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Bis-styrylpyridine and bis-styrylbenzene derivatives as inhibitors for Aβ fibril formation

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Abstract—New bis-styrylpyridine and bis-styrylbenzene derivatives were designed and synthesized. These 34 compounds were evaluated by Aβ fibril formation inhibitory assay using thioflavin T as a dye (named ThT assay). Most of them showed excellent inhibitory activities for Aβ fibril formation at IC_{50} of 0.1–2.7 μM which is comparable to curcumin (IC_{50} of 0.8 μM). Among them, nine compounds were screened for their cytotoxicities on HT-22 cell by MTT assay at 1, 10, and 50 μM. In particular, **I-7** and **II-2** exhibited the best combination of inhibitory activity and compound cytotoxicity.

Alzheimer disease (AD) is a sort of dementia and Alzheimer patients progressively undergo brain disorder that destroys cognitive functions and daily activities.

Since cholinergic deficit in the central nervous system has been observed in AD patients, inhibitors for acetylcholine esterase (AchE) such as tacrin, donepezil, and rivastigmine are used for the clinical treatment of AD symptoms in current therapeutic approaches. Although these are the only FDA-approved AD therapies on the market, they cannot solve fundamental problems but can be only palliative solutions.[1]

Pathomorphologically important hallmarks in AD are senile plaques (SP) composed of amyloid β (Aβ)[2–4] and neurofibrillary tangles (NFTs). Therefore, reducing deposition of Aβ plaques in the brain is believed to be pivotal to treat the AD patients.[5] Based on Aβ hypothesis,[6] Aβ 42 monomers divided by β-, γ-secretases from APP are gradually aggregated to oligomers, protofibrils, fibrils, and plaques. Aβ 42 aggregates such as oligomers and fibrils deposit in the brain and cause strong neuronal toxicity. On the other hand, it was recently reported that the precursors of fibrils, oligomers might be more toxic than fibrils.[7]

Consequently, we attempted to design and synthesize small molecules that prevent the formation of insoluble Aβ fibrils by specific binding with Aβ 42 monomers or relatively smaller oligomers. Based on the previous results,[8] we synthesized (*E*,*E*)-1,3-bis-3′-methoxy-4′-hydroxystyrylbenzene which was suggested to shift the equilibrium of multimeric Aβ, inhibiting the pathogenic oligomer or fibril formation. In this research, we designed and synthesized various new bis-styrylpyridine and bis-styrylbenzene derivatives following the below Scheme (Fig. 1).

All the synthesized compounds were evaluated by Aβ fibril formation inhibitory assay using thioflavin T as a dye (named ThT assay).[9] IC_{50} value was estimated after % inhibition screening at 20 μM, then 2 μM of the compounds. Among them, nine compounds were screened for their cytotoxicities on HT-22 cell by MTT assay at 1 and 10 μM (Fig. 4).[10]

Keywords: Aβ fibril formation inhibitor; Alzheimer disease; Bis-styrylpyridine; Bis-styrylbenzene; Amyloid β.
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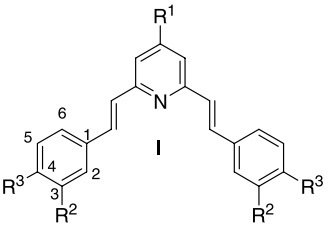

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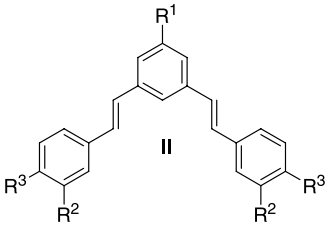
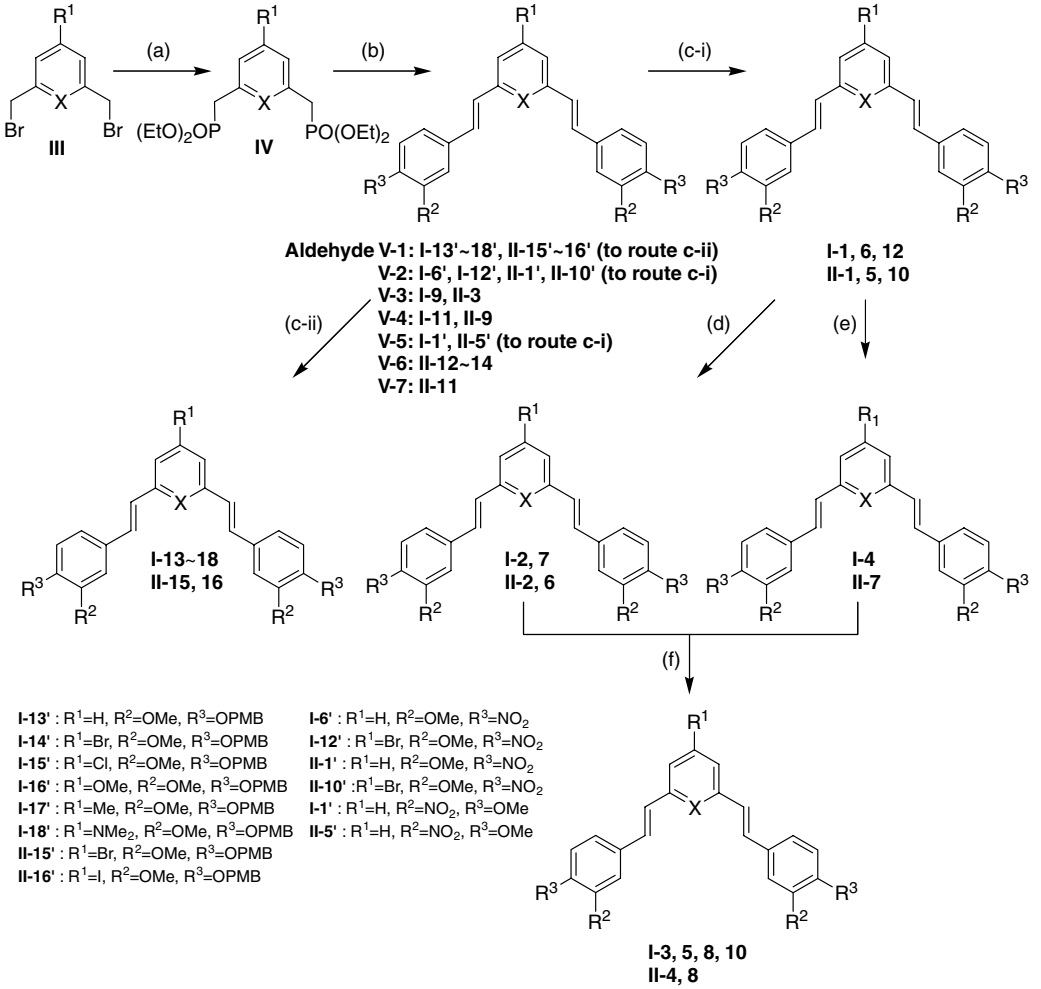


II

I-1: R^1=H, R^2=NH_2, R^3=OMe
I-2: R^1=H, R^2=NHMe, R^3=OMe
I-3: R^1=H, R^2=NHMe, R^3=OH
I-4: R^1=H, R^2=NMe_2, R^3=OMe
I-5: R^1=H, R^2=NMe_2, R^3=OH
I-6: R^1=H, R^2=OMe, R^3=NH_2
I-7: R^1=H, R^2=OMe, R^3=NHMe
I-8: R^1=H, R^2=OH, R^3=NHMe
I-9: R^1=H, R^2=OMe, R^3=NMe_2
I-10: R^1=H, R^2=OH, R^3=NMe_2
I-11: R^1=H, R^2=OMe, R^3=$N(CH_2CH_2)_2$
I-12: R^1=Br, R^2=OMe, R^3=NH_2
I-13: R^1=H, R^2=OMe, R^3=OH
I-14: R^1=Br, R^2=OMe, R^3=OH
I-15: R^1=Cl, R^2=OMe, R^3=OH
I-16: R^1=OMe, R^2=OMe, R^3=OH
I-17: R^1=Me, R^2=OMe, R^3=OH
I-18: R^1=NMe_2, R^2=OMe, R^3=OH

II-1: R^1=H, R^2=OMe, R^3=NH_2
II-2: R^1=H, R^2=OMe, R^3=NHMe
II-3: R^1=H, R^2=OMe, R^3=NMe_2
II-4: R^1=H, R^2=OH, R^3=NMe_2
II-5: R^1=H, R^2=NH_2, R^3=OMe
II-6: R^1=H, R^2=NHMe, R^3=OMe
II-7: R^1=H, R^2=NMe_2, R^3=OMe
II-8: R^1=H, R^2=NMe_2, R^3=OH
II-9: R^1=H, R^2=OMe, R^3=$N(CH_2CH_2)_2$
II-10: R^1=Br, R^2=OMe, R^3=NH_2
II-11: R^1=Br, R^2=OMe, R^3=OMe
II-12: R^1=H, R^2=H, R^3=NMe_2
II-13: R^1=Br, R^2=H, R^3=NMe_2
II-14: R^1=I, R^2=H, R^3=NMe_2
II-15: R^1=Br, R^2=OMe, R^3=OH
II-16: R^1=I, R^2=OMe, R^3=OH

Figure 1. Structures of bis-styrylpyridine and bis-styrylbenzene derivatives.



I-13' : R^1=H, R^2=OMe, R^3=OPMB
I-14' : R^1=Br, R^2=OMe, R^3=OPMB
I-15' : R^1=Cl, R^2=OMe, R^3=OPMB
I-16' : R^1=OMe, R^2=OMe, R^3=OPMB
I-17' : R^1=Me, R^2=OMe, R^3=OPMB
I-18' : R^1=NMe_2, R^2=OMe, R^3=OPMB
II-15' : R^1=Br, R^2=OMe, R^3=OPMB
II-16' : R^1=I, R^2=OMe, R^3=OPMB

I-6' : R^1=H, R^2=OMe, R^3=NO_2
I-12' : R^1=Br, R^2=OMe, R^3=NO_2
II-1' : R^1=H, R^2=OMe, R^3=NO_2
II-10' :R^1=Br, R^2=OMe, R^3=NO_2
I-1' : R^1=H, R^2=NO_2, R^3=OMe
II-5' : R^1=H, R^2=NO_2, R^3=OMe

Scheme 1. Synthesis of bis-styrylpyridine and bis-stylrylbenzene derivatives. Reagents and conditions: (a) triethylphosphite, 135 °C, 2.5 h; (b) 1 M ^{t}KOBu in THF, aldehyde (**V-1**–**7**), THF, rt, 0.5 h; (c—i) $SnCl_2$, EtOH, reflux, 3 h; (c—ii) 1 N HCl, EtOH, reflux, 3 h; (d) NaOMe, $(CH_2O)_n$, MeOH, $NaBH_4$, DMSO, reflux, 2 h; (e) AcOH, $(CH_2O)_n$, $NaCNBH_3$; (f) BBr_3, CH_2Cl_2, rt, 8 h.

Regarding the chemicals, new bis-styrylpyridine and bis-styrylbenzene derivatives were synthesized as shown in Scheme 1. Various synthetic 1,3-bis(bromomethyl)pyridines (or benzenes) (**III**) were reacted with triethyl phosphite by Arbuzov reaction to give the corresponding 1,3-bis(diethylphosphono-methyl)pyridines (or benzenes) (**IV**). The Horner–Emmons reaction of phosponates (IV) with synthetic aldehydes (V) (Fig. 2) is the key step to obtain only *trans–trans* (*E*, *E*) isomer in the presence of potassium *tert*-butoxide as a base, respectively.[11,12] To obtain a series of styryl compounds having hydroxyl group at C-4 and methoxy at C-3 in the terminal aromatic ring (Fig. 1), PMB-protected intermediates were consecutively deprotected (by c-ii in Scheme 1). [8] To obtain C-3 (or C-4) mono or dimethylaminostyrylpyridine (or benzene) derivatives, the intermediates bearing nitro group at C-3 (or C-4) position were reduced (by c-i in Scheme 1), then subsequently were served in reductive amination (by d or e in Scheme 1).[13] Finally, styryl compounds with both alkylamine and hydroxyl groups were prepared by demethylation using BBr_3 (by f in Scheme 1).[11,12]

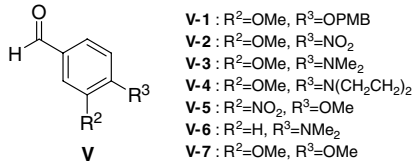


Figure 2. Synthetic aldehydes (**V-1**–**7**).

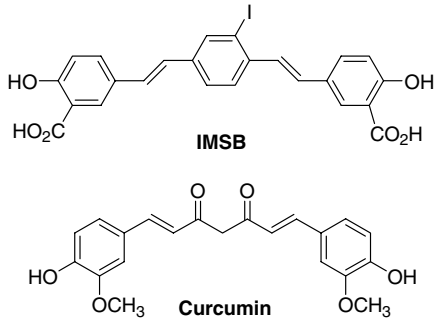


Figure 3. Structures of IMSB and curcumin.

In the design of the target compounds, the features of IMSB (Fig. 3)[11,12] were considered. Although IMSB has the excellent binding affinity for Aβ fibrils, its inhibitory activity for Aβ fibril formation was relatively low (IC_{50} of 8 μM) and brain uptake was hampered by the polar carboxylic acid group. Therefore, we replaced the carboxylic acid with a hydroxyl or alkylamine group to increase lipophilicity for penetration of blood–brain barrier (BBB) and enhance in vivo pharmacokinetic stability with high solubility. We then changed *p*-position of the styryl conjugated system to *m*-position as in curcumin.[15] It was reported[16] that low dose of curcumin effectively disaggregates Aβ as well as prevents fibril and oligomer formation in vitro and in vivo (aged Tg 2576 mice). Especially it is widely used as a food additive and has undergone extensive toxicological screening and preclinical investigation in vivo. Therefore, structural similarity of the synthetic compounds with curcumin possesses good opportunity to be a potent AD drug candidate.

Shown in Table 1, 27 compounds have an excellent inhibitory activity with IC_{50} of 0.1–2.7 μM and the curcumin, a reference compound, was assessed with IC_{50} of 0.8 μM.

Table 1. Biological activity of bis-styrylpyridines (**I-1**–**18**) and bis-styrylbenzenes (**II-1**–**16**) for inhibition of Aβ 42 fibril formation

Compound	Inhibition of fibril formation IC_{50} (μM)[a]	Compound	Inhibition of fibril formation IC_{50} (μM)[a]
I-1	0.6	**II-1**	0.6
I-2	1.1	**II-2**[17]	0.6
I-3	0.5	**II-3**	1.0
I-4	0.9	**II-4**	0.7
I-5	2.3	**II-5**	0.3
I-6	0.7	**II-6**	1.3
I-7[17]	0.5	**II-7**	1.1
I-8	0.9	**II-8**	2.7
I-9	0.8	**II-9**	>20
I-10	0.9	**II-10**	1.8
I-11[b]	-	**II-11**[b]	≃20
I-12	1.1	**II-12**	>20
I-13	0.8	**II-13**	>20
I-14	1.1	**II-14**	>20
I-15	1.1	**II-15**	0.1
I-16	1.7	**II-16**[b]	—
I-17	1.4	Curcumin	0.8
I-18	1.9	Acridine orange[14]	0.6
		IMSB	8

IC_{50} was calculated from nonlinear regression by Graphpad Prism software.
[a] ThT assay.
[b] These were not suitable to ThT assay because of their own high-fluorescence interference effect.

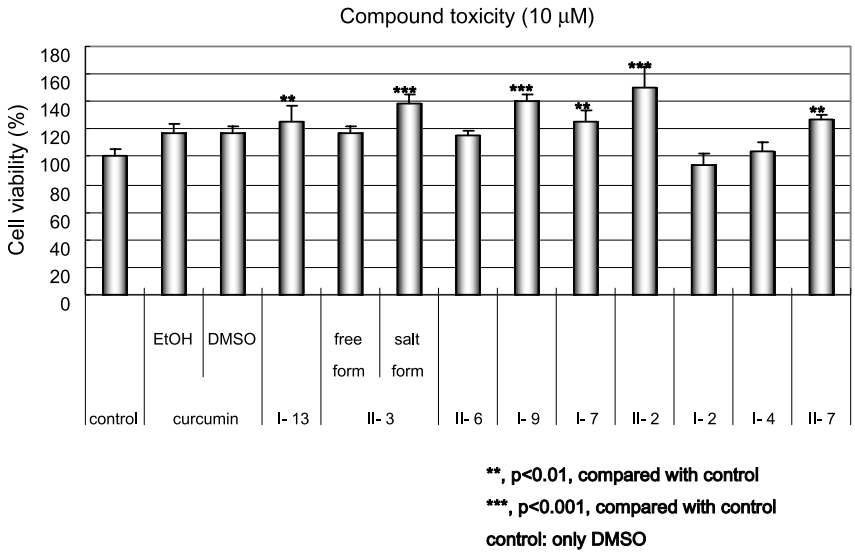


Figure 4. Compound (finally 10 μM) cytotoxicities by MTT assay on HT-22 cell. The cells were treated with the compounds in media with 10% fetal bovine serum and 1% penicillin/streptomycin (totally 0.5% DMSO). In control, the cells were only treated with the same media (0.5% DMSO).

In the structure–activity relationship, the central pyridine or benzene did not affect the IC_{50} and R^1 substituents in the central ring also did not show a meaningful effect (**I-13**–**18** in Table 1). The value of IC_{50} was increased slightly in order of halogen < Me < OMe < NMe_2. The compounds (**II-12**, **13**, and **14**) with only one substituent (dimethylamine) at C-4 position in the terminal aromatic ring showed lower activity than compound (**II-3**) with two substituents (dimethylamine and methoxy) both at C-3 and C-4.

Changing –OMe at C-4 (**II-11**) to –OH (**II-15**) or –NH_2 (**II-10**) significantly enhanced the inhibitory activity. These results suggest that the presence of two electron donating groups both at C-3 and C-4 positions probably plays a critical role to display higher activity, and at least one of them at C-3 or C-4 is necessary to be –NH_2, –NHMe, –NMe_2 or OH.[12] When R^3 substituent was replaced with NH_2, NMe_2 or $N(CH_2\ CH_2)_2$, inhibitory activity was decreased from 0.6, 1.0 to >20 μM (in **II-1**, **3**, and **9**).

Selected nine compounds were initially screened at low concentration of 1 μM for the compound cytotoxicity and then at high concentration of 10 μM. In Figure 4, **II-2** (150%) showed a predominant relative cell viability compared with control (100%) and curcumin (116%) at 10 μM. And HT-22 neuronal cells are mostly safe to the other compounds at high dose.

In conclusion, most of the synthesized compounds in this study inhibit Aβ 42 fibril formation near sub-μM range of IC_{50} and most of the compounds do not show their own cytotoxicities on HT-22 neuronal cell even at high concentration. In particular, **I-7** and **II-2** exhibited the best combination of inhibitory activity and compound cytotoxicity. Based on these results, they are more potent Aβ fibril formation inhibitors than curcumin.

Additional assays including in vitro BBB penetration, protection effect of toxicity induced by Aβ 42 fibrils, in vivo pharmacokinetics, and behavior tests on APP/PSI mice are currently in progress.

Acknowledgments

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9. Revine, H., III *Arch. Biochem. Biophys.* **2002**, *404*, 106, In vitro ThT assay: the final concentration of Aβ 42 (Bachem) was 25 μM. The excitation and emission wavelengths were 450 and 482 nm with 10 nm slit (LS-55 luminescence spectrometer: Perkin-Elmer).
10. MTT assay: MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added into the HT-22 cell.

Cell viability was estimated by measuring the absorbance of MTT formazan at 570 nm with a microplate reader. All compounds were tested as the HCl salt form. Compound **II-3** was tested in the state of a free-form and a HCl-salt form as an example.

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17. *Selected data*. Compound **I-7**: ^{1}H NMR (CD_3OD, 300 MHz) δ 2.86 (s, 6H), 3.93 (s, 6H), 6.59 (d, J = 7.83, 2H), 7.03 (d, J = 16.15, 2H), 7.12 (d, J = 8.35, 2H), 7.07 (s, 2H), 7.37 (d, J = 7.81, 2H), 7.52 (d, J = 16.14, 2H), 7.67 (t, J = 7.51, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 30.16, 55.46, 106.78, 108.65, 118.45, 122.29, 124.05, 125.33, 133.35, 136.58, 139.98, 146.87, 156.18; HRMS *m/z* Calcd for $C_{25}H_{27}N_3O_2$ $(M)^+$ 401.2103. Found: 401.2182. Anal. Calcd for $C_{25}H_{27}N_3O_2$: C, 74.79; H, 6.78; N, 10.47. Found: C, 74.51; H, 6.77; N, 10.12. **II-2**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.91 (s, 6H), 3.93 (s, 6H), 6.58 (d, J = 8.09, 2H), 6.94 (d, J = 16.23, 2H), 7.01 (d, J = 1.64, 2H), 7.07 (dd, J = 8.20, 1.71, 2H), 7.11 (d, J = 16.25, 2H), 7.33 (t, J = 4.63, 1H), 7.34 (d, J = 1.18, 2H), 7.61 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 30.44, 55.49, 106.66, 109.30, 121.11, 123.10, 124.40, 124.52, 127.36, 128.84, 129.39, 138.63, 139.11, 147.12; HRMS *m/z* Calcd for $C_{26}H_{28}N_2O_2$ $(M)^+$ 400.2151, Found 400.2151; Anal. Calcd for $C_{26}H_{28}N_2O_2$: C, 77.97; H, 7.05; N, 6.99. Found: C, 78.41; H, 7.18; N, 6.57.