+$), 403.2132. Mp 189 °C.

4.2.41. (*E*,*E*)-4,6-Bis-styrylpyrimidine (1g)

Compound **1g** was prepared from 4,6-dimethylpyrimidine and benzaldehyde according to general procedure A. The crude product was purified by recrystallization from EtOAc to give **1g** (0.43 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.91 (d, J = 15.9 Hz, 2H), 7.63–7.61 (m, 4H), 7.43–7.34 (m, 6H), 7.30 (s, 1H), 7.08 (d, J = 15.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 158.7, 137.0, 135.7, 129.4, 128.9, 127.6, 125.8, 116.4.

4.2.42. 2-Methoxy-(*E*,*E*)-4,6-bis-(4′-hydroxystyryl)pyrimidine (2a)

Compound **2a′** (0.57 g, 0.97 mmol) was deprotected with TFA (6 mL) in CH_2Cl_2 (60 mL) according to the general procedure D. The crude product was purified by recrystallization from EtOAc to give **2a** (0.17 g, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (br s, 2H), 7.79 (d, J = 15.9 Hz, 2H), 7.54 (d, J = 8.5 Hz, 4H), 7.20 (s, 1H), 6.98 (d, J = 15.9 Hz, 2H), 6.81 (d, J = 8.5 Hz, 4H), 3.96 (s, 3H);

^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 165.0, 159.5, 137.4, 130.0, 126.9, 122.6, 116.3, 110.5, 54.6. HRMS: calcd for $C_{21}H_{18}N_2O_3 + H^+$, 347.1396; found (ESI, $[M+H]^+$), 347.1393. Mp 162 °C.

4.2.43. 2-Methoxy-(*E*,*E*)-4,6-bis-(3′-hydroxystyryl)pyrimidine (2b)

Compound **2b′** (0.10 g, 0.17 mmol) was deprotected in EtOH/1 N HCl (8 mL) according to the general procedure C. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:2) to give **2b** (0.02 g, 34%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (br s, 2H), 7.79 (d, J = 15.9 Hz, 2H), 7.37 (s, 1H), 7.22 (dd, J = 7.8, 7.8 Hz, 2H), 7.12 (d, J = 6.0 Hz, 2H), 7.11 (d, J = 15.9 Hz, 2H), 7.05 (s, 2H), 7.78 (dd, J = 6.0, 1.8 Hz, 2H), 3.98 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.6, 165.2, 158.2, 137.1, 137.0, 130.4, 126.3, 119.2, 117.1, 114.4, 111.5, 54.6. HRMS: calcd for $C_{21}H_{18}N_2O_3 + H^+$, 347.1396; found (ESI, $[M+H]^+$), 347.1394. Mp 229 °C.

4.2.44. 2-Methoxy-(*E*,*E*)-4,6-bis-(4′-hydroxy-3′-methoxystyryl) pyrimidine (2c)

Compound **2c′** (0.63 g, 0.97 mmol) was deprotected with TFA (7 mL) in CH_2Cl_2 (70 mL) according to the general procedure D. The crude product was purified by recrystallization from EtOAc to give **2c** (0.28 g, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (br s, 2H), 7.79 (d, J = 15.9 Hz, 2H), 7.30 (d, J = 1.7 Hz, 2H), 7.22 (s, 1H), 7.12 (dd, J = 8.1, 1.7 Hz, 2H), 7.04 (d, J = 15.9 Hz, 2H), 6.81 (d, J = 8.1 Hz, 2H), 3.97 (s, 3H), 3.84 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.2, 164.9, 149.0, 148.4, 138.0, 127.4, 122.8, 122.6, 116.1, 111.3, 110.3, 56.0, 54.7. HRMS: calcd for $C_{23}H_{22}N_2O_5 + H^+$, 407.1607; found (ESI, $[M+H]^+$), 407.1606. Mp 121 °C.

4.2.45. 2-Methoxy-(*E*,*E*)-4,6-bis-(3′-hydroxy-4′-methoxystyryl) pyrimidine (2d)

Compound **2d′** (0.22 g, 0.03 mmol) was deprotected with TFA (1.5 mL) in CH_2Cl_2 (15 mL) according to the general procedure D. The crude product was purified by column chromatography ($MeOH/CH_2Cl_2$ = 1:50) to give **2d** (0.11 g, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (br s, 2H), 7.75 (d, J = 15.9 Hz, 2H), 7.28 (s, 1H), 7.13–7.09 (m, 4H), 6.98–6.92 (m, 4H), 3.96 (s, 3H), 3.80 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.5, 165.3, 149.7, 147.2, 137.1, 128.8, 123.8, 120.8, 114.1, 112.5, 110.8, 56.0, 54.5. HRMS: calcd for $C_{23}H_{22}N_2O_5 + H^+$, 407.1607; found (ESI, $[M+H]^+$), 407.1607. Mp 140 °C.

4.2.46. 2-Methoxy-(*E*,*E*)-4,6-bis-(4′-hydroxy-3′-*N*,*N*-dimethylaminostyryl)pyrimidine (2e)

Compound **2e′** (0.58 g, 1.11 mmol) was deprotected in EtOH/1 N HCl (40 mL) according to the general procedure C. The crude product was purified by column chromatography ($MeOH/CH_2Cl_2$ = 1:40) to give **2e** (0.41 g, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (br s, 2H), 7.76 (d, J = 15.9 Hz, 2H), 7.24 (s, 1H), 7.17 (d, J = 1.8 Hz, 2H), 7.13 (dd, J = 8.0, 1.8 Hz, 2H), 6.94 (d, J = 15.9 Hz, 2H), 6.79 (d, J = 8.0 Hz, 2H), 3.95 (s, 3H), 2.71 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.6, 165.5, 152.1, 141.6, 137.5, 127.4, 123.0, 122.9, 117.8, 116.3, 110.3, 54.5, 43.0. HRMS: calcd for $C_{25}H_{28}N_4O_3 + H^+$, 433.2240; found (ESI, $[M+H]^+$), 433.2237. Mp 115 °C.

4.2.47. 2-Methoxy-(*E*,*E*)-4,6-bis-(3′-hydroxy-4′-*N*,*N*-dimethylaminostyryl)pyrimidine (2f)

Compound **2f′** (0.53 g, 1.01 mmol) was deprotected in EtOH/1 N HCl (40 mL) according to the general procedure C. The crude product was purified by column chromatography ($MeOH/CH_2Cl_2$ = 1:40) to give **2f** (0.32 g, 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (br s, 2H), 7.71 (d, J = 15.8 Hz, 2H), 7.29 (s, 1H), 7.06–7.05 (m, 4H), 6.85 (d, J = 15.8 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 3.95 (s, 3H), 2.74 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.6, 165.2, 149.8, 143.0, 137.1, 129.0, 123.3, 120.8, 118.1, 114.2, 110.6, 54.5, 42.6. HRMS: calcd for $C_{25}H_{28}N_4O_3 + H^+$, 433.2240; found (ESI, $[M+H]^+$), 433.2234. Mp 102 °C.

4.2.48. 2-Methoxy-(*E*,*E*)-4,6-bis-styrylpyrimidine (2g)

Compound **2g** was prepared from **7a** and benzaldehyde according to general procedure A. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:10) to give **2g** (0.50 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 15.9 Hz, 2H), 7.61–7.60 (m, 4H), 7.42–7.33 (m, 6H), 7.03 (d. J = 15.9 Hz, 2H), 6.94 (s, 1H), 4.12 (s. 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 165.0, 136.9, 135.8, 129.3, 128.8, 127.6, 125.7, 111.3, 54.6.

4.2.49. 2-Methylthio-(*E*,*E*)-4,6-bis-(4′-hydroxystyryl)pyrimidine (3a)

Compound **3a′** (0.52 g, 0.86 mmol) was deprotected with TFA (4 mL) in CH_2Cl_2 (40 mL) according to the general procedure D. The crude product was purified by recrystallization from diethyl ether to give **3a** (0.29 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (br s, 2H), 7.79 (d, J = 15.9 Hz, 2H), 7.55 (d, J = 8.6 Hz, 4H), 7.24 (s, 1H), 6.96 (d, J = 15.9 Hz, 2H), 6.81 (d, J = 8.6 Hz, 4H), 2.58 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.9, 163.2, 159.4, 137.2, 130.0, 126.9, 122.7, 116.2, 111.9, 13.9. HRMS: calcd for $C_{21}H_{18}N_2O_2S + H^+$, 363.1167; found (ESI, $[M+H]^+$), 363.1159. Mp 219 °C.

4.2.50. 2-Methylthio-(*E*,*E*)-4,6-bis-(3′-hydroxystyryl)pyrimidine (3b)

Compound **3b′** (0.79 g, 1.30 mmol) was deprotected in EtOH/1 N HCl (100 mL) according to the general procedure C. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1.5:1) to give **3b** (0.24 g, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (br s, 2H), 7.89 (d, J = 15.9 Hz, 2H), 7.81 (s, 1H), 7.27–7.09 (m, 8H), 6.81 (d, J = 8.0 Hz, 2H), 2.94 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.0, 167.8, 163.0, 142.3, 141.8, 135.1, 130.7, 124.0, 121.9, 119.2, 117.4, 18.7. HRMS: calcd for $C_{25}H_{18}N_4O_2S + H^+$, 363.1167; found (ESI, $[M+H]^+$), 363.1170. Mp 205 °C.

4.2.51. 2-Methylthio-(*E*,*E*)-4,6-bis-(4′-hydroxy-3′-methoxystyryl)pyrimidine (3c)

Compound **3c′** (0.75 g, 1.10 mmol) was deprotected with TFA (5 mL) in CH_2Cl_2 (50 mL) according to the general procedure D. The crude product was purified by recrystallization from Et_2O to give **3c** (0.21 g, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.47 (s, 2H), 7.78 (d, J = 15. 9 Hz, 2H), 7.30 (d, J = 1.6 Hz, 2H), 7.25 (s, 1H), 7.12 (dd, J = 8.1, 1.6 Hz, 2H), 7.02 (d, J = 15.9 Hz, 2H), 6.80 (d, J = 8.1 Hz, 2H), 3.83 (s, 6H), 2.58 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.0, 163.3, 148.9, 148.4, 137.6, 127.5, 123.1, 122.5, 116.1, 111.7, 111.3, 56.0, 13.9. HRMS: calcd for $C_{23}H_{22}N_2O_4S + H^+$, 423.1379; found (ESI, $[M+H]^+$), 423.1372. Mp 145 °C.

4.2.52. 2-Methylthio-(*E*,*E*)-4,6-bis-(3′-hydroxy-4′-methoxystyryl)pyrimidine (3d)

Compound **3d′** (1.25 g, 1.90 mmol) was deprotected in EtOH/1 N HCl (100 mL) according to the general procedure C. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1.5:1) to give **3d** (0.24 g, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (br s, 2H), 7.74 (d, J = 15.9 Hz, 2H), 7.31 (s, 1H), 7.13 (d, J = 1.9 Hz, 2H), 7.10 (dd, J = 8.3, 1.9 Hz, 2H), 6.96 (d, J = 8.3 Hz, 2H), 6.92 (d, J = 15.9 Hz, 2H), 3.80 (s, 6H), 2.57 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.0, 163.2, 149.7, 147.1, 137.2, 128.8, 123.7, 120.9, 114.1, 112.5, 112.1, 56.0, 13.9. HRMS: calcd for $C_{23}H_{22}N_2O_4S + H^+$, 423.1379; found (ESI, $[M+H]^+$), 423.1376. Mp 216 °C.

4.2.53. 2-Methylthio-(*E*,*E*)-4,6-bis-(4′-hydroxy-3′-*N*,*N*-dimethylaminostyryl)pyrimidine (3e)

Compound **3e**′ (0.50 g, 0.90 mmol) was deprotected in EtOH/1 N HCl (60 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **3e** (0.17 g, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60 (br s, 2H), 7.76 (d, J = 15.9 Hz, 2H), 7.29 (s, 1H), 7.17 (d, J = 1.7 Hz, 2H), 7.15 (dd, J = 8.1, 1.7 Hz, 2H), 6.94 (d, J = 15.9 Hz, 2H), 6.79 (d, J = 8.1 Hz, 2H), 2.71 (s, 12H), 2.57 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.0, 163.3, 152.2, 141.6, 137.7, 127.3, 123.1, 122.7, 117.8, 116.3, 111.6, 43.0, 13.9. HRMS: calcd for $C_{25}H_{28}N_4O_2S + H^+$, 449.2011; found (ESI, $[M+H]^+$), 449.2009. Mp 168 °C.

4.2.54. 2-Methylthio-(*E*,*E*)-4,6-bis-(3′-hydroxy-4′-*N*,*N*-dimethylaminostyryl)pyrimidine (3f)

Compound **3f**′ (0.54 g, 1.00 mmol) was deprotected in EtOH/1 N HCl (60 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **3f** (0.26 g, 61%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (br s, 2H), 7.73 (d, 2H), 7.35 (s, 1H), 7.08–7.06 (m, 4H), 6.88–6.84 (m, 4H), 2.75 (s, 12H), 2.65 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.0, 163.1, 149.8, 142.7, 137.3, 129.2, 123.2, 120.8, 118.2, 114.3, 111.8, 42.7, 13.9. HRMS: calcd for $C_{25}H_{28}N_4O_2S + H^+$, 449.2011; found (ESI, $[M+H]^+$), 449.2007. Mp 123 °C.

4.2.55. 2-Methylthio-(*E*,*E*)-4,6-bis-styrylpyrimidine (3g)

Compound **3g** was prepared from **7b** and benzaldehyde according to general procedure A. The crude product was purified by crystallization from EtOAc to give **3g** (0.37 g, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 15.9 Hz, 2H), 7.61–7.59 (m, 4H), 7.42–7.35 (m, 6H), 7.01 (d, J = 15.9 Hz, 2H), 6.96 (s, 1H), 2.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 162.7, 137.0, 135.8, 129.3, 128.8, 127.7, 125.8, 112.0, 14.2.

4.2.56. (*E*,*E*)-3,5-Bis-(4′-hydroxystyryl)benzene (4a)

Compound **4a**′ (0.50 g, 1.20 mmol) was deprotected in EtOH/1 N HCl (60 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **4a** (0.37 g, 100%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (br s, 2H), 7.70 (s, 1H), 7.42 (d, J = 8.5 Hz, 4H), 7.36–7.28 (m, 3H), 7.18 (d, J = 16.4 Hz, 2H), 7.00 (d, J = 16.4 Hz, 2H), 6.76 (d, J = 8.5 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.8, 138.3, 129.4, 129.1, 128.5, 128.3, 125.4, 125.2, 124.0, 116.0. HRMS: calcd for $C_{22}H_{19}O_2 + H^+$, 315.1385; found (ESI, $[M+H]^+$), 315.1371. Mp 243 °C.

4.2.57. (*E*,*E*)-3,5-Bis-(3′-hydroxystyryl)benzene (4b)

Compound **4b**′ (0.71 g, 1.70 mmol) was deprotected in EtOH/1 N HCl (80 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **4b** (0.31 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.44 (s, 2H), 7.82 (s, 1H), 7.48–7.33 (m, 3H), 7.23 (d, J = 16.4 Hz, 2H), 7.17 (dd, J = 7.7, 7.7 Hz, 2H), 7.15 (d, J = 16.4 Hz, 2H), 7.01 (d, J = 7.7 Hz, 2H), 6.98 (s, 2H), 6.68 (dd, J = 7.7, 2.1 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.1, 138.7, 137.8, 130.1, 129.5, 129.4, 128.4, 126.3, 124.8, 117.9, 115.4, 113.5. HRMS: calcd for $C_{22}H_{18}O_2 + H^+$, 315.1385; found (ESI, $[M+H]^+$), 315.1389. Mp 189 °C.

4.2.58. (*E*,*E*)-3,5-Bis-(4′-hydroxy-3′-methoxystyryl)benzene (4c)

Compound **4c**′ (3.13 g, 6.70 mmol) was deprotected in EtOH/1 N HCl (320 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **4c** (2.14 g, 85%) without purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 2H), 7.73 (s, 1H), 7.39–7.29 (m, 3H), 7.20 (d, J = 1.8 Hz, 2H), 7.19 (d, J = 16.3 Hz, 2H), 7.06 (d, J = 16.3 Hz, 2H), 6.99 (dd, J = 8.1, 1.8 Hz, 2H), 6.76 (d, J = 8.1 Hz, 2H), 3.82 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.3, 147.2, 138.3, 129.4, 129.3, 129.1, 125.7, 125.3, 123.9, 120.6, 116.0, 110.2, 56.0. HRMS: calcd for $C_{24}H_{22}O_4 + H^+$, 375.1596; found (ESI, $[M+H]^+$), 375.1588. Mp 181 °C.

4.2.59. (*E*,*E*)-3,5-Bis-(3′-hydroxy-4′-methoxystyryl)benzene (4d)

Compound **4d**′ (0.69 g, 1.80 mmol) was deprotected in EtOH/1 N HCl (80 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **4d** (0.52 g, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 2H), 7.74 (s, 1H), 7.41–7.29 (m, 3H), 7.16 (d, J = 16.3 Hz, 2H), 7.04 (d, J = 1.9 Hz, 2H), 6.98 (dd, J = 8.3, 1.9 Hz, 2H), 6.98 (d, J = 16.3 Hz, 2H), 6.91 (d, J = 8.3 Hz, 2H), 3.77 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.2, 147.1, 138.1, 130.5, 129.4, 129.1, 126.3, 125.5, 124.3, 118.9, 113.4, 112.6, 56.0. HRMS: calcd for $C_{24}H_{22}O_4 + H^+$, 375.1596; found (ESI, $[M+H]^+$), 375.1593. Mp 255 °C.

4.2.60. (*E*,*E*)-3,5-Bis-(4′-hydroxy-3′-*N*,*N*-dimethylaminostyryl) benzene (4e)

Compound **4e**′ (0.35 g, 0.70 mmol) was deprotected in EtOH/1 N HCl (40 mL) according to the general procedure C. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:1) to **4e** (0.18 g, 64%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (br s, 2H), 7.74 (s, 1H), 7.37–7.27 (m, 3H), 7.18 (d, J = 15.6 Hz, 2H), 7.10 (s, 2H), 7.03 (d, J = 8.1 Hz, 2H), 7.00 (d, J = 15.6 Hz, 2H), 6.75 (d, J = 8.1 Hz, 2H), 2.71 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.5, 141.5, 138.3, 129.6, 129.3, 125.3, 125.2, 123.8, 121.3, 116.7, 116.2, 43.1. HRMS: calcd for $C_{26}H_{28}N_2O_2 + H^+$, 401.2229; found (ESI, $[M+H]^+$), 401.2230. Mp 94 °C.

4.2.61. (*E*,*E*)-3,5-Bis-(3′-hydroxy-4′-*N*,*N*-dimethylaminostyryl) benzene (4f)

Compound **4f**′ (0.90 g, 1.80 mmol) was deprotected in EtOH/1 N HCl (80 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **4f** (0.54 g, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 2H), 7.73 (s, 1H), 7.39–7.28 (m, 3H), 7.15 (d, J = 16.3 Hz, 2H), 6.99 (d, J = 1.7 Hz, 2H), 6.96 (dd, J = 8.1, 1.7 Hz, 2H), 6.94 (d, J = 16.3 Hz, 2H), 6.82 (d, J = 8.1 Hz, 2H), 2.69 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.1, 141.2, 138.1, 131.3, 129.4, 129.2, 126.0, 125.4, 124.2, 118.9, 118.4, 113.5, 43.0. calcd for $C_{26}H_{28}N_2O_2 + H^+$, 401.2229; found (ESI, $[M+H]^+$), 401.2225. Mp 167 °C.

4.2.62. (*E*,*E*)-3,5-Bis-styrylbenzene (4g)

Compound **4g** was prepared from **10a** and benzaldehyde according to the general procedure B to give **4g** (0.33 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.55–7.53 (m, 4H), 7.44–7.33 (m, 7H), 7.29–7.25 (m, 2H), 7.19–7.10 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 137.2, 129.0 (overlapped), 128.7, 128.5, 127.7, 126.5, 125.7, 124.7.

4.2.63. (*E*,*E*)-3,5-Bis-(4′-hydroxystyryl)phenol (5a)

Compound **5a**′ (0.48 g, 1.10 mmol) was deprotected in EtOH/1 N HCl (60 mL) according to the general procedure C. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:1) to give **5a** (0.34 g, 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (br s, 2H), 9.38 (br s, 1H), 7.41 (d, J = 8.5 Hz, 4H), 7.16 (s, 1H), 7.07 (d, J = 16.3 Hz, 2H), 6.91 (d, J = 16.3 Hz, 2H), 6.78 (s, 2H), 6.75 (d, J = 8.5 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.2, 157.7, 139.4, 128.8, 128.5, 128.3, 125.7, 116.0, 115.8, 112.1. HRMS: calcd for $C_{22}H_{19}O_3 + H^+$, 331.1334; found (ESI, $[M+H]^+$), 331.1335. Mp 204 °C.

4.2.64. (*E*,*E*)-3,5-Bis-(3′-hydroxystyryl)phenol (5b)

Compound **5b**′ (0.38 g, 1.00 mmol) was deprotected in EtOH/1 N HCl (40 mL) according to the general procedure C. The crude

product was purified by recrystallization from EtOH/H_2O to give **5b** (0.25 g, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 9.42 (s, 2H), 7.28 (s, 1H), 7.15 (dd, J = 7.8, 7.8 Hz, 2H), 7.12 (d, J = 16.2 Hz, 2H), 7.05 (d, J = 16.2 Hz, 2H), 7.02 (d, J = 7.8 Hz, 2H), 6.95 (s, 2H), 6.86 (s, 2H), 6.68–6.65 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.3, 158.0, 138.9, 138.7, 130.1, 129.1, 128.7, 118.0, 116.5, 115.3, 113.5, 113.1. HRMS: calcd for $C_{22}H_{19}O_3 + H^+$, 331.1334; found (ESI, $[M+H]^+$), 331.1334. Mp 190 °C.

4.2.65. (*E*,*E*)-3,5-Bis-(4′-hydroxy-3′-methoxystyryl)phenol (5c)

Compound **5c′** (0.61 g, 1.20 mmol) was deprotected in EtOH/1 N HCl (60 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **5c** (0.42 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 9.13 (br s, 2H), 7.19 (s, 1H), 7.18 (d, J = 1.5 Hz, 2H), 7.08 (d, J = 16.3 Hz, 2H), 6.97 (dd, J = 8.1, 1.5 Hz, 2H), 6.97 (d, J = 16.3 Hz, 2H), 6.78 (s, 2H), 6.75 (d, J = 8.1 Hz, 2H), 3.81 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.2, 148.2, 147.1, 139.4, 129.1, 129.1, 125.9, 120.6, 116.0, 115.6, 112.2, 110.2, 56.0. HRMS: calcd for $C_{24}H_{22}O_5 + H^+$, 391.1545; found (ESI, $[M+H]^+$), 391.1540. Mp 119 °C.

4.2.66. (*E*,*E*)-3,5-Bis-(3′-hydroxy-4′-methoxystyryl)phenol (5d)

Compound **5d′** (0.50 g, 1.00 mmol) was deprotected in EtOH/1 N HCl (40 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **5d** (0.37 g, 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (br s, 1H), 9.00 (br s, 2H), 7.20 (s, 1H), 7.05 (d, J = 16.4 Hz, 2H), 7.02 (d, J = 1.9 Hz, 2H), 6.97 (dd, J = 8.3, 1.9 Hz, 2H), 6.90 (d, J = 8.3 Hz, 2H), 6.99 (d, J = 16.4 Hz, 2H), 6.79 (d, J = 1.0 Hz, 2H), 3.77 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.6, 147.6, 146.5, 138.6, 129.9, 128.3, 125.9, 118.3, 115.4, 112.8, 112.0, 111.9, 55.5. HRMS: calcd for $C_{24}H_{22}O_5 + H^+$, 391.1545; found (ESI, $[M+H]^+$), 391.1544. Mp 163 °C.

4.2.67. (*E*,*E*)-3,5-Bis-(4′-hydroxy-3′-*N*,*N*-dimethylaminostyryl) phenol (5e)

Compound **5e′** (0.43 g, 0.85 mmol) was deprotected in EtOH/1 N HCl (40 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **5e** (0.29 g, 67%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (br s, 1H), 9.24 (br s, 2H), 7.20 (s, 1H), 7.08 (d, J = 1.7 Hz, 2H), 7.06 (d, J = 16.3 Hz, 2H), 7.01 (dd, J = 8.0, 1.7 Hz, 2H), 6.90 (d, J = 16.3 Hz, 2H), 6.76 (s, 2H), 6.74 (d, J = 8.0 Hz, 2H), 2.69 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.2, 150.4, 141.4, 139.4, 129.3, 128.9, 125.5, 121.3, 116.7, 116.1, 115.6, 112.1, 43.1. HRMS: calcd for $C_{26}H_{28}N_2O_3 + H^+$, 417.2178; found (ESI, $[M+H]^+$), 417.2170. Mp 127 °C.

4.2.68. (*E*,*E*)-3,5-Bis-(3′-hydroxy-4′-*N*,*N*-dimethylaminostyryl) phenol (5f)

Compound **5f′** (0.69 g, 1.30 mmol) was deprotected in EtOH/1 N HCl (80 mL) according to the general procedure C. The crude product was purified by recrystallization from EtOH/H_2O to give **5f** (0.35 g, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (br s, 1H), 9.34 (s, 2H), 7.23 (s, 1H), 7.07 (d, J = 16.3 Hz, 2H), 7.02–7.00 (m, 6H), 6.90 (d, J = 16.3 Hz, 2H), 6.82 (s, 2H), 2.77 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.3, 150.2, 139.1, 138.9, 132.9, 128.7, 127.1, 119.1, 118.8, 116.1, 113.8, 112.6, 43.4. HRMS: calcd for $C_{26}H_{28}N_2O_3 + H^+$, 417.2178; found (ESI, $[M+H]^+$), 417.2173. Mp 148 °C.

4.2.69. (*E*,*E*)-3,5-Bis-styrylphenol (5g)

Compound **5g** was prepared from **10b** and benzaldehyde according to the general procedure B. The crude product was purified by flash column chromatography (EtOAc/*n*-hexane = 1:10) to give **5g** (0.41 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.50 (m, 4H), 7.38–7.34 (m, 4H), 7.29–7.22 (m, 3H), 7.14–7.03 (m, 4H), 6.90 (s, 2H), 4.81 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 139.3, 137.1, 129.5, 128.7, 128.1, 127.8, 126.6, 118.3, 112.3.

4.2.70. 2-Methoxy-4,6-dimethylpyrimidine (7a)

Acetylacetone (50.0 g, 0.5 mol) and K_2CO_3 (138.0 g, 1.0 mol) were dissolved in H_2O (250 mL). *O*-Methylisourea sulfate (61.5 g, 0.25 mol) was added and the reaction mixture was refluxed with stirring. After cooling, more K_2CO_3 (70 g) was added and the reaction mixture was left at 25 °C for 48 h. The formed oil was separated, combined with the ether, extracted with the aqueous phase, and dried over $MgSO_4$. The organic layer was concentrated in vacuo. Vacuum distillation gave **7a** (48.3 g, 70%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (s, 1H), 3.97 (s, 3H), 2.40 (s, 6H).

4.2.71. 4,6-Dimethyl-2-methyl-mercapyrimidine (7b)

Compound **7b** was prepared from methylisothiourea sulfate (69.6 g, 0.25 mol) and acetylacetone (50.0 g, 0.5 mol) as described for **7a** (57.8 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (s, 1H), 2.56 (s, 3H), 2.40 (s, 6H).

4.2.72. 3,5-Di(iodomethyl)phenol (9a)

To a solution of 3,5-di(hydroxymethyl)phenol **8** (38.5 g, 0.25 mol) in dry 1,4-dioxane (300 mL) were added $BF_3{\cdot}Et_2O$ (61.7 mL, 0.50 mol) and KI (83.0 g, 0.5 mol) were added and the resulting mixture was stirred at room temperature. After 25 h, the reaction mixture was poured into cold water and extracted with diethyl ether. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/*n*-hexane = 1:10) to give **9b** (81.3 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90 (s, 1H), 6.67 (d, J = 1.0 Hz, 2H), 5.24 (s, 1H), 4.29 (s, 4H).

4.2.73. 1,3-Bis(diethylphosphonomethyl)benzene (10a)

To a solution of α,α'-dibromo-*m*-xylene **9a** (59.4 g, 0.225 mol) in toluene (500 mL) was added triethylphosphite (77.1 mL, 0.4 mol) and the reaction mixture was refluxed overnight. The excess toluene was removed by evaporation to give **10a** (72.3 g, 85%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.17 (m, 4H), 4.04–3.96 (m, 8H), 3.12 (d, J = 21.9 Hz, 4H), 1.23 (t, J = 7.0 Hz, 12H).

4.2.74. 3,5-Bis(diethylphosphonomethyl)phenol (10b)

Compound **10b** was prepared from **9b** (81.3 g, 0.217 mol) and triethylphosphite (74.4 mL, 0.434 mol) as described for **10a** (76.1 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (d, J = 1.5 Hz, 2H), 6.63 (s, 1H), 4.04–3.96 (m, 8H), 3.07 (d, J = 21.6 Hz, 4H), 1.24 (t, J = 7.0 Hz, 12H).

4.2.75. General procedure E

4.2.75.1. Protection of hydroxyl group with *p*-methoxybenzyl group. A mixture of hydroxybenzaldehyde (1.0 equiv) and *p*-methoxybenzyl chloride(1.1 equiv) in DMF containing K_2CO_3 (1.2 equiv) was heated at 60 °C overnight. The reaction mixture was poured into cold H_2O and the precipitate was collected by filteration to give the product.

4.2.76. General procedure F

4.2.76.1. Protection of hydroxyl group with methoxymethyl group. To a stirred solution of phenol (1.0 equiv) and triethylamine (1.2 equiv) in dry THF was added chloromethyl methyl ether (1.1 equiv) dropwise at 0 °C under N_2. When the reaction was completed, the reaction mixture was extracted with H_2O and EtOAc. The organic layer was washed with aqueous 1 N HCl, 1 N NaOH, and brine, dried over $MgSO_4$, and concentrated in vacuo to give the product.

4.2.77. 4-(4-Methoxybenzyloxy)benzaldehyde (12a)

Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.84 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 5.08 (s, 2H), 3.83 (s, 3H).

4.2.78. 3-(4-Methoxybenzyloxy)benzaldehyde (12b)

Yield: 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.98 (s, 1H), 7.48–7.43 (m, 3H), 7.38 (d, J = 8.5 Hz, 2H), 7.25–6.95 (m, 1H), 6.94 (d, J = 8.5 Hz, 2H), 5.06 (s, 2H), 3.83 (s, 3H).

4.2.79. 3-Methoxy-4-(4-methoxybenzyloxy)benzaldehyde (12c)

Yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (s, 1H), 7.36 (d, J = 1.7 Hz, 1H), 7.34 (dd, J = 8.1, 1.7 Hz, 1H), 7.31 (d, J = 8.6 Hz, 2H), 6.95 (d, J = 8.1 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 5.11 (s, 2H), 3.88 (s, 3H), 3.75 (s, 3H).

4.2.80. 4-Methoxy-3-(4-methoxybenzyloxy)benzaldehyde (12d)

Yield: 91%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.83 (s, 1H), 7.47 (d, J = 1.8 Hz, 1H), 7.46 (dd, J = 8.7, 1.8 Hz, 1H), 7.38 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 8.7 Hz, 1H), 6.91 (d, J = 8.7 Hz, 2H), 5.12 (s, 2H), 3.95 (s, 3H), 3.81 (s, 3H).

4.2.81. 4-(Methoxymethoxy)benzaldehyde (13a)

Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.83 (d, J = 8.7 Hz, 2H), 7.14 (d, J = 8.7 Hz, 2H), 5.25 (s, 2H), 3.49 (s, 3H).

4.2.82. 3-(Methoxymethoxy)benzaldehyde (13b)

Yield: 54%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.98 (s, 1H), 7.55–7.43 (m, 3H), 7.30 (ddd, J = 8.0, 2.5, 1.2 Hz, 1H), 5.23 (s, 2H), 3.49 (s, 3H).

4.2.83. 3-Methoxy-4-(methoxymethoxy)benzaldehyde (13c)

Yield: 86%. ^{1}H NMR (400 MHz DMSO-d_6) δ 9.84 (d, J = 1.0 Hz, 1H), 7.50 (dd, J = 8.2, 1.7 Hz, 1H), 7.43 (d, J = 1.4 Hz, 1H), 7.24 (d, J = 8.2 Hz, 1H), 5.28 (s, 2H), 3.83 (s, 3H), 3.38 (s, 3H).

4.2.84. 4-Methoxy-3-(methoxymethoxy)benzaldehyde (13d)

Yield: 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 7.60 (dd, J = 8.3, 1.8 Hz, 1H), 7.52 (d, J = 1.8 Hz, 1H), 7.20 (d, J = 8.3 Hz, 1H), 5.22 (s, 2H), 3.87 (s, 3H), 3.38 (s, 3H).

4.2.85. 4-Hydroxy-3-nitrobenzoic acid methyl ester (15a)

To a solution of 4-hydroxy-3-nitrobenzoic acid (25.0 g, 0.13 mol) in MeOH (150 mL) was added concd H_2SO_4 (5 mL) and the mixture was refluxed overnight. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. The residue was poured into cold H_2O and the precipitate was collected by filteration to give **15a** (23.2 g, 88%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.89 (s, 1H), 8.82 (d, J = 2.1 Hz, 1H), 8.24 (dd, J = 8.7, 2.1 Hz, 1H), 7.22 (d, J = 8.7 Hz, 1H), 3.94 (s, 3H).

4.2.86. 3-Hydroxy-4-nitrobenzoic acid methyl ester (15b)

Compound **15b** was prepared from 3-hydroxy-4-nitrobenzoic acid (25 g, 0.13 mol) as described for **15a** (25.0 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.48 (br s, 1H), 8.16 (d, J = 8.8 Hz, 1H), 7.81 (d, J = 1.8 Hz, 1H), 7.60 (dd, J = 8.8, 1.8 Hz, 1H), 3.95 (s, 3H).

4.2.87. 4-(Methoxymethyl)-3-nitrobenzoic acid methyl ester (16a)

Compound **16a**, obtained as a yellow solid, was prepared from **15a** (23.2 g, 0.11 mol) as described in general procedure F (96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, J = 2.1 Hz, 1H), 8.17 (dd, J = 8.8, 2.1 Hz, 1H), 7.37 (d, J = 8.8 Hz, 1H), 5.36 (s, 2H), 3.94 (s, 3H), 3.54 (s, 3H).

4.2.88. 3-(Methoxymethyl)-4-nitrobenzoic acid methyl ester (16b)

Compound **16b**, obtained as a yellow solid, was prepared from **15a** (27.0 g, 0.13 mol) as described in general procedure F (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 1.5 Hz, 1H), 7.80 (d, J = 8.3 Hz, 1H), 7.75 (dd, J = 8.3, 1.5 Hz, 1H), 5.34 (s, 2H), 3.95 (s, 3H), 3.53 (s, 3H).

4.2.89. *N,N*-3-(Dimethylamino)-4-(methoxymethyl)benzoic acid methyl ester (17a)

To a solution of **16a** (5.55 g, 23.0 mmol) in MeOH (150 mL) was added 10% Pd/C (1.0 g) and the mixture was stirred at room temperature under H_2. When reduction of nitro group was complete, formaldehyde (37% solution in H_2O, 30.0 mL, 0.37 mol) was added. The mixture was stirred under H_2 overnight. The reaction mixture was filtered throughout Celite 545 and the filtrate was concentrated in vacuo to give the product (5.28 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67–7.63 (m, 2H), 7.13 (d, J = 2.2 Hz, 1H), 5.30 (s, 2H), 3.88 (s, 3H), 3.52 (s, 3H), 2.83 (s, 6H).

4.2.90. *N,N*-4-(Dimethylamino)-3-(methoxymethyl)benzoic acid methyl ester (17b)

Compound **17b** was prepared from **16b** (5.33 g, 22.1 mmol) as described for **17a** (4.54 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 1.9 Hz, 1H), 7.66 (dd, J = 8.3, 1.9 Hz, 1H), 6.90 (d, J = 7.2 Hz, 1H), 5.25 (s, 2H), 3.86 (s, 3H), 3.52 (s, 3H), 2.90 (s, 6H).

4.2.91. *N,N*-3-(Dimethylamino)-4-(methoxymethyl)benzyl alcohol (18a)

To a suspension of $LiAlH_4$ (1.1 g, 27.6 mmol) in dry THF (50 mL) was added dropwise **17a** (5.0 g, 20.9 mmol) in dry THF (50 mL) at 0 °C. The reaction mixture was stirred for 1 h, and quenched with H_2O (2 mL). Then 15% NaOH solution (2 mL) and H_2O (6 mL) were added and the reaction mixture was stirred at ambient temperature for 1 h. The precipitate was removed by filter and the filtrate was evaporated to give **18a** (4.23 g, 96%) as a oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, J = 8.1 Hz, 1H), 6.98 (s, 1H), 6.92 (dd, J = 8.1, 1.6 Hz, 1H), 5.25 (s, 2H), 4.61 (s, 2H), 3.52 (s, 3H), 2.81 (s, 6H).

4.2.92. *N,N*-4-(Dimethylamino)-3-(methoxymethyl)benzyl alcohol (18b)

Compound **18b** was prepared from **17b** (5.0 g, 20.9 mmol) as described for **18a** (4.28 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18–6.96 (m, 3H), 5.27 (s, 3H), 4.61 (s, 2H), 3.53 (s, 3H), 2.83 (s, 6H).

4.2.93. *N,N*-3-(Dimethylamino)-4-(methoxymethyl)benzaldehyde (13e)

To a solution of **18a** (18.0 g, 0.085 mol) in CH_2Cl_2 (500 mL) was added MnO_2 (66 g, 0.759 mol) and the mixture was refluxed overnight. MnO_2 was removed by filtration throughout Celite 545 and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/*n*-hexane = 1:5) to give **13e** (9.96 g, 56%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (s, 1H), 7.47 (dd, J = 8.2, 1.9 Hz, 1H), 7.44 (d, J = 1.9 Hz, 1H), 7.22 (d, J = 8.2 Hz, 1H), 5.33 (s, 2H), 3.53 (s, 3H), 2.84 (s, 6H).

4.2.94. *N,N*-4-(Dimethylamino)-3-(methoxymethyl)benzaldehyde (13f)

Compound **13f** was prepared from **18b** (20.2 g, 0.095 mol) as described for **13e** (18.6 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 7.57 (d, J = 1.6 Hz, 1H), 7.47 (dd, J = 8.2, 1.7 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 5.26 (s, 2H), 3.53 (s, 3H), 2.96 (s, 6H).

4.3. Preparation of Aβ protein for fibrillization studies

Purified Aβ(1–42) was purchased from Bachem (United states). Aβ(1–42) stock solutions (250 μM) were prepared by completely dissolving the peptide in DMSO. Aβ(25–35) purchased from US peptide was prepared as 10 mM stock solution in DMSO and diluted 10-fold with phosphate-buffered saline (PBS) to induce aggregation for 1 h at room temperature.

4.4. ThT fluorescence assay for Aβ fibril formation

Aggregation of Aβ(1–42) peptide was measured by the ThT assay, in which the fluorescence intensity reflects the degree of Aβ fibril formation. To each well of a 96-well microplate (black) were added 250 μM Aβ(1–42) (5 μL) and PBS buffer (45 μL). The mixed solution was added to compounds and incubated for 1 h at room temperature. ThT was diluted with 50 mM glycine buffer solution to be 5 μM. To each well was added diluted ThT solution (150 μL). After shaking together for 10 s, the ThT fluorescence was measured by using TECAN spectrofluorometer (Safire, TECAN) with excitation at 450 nm and emission at 480 nm.

4.5. Cell culture

HT22, a murine cell line of hippocampal origin, was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin (Sigma) at 37 °C and 5% CO_2. At the outset, 90% confluent cells were dissociated and plated at a density of 5×10^3 cells/well in a 96-well plate. When cells were attached to the plate, the medium was replaced with plain DMEM.

4.6. Cytotoxicity assay and Aβ burden assay

The innate cytotoxicity of compounds was measured by using MTT assay. Before compound treatment, HT22 cells were incubated in DMEM without serum for 1 h. Following treatment, cells were incubated for 18 h. 5 mg/mL of MTT solution was added to the culture medium and the culture further incubated for 4 h at 37 °C. Cells were then solubilized in 50% dimethylformamide and 10% sodium dodecyl sulfide (pH 4.7) for 18 h. The degree of cell survival was determined by checking conversion of MTT to formazan. The absorbance was measured at 570/630 nm using a plate reader (Sunrise, TECAN) and the percentage cytotoxicity was calculated. For Aβ burden assay, 1 h after compounds treatment, aggregated Aβ(25–35) was added to the concentration of 25 μM and cells were incubated for 18 h to induce cell necrosis. Cell viability was checked in the same manner as described above for MTT assay.

4.7. SDS–PAGE with PICUP technique

Aβ(1–42) peptide was treated in pre-chilled 1,1,1,3,3,3,-hexafluoro-2-propanol (HFIP) to a final concentration of 0.5 mM for monomerization of Aβ(1–42) peptides. After the sonication for 5 min at room temperature, it was vortexed gently and then incubated for 30 min at room temperature. To remove HFIP, the tubes were left opened in a clean hood overnight. The monomerized Aβ(1–42) peptides were dissolved in anhydrous DMSO to prepare Aβ stock solution (0.5 mM). The Aβ aggregation reaction was initiated by adding Aβ stock solution into phosphate buffered saline (PBS, pH 7.3) in the absence or presence of compounds (25 μM final Aβ(1–42) concentration and 2 μM final compound concentration). The assay solutions were incubated for 2 h at 37 °C, followed by photo-induced cross-linking of unmodified proteins (PICUP) technique. 1.2 μM of ammonium persulfate (20 mM, Sigma–Aldrich) and tris(2,2'-bipyridyl)dichlororuthenium(II) (1 mM, Sigma–Aldrich) in sodium phosphate buffer (10 mM, pH 7.4) were added into assay solution. The mixtures were irradiated 3 times (1 s each) using a 200 W incandescent lamp and quenched with a reducing PAGE sample buffer. The mixtures were boiled for 5 min, then loaded onto a 1.0 mm-thick 10–20% tris–tricine gradient gel (Criterion™ Precast Gel, Biorad), followed by the electrophoresis at 130 V in tris/tricine/SDS running buffer. Separated Aβ bands were stained via silver staining kit (PlusOne silver staining kit, Amersham Biosciences) to visualize.

4.8. Experimental design for in vivo tests

Male ICR mice weighing 25 g purchased from OrientBio Co. (Korea) were bred as five mice per cage and the cage was kept with following condition maintaining the temperature of 22 ± 2 °C and the relative humidity of 50 ± 5% under the regularly controlled light/dark condition with an interval of 12 h.

Aβ(1–42) (Bachem) was dissolved in DMSO in order to be 250 μM solution, diluted with PBS to 10 nM, and aggregated at 37 °C for 4 days (Passive avoidance task) or 6 days (Y-maze task). Aggregated Aβ(1–42) was administrated into the mice according to the procedure disclosed in the literature.[27] 50 μL of pre-aggregated Aβ(1–42) was injected into the 2.4 mm depth of bregma region with Hamilton micro-syringe equipped with 26-gauge needle.

After the injection of Aβ(1–42), Y-maze, object recognition, passive avoidance, and watermaze task were performed. Y-maze task and object recognition task were performed 2 days after the administration of Aβ and passive avoidance and watermaze task were performed 3 days after the administration of Aβ. The compounds were administered orally at 100 mg/kg into the mice once a day. In case of watermaze task, the feed was administered by pellet feed for 7 days and one pellet was designed to contain 3 mg of the compound.

4.9. Y-maze task

The Y-maze apparatus was made of black acrylic and was composed three arms (L: 40 cm, W: 5 cm, H: 10 cm) having identical angle each other. Mice were positioned at the center of the maze and let to move freely within the maze for 8 min. Thereafter, the entering order of the mice into the pathway was observed and the entering was determined when 4 limbs of the mice were entered within the pathway. To determine the spatial memory, the determined spontaneous alternation behavior was calculated by following empirical formula and the actual alternation was assigned to one time at the time that mice was entered three pathways continuously.

4.10. Object recognition task

The object recognition task was performed by using an open-field box consisting of black acrylic (L: 50 cm, W: 50 cm, H: 30 cm). Two objects were placed in a symmetrical position about 5 cm away from the wall. During the acquisition trial (first trial), the mouse was exposed to two identical objects for 3 min. The mouse was then returned to its home cage. After a delay of 24 h, the mouse was exposed to one of the familiar objects and a novel object for 3 min. All combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects. Care was taken to avoid olfactory stimuli by cleaning the objects carefully. Exploration behavior was calculated by following formula and exploration was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose.

$$\text{Preference index} = \frac{\text{exploration time for new object}}{\text{exploration time for total object}}$$

4.11. Passive avoidance task

Black avoidance shuttle box was divided into two chambers of equal size (L: 18 cm, W: 9.5 cm, H: 17 cm) partitioned by compartment door (L: 4 cm, W: 3.5 cm) allowing electricity to run on the floor of the dark compartment. A light chamber is equipped with a 20 W lamp on the hinged plexiglass lid and the mice were allowed to enter dark chamber through compartment door.

The experiments consisted of training and test sessions: In the training session, the mouse was initially placed in the light chamber and allowed for habituation, and the door was then opened. As soon as mice preferring darkness went out from light chamber and entered the dark chamber, the door was closed immediately, and an electric shock (0.25 mA, 3 s, once) was delivered to the mouse through the grid floor for 3 s. 24 h after the training session, the identical experiment was performed again with mouse to measure the latency time staying at the light chamber. The data was regarded as the index which meant the memory on previous training by 0.25 mA of electronic shock for 3 s. Latency to enter the dark compartment from the light compartment was measured as a step-through latency. If it did not enter the dark chamber within the cut-off time (300 s), it was assigned a value of 300 s as its latency.

4.12. Watermaze task

The Morris water maze consisting of a large circular tank (diameter 120 cm) containing water at 25 ± 1 °C. The water was rendered opaque by the addition of white, non-toxic paint. To escape from the water, the mice had to find a hidden escape platform (diameter 10 cm) submerged approximately 1 cm below the water surface. The platform was located at the center of one of the four quadrants of the pool (arbitrarily designated NE, NW, SE, SW). Two- and three-dimensional visual cues were positioned around the tank.

The mice were placed into the pool facing the side wall at one of start locations (chosen randomly across trials), and allowed to swim until they found the platform, or for a maximum of 60 s. They received 4 trials per day for 5 days, with an ITI (inter test interval) of approximately 30 s.

4.13. Statistical analysis

Data were expressed as mean ± standard error of the mean. Data were analyzed by Student's *t*-test or ANOVA with repeated measures (multiple comparisons). Planned comparisons were used for post-hoc analysis. A P-value < 0.05 was considered significant.

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