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SYNTHESIS AND EVALUATION OF 4-ARYL-2(1*H*)-QUINOLINONES AS POTENT AMYLOID β FIBRILLOGENESIS INHIBITORS

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Abstract – 4-Aryl-2(1*H*)-quinolinones were synthesized and evaluated *in vitro* as inhibitors of $A\beta_{1-42}$ fibrillogenesis using a thioflavin T fluorescence method. The most potent anti-aggregating molecules (**4b** and **5c**) were found among the derivatives bearing OH and/or OMe groups at C-4' (R^4) and/or C-6 (R^2) of the 4-aryl-2(1*H*)-quinolinone moiety. Furthermore, the derivative bearing 4'-F substituent (**4f**) proved to be a very active inhibitor.

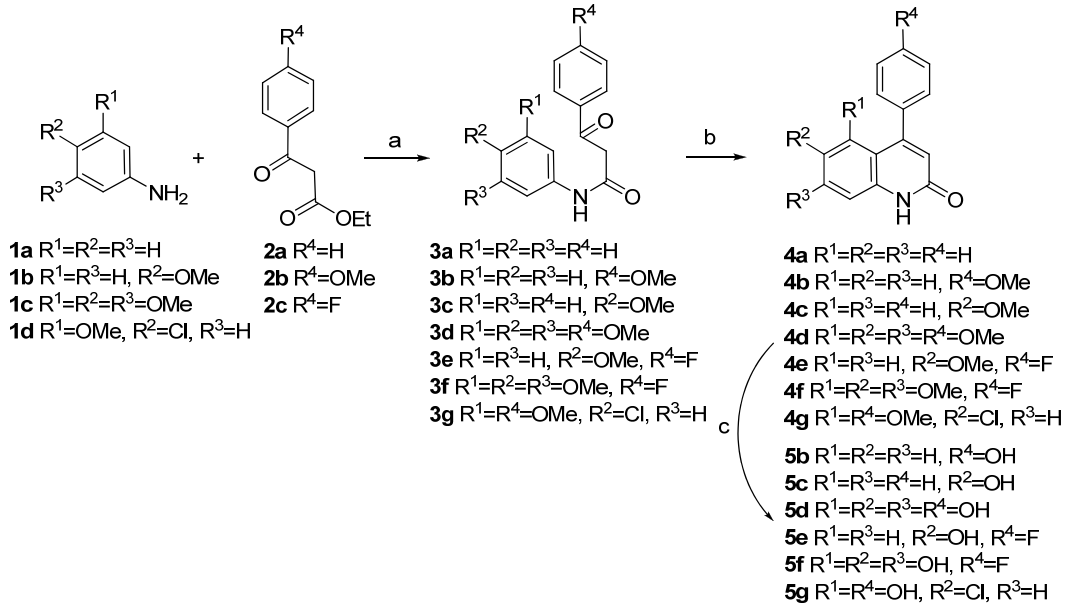
INTRODUCTION

Alzheimer's disease (AD) is a progressive, degenerative disease of the brain and recognized as the leading cause of dementia in the aging population. AD is characterized by the aggregation of amyloid β peptides (Aβ) into amyloid plaques in selected areas of brain.[1] Aβ is a 39- to 43-residue secretase cleavage product of the amyloid precursor protein (APP). The most common forms in extracellular fluids are a 40-residue fragment ($A\beta_{1-40}$, *ca* 90%) and a more aggregation-prone and toxic 42-residue fragment ($A\beta_{1-42}$, *ca* 10%). The accumulation of Aβ as amyloid in the brain is caused by an imbalance in the production and clearance of these peptides.[2]

A number of efforts in searching for inhibitors of Aβ aggregation has demonstrated that small molecules, containing aromatic rings often bearing hydroxy groups, can inhibit protein–protein interactions.[3] Recently we have found two types of inhibitors of Aβ, which would be distyrylbenzene derivatives[4] and 2-substituted benzofurans.[5] In continuation of our focus on developing low-molecular weight inhibitors of Aβ, we were interested in the synthesis of 4-aryl-2(1*H*)-quinolinones, as structural analogs of recently reported 2-arylbenzofurans.[5] Although the quinolinones have wide-ranging biological activities,[6] such as anti-malarial[6b] and anti-bacterial[6c] activities, there is no report on inhibition of Aβ fibril formation by the quinolinones. We here report the synthesis of 4-aryl-2(1*H*)-quinolinones and their activity as inhibitors of $A\beta_{1\text{-}42}$ fibirillogenesis using a thioflavin T (ThT) fluorescence assay.[7]

RESULTS AND DISCUSSION

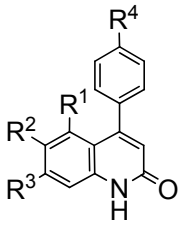
There are many methods for the synthesis of the 4-aryl-2(1*H*)-quinolinones based on cyclization approaches,[8-10] including Friedländer cyclization,[8] Knorr cyclization,[9] and transition metal-catalyzed cyclizations.[10] We prepared 4-aryl-2(1*H*)-quinolinones employing the Knorr cyclization of the corresponding benzoylacetoanilides **3a-g** as shown in Scheme 1. Amidation between anilines **1a-d** and benzoylacetate **2a-c** in pyridine gave benzoylacetoanilides **3a-g**, which were treated with polyphosphoric acid (PPA) at 95 °C to afford 4-aryl-2(1*H*)-quinolinones **4a-g** in moderate to good yields. Demethylation of **4b-g** was achieved by heating with pyridine hydrochloride in either an oil bath or a microwave oven to give phenols **5b-g** in 78-98% yields.



Scheme 1. Reagents and conditions: a. pyridine, reflux; b. polyphosphoric acid, 95 °C; c. pyridine hydrochloride, heat

The inhibitory effects on the $A\beta_{1-42}$ fibrillogenesis of 4-aryl-2(1*H*)-quinolinones **4a-g** and **5b-g** were evaluated by ThT fluorescence assay. For all the synthesized compounds we measured the percent of fibril formation inhibition. The inhibition data are summarized in Table 1. The core structure, 4-phenyl-2(1*H*)-quinolinone **4a** lacking hydroxyl or methoxy groups, is inactive. With regard to monosubstituted derivatives, the derivatives bearing 4'-OMe (**4b**), 4'-OH (**5b**) and 6-OH (**5c**) groups showed the most potent activities. In general, the derivatives bearing hydroxyl group (**5c** and **5d**) showed higher activities compared to the corresponding methoxy congeners (**4c** and **4d**). It is worth noting that the introduction of 4'-F substituent (**5e** and **5f**) on the phenyl ring of the potent inhibitors (**5c** and **5d**) lost their activities, whereas the respective methoxy derivatives (**4e** and **4f**) improved their activities. The introduction of 6-Cl substituent (**4g** and **5g**) on the phenyl ring of the quinolinone did not exhibit any effect of interest.

Table 1. Inhibition data of $A\beta_{1-42}$ fibrillogenesis by 4-aryl-2(1*H*)-quinolinones **4a-g** and **5b-g**



compound	R^1	R^2	R^3	R^4	inhibition ±SD [a] (%)
4a	H	H	H	H	— [c]
4b	H	H	H	OMe	84.4±1.5 ** [b]
4c	H	OMe	H	H	— [c]
4d	OMe	OMe	OMe	OMe	— [c]
4e	H	OMe	H	F	74.7±2.4 ** [b]
4f	OMe	OMe	OMe	F	85.5±2.4 ** [b]
4g	OMe	Cl	H	OMe	8.9±13.0
5b	H	H	H	OH	71.3±6.3 ** [b]
5c	H	OH	H	H	81.8±0.6 ** [b]
5d	OH	OH	OH	OH	60.4±15.7 ** [b]
5e	H	OH	H	F	— [c]
5f	OH	OH	OH	F	— [c]
5g	OH	Cl	H	OH	4.7±4.5

[a] Data are means±SD (n=5). [b] Dunnett test: **p<0.01
[c] — : not active

The inhibitory behavior of compound **5c** was then characterized with transmission electron microscopy (TEM) to assess quantity and shape of fibril. TEM images confirmed the conformational changes observed in ThT fluorescence spectroscopy studies above described (Figure 1).

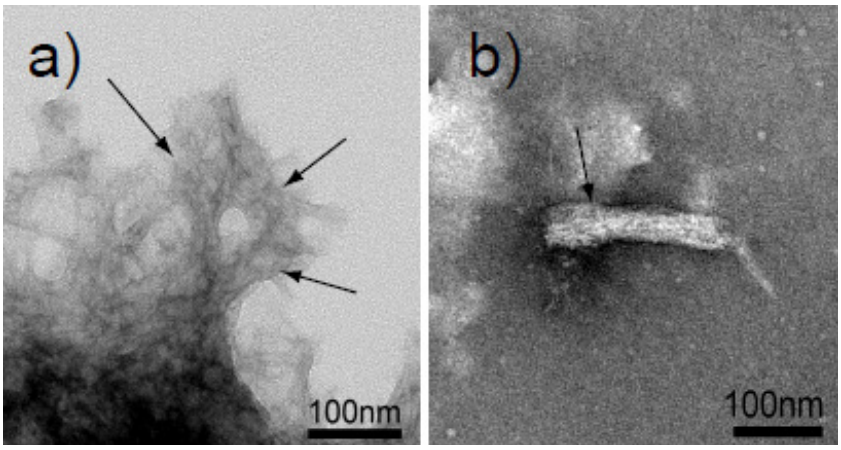


Figure 1. Representative transmission electron images indicating the inhibition of $A\beta_{1-42}$ fibrillogenesis by compound **5c**
(a) TEM image of $A\beta_{1-42}$ fibrils formed without any added inhibitor compound.
(b) TEM image of $A\beta_{1-42}$ peptide incubated with compound **5c**.

TEM images provide strong support for the ThT data shown above. They indicate significant difference between inhibitor-free Aβ control sample (Figure-1a) and sample incubated with inhibitor **5c** (Figure-1b). While inhibitor-free peptide formed the expected network of long fibers, drastic lack of such aggregates can be observed in the presence of **5c**. Thus, we confirmed the antifibrillogenic activity for Aβ fibrillogenesis inhibitors, 4-aryl-2(1*H*)-quinolinones **4b**, **4e**, **4f**, **5b-d**.

CONCLUSION

4-Aryl-2(1*H*)-quinolinones were synthesized and evaluated *in vitro* as inhibitors of $A\beta_{1-42}$ fibrillogenesis using a ThT fluorescence method. The most potent anti-aggregating molecules (**4b** and **5c**) were found among the derivatives bearing OH and/or OMe groups at C-4' (R^4) and/or C-6 (R^2) of the 4-aryl-2(1*H*)-quinolinone moiety. Furthermore, the derivative bearing 4'-F substituent (**4f**) proved to be a very active inhibitor. Thus, the 4-aryl-2(1*H*)-quinolinone would be a scaffold for a new class of low-molecular weight inhibitors of $A\beta_{1-42}$ aggregation. Efforts to understand inhibition mechanism of this class of compounds will be the focus of future efforts.

EXPERIMENTAL

Chemistry

IR spectra were obtained using a Shimadzu FT/IR Prestige-21 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AV 400 spectrometer, and chemical shifts were referenced to the residual solvent peaks (δ_H 7.26 and δ_C 77.0 for $CDCl_3$, and δ_H 2.49 and δ_C 39.7 for DMSO-d_6). MS spectra were measured with a JEOL JMS-T100LP spectrometer. Melting points were determined on a Yanaco micro melting apparatus MP-J3 and are uncorrected.
Known 4-aryl-2(1*H*)-quinolinones (**4a**,[11] **4b**,[9b] **4c**[12]) were prepared from **3a-c** according to the literature.[9]

General procedure for amidation between anilines 1a-d and 3-aryl-oxopropanoates 2a-c

A mixture of aniline **1** (1.0 mmol) and ethyl 3-aryl-3-oxopropanoate **2** (1.0－1.8 mmol) in pyridine (0.25 mmol) was refluxed for 4－24 h. Evaporation of solvent gave a residue, which was purified by silica gel column chromatography (hexane/EtOAc) to afford solid. The solid was recrystallized from EtOAc.

[*N*-(3,4,5-Trimethoxyphenyl)-*β*-(4-methoxyphenyl)-*β*-oxo]propanamide 3d

Reaction of **1c** and **2b** gave **3d** in 53% yield; mp 128-129 °C (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 3.81 (s, 3H), 3.86 (s, 6H), 3.90 (s, 3H), 4.05 (s, 2H), 6.89 (s, 2H), 6.99 (ddd, 2H, J = 9.0, 2.8, 2.0 Hz), 8.02 (ddd, 2H, J = 9.0, 2.8, 2.0 Hz), 9.38 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 45.1, 55.6, 56.0, 60.9, 97.6, 114.1, 128.9, 131.1, 133.8, 134.6, 153.2, 164.0, 164.6, 194.8. IR (thin film) ν_{max} 3327, 2939, 1602, 1508, and 1129 cm^{-1}; HRESITOFMS *m*/*z* 360.1489 (M^++H; calcd for $C_{19}H_{22}NO_6$, 360.1447).

[*N*-(*p*-Methoxyphenyl)-*β*-(*p*-fluorophenyl)-*β*-oxo]propanamide 3e

Reaction of **1b** and **2c** gave **3e** in 53% yield; mp 141 °C (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 3.79 (s, 3H), 4.07 (s, 2H), 6.86 (ddd, 2H, J = 8.8, 3.6, 2.0 Hz), 7.18 (dddd, 2H, J = 9.0, 8.4, 2.8, 2.0 Hz), 7.47 (ddd, 2H, J = 8.8, 3.6, 2.0 Hz), 8.08 (dddd, 2H, J = 9.0, 5.4, 2.8, 2.0 Hz), 9.04 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 45.6, 55.5, 114.1, 116.2 (d, J = 87.6 Hz), 121.9, 130.6, 131.5 (d, J = 38.4 Hz), 132.5 (d, J = 11.6 Hz), 156.6, 167.7, 194.8. IR (thin film) ν_{max} 3311 and 1509 cm^{-1}; HRESITOFMS *m*/*z* 288.1053 (M^++H; calcd for $C_{16}H_{15}FNO_3$, 288.1036).

[*N*-(3,4,5-Trimethoxyphenyl)-*β*-(*p*-fluorophenyl)-*β*-oxo]propanamide 3f

Reaction of **1c** and **2c** gave **3f** in 78% yield; mp 160 °C (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 3.81 (s, 3H), 3.86 (s, 6H), 4.08 (s, 2H), 6.87 (s, 2H), 7.20 (dddd, 2H, J = 9.0, 8.4, 2.8, 2.0 Hz), 8.08 (dddd, 2H, J = 9.0, 5.4, 2.8, 2.0 Hz), 9.12 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 45.7, 56.1, 60.9, 97.6, 116.2 (d, J = 87.6 Hz), 131.4 (d, J = 38.4 Hz), 132.4 (d, J = 11.2 Hz), 133.6, 134.7, 153.2, 163.5, 165.2, 167.7, 194.7. IR (KBr) ν_{max} 3352, 1669, 1510, and 1131 cm^{-1}; HRESITOFMS *m*/*z* 348.1251 (M^++H; calcd for $C_{18}H_{19}FNO_5$, 348.1247).

[*N*-(4-Chloro-3-methoxyphenyl)-*β*-(*p*-methoxyphenyl)-*β*-oxo]propanamide 3g

Reaction of **1d** and **2b** gave **3g** in 73% yield; mp 134-136 °C (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 3.90 (s, 3H), 3.91 (s, 3H), 4.05 (s, 2H), 6.98 (d, 2H, J = 8.8 Hz), 7.00 (dd, 8.8, 2.4 Hz), 7.28 (d, 8.8 Hz), 7.48 (d, 2.4 Hz), 8.01 (d, 8.8 Hz), 9.58 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 44.7, 55.6, 56.2, 104.5, 112.4, 114.2, 117.5, 128.9, 130.0, 131.1, 137.5, 155.1, 164.1, 164.7, 194.9. IR (KBr) ν_{max} 3339, 2926, 1695, and 1604 cm^{-1}; HRESITOFMS *m*/*z* 334.0880 (M^++H; calcd for $C_{17}H_{17}ClNO_4$, 334.0846).

General procedure for cyclization of propanamides 3d-g

A mixture of propanamide **3** (1.0 mmol) and polyphosphoric acid (4.0 g) was heated at 95 °C for 1.5-24 h. The mixture was poured into ice water and the precipitated solid was filtered and washed with ice water. The solid was recrystallized from EtOH.

5,6,7-Trimethoxy-4-(*p*-methoxyphenyl)-2(1*H*)-quinolinone 4d

Cyclization of **3d** gave quinolinone **4d** in a quantitative yield; mp 278 °C (EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 3.27 (s, 3H), 3.81 (s, 3H), 3.87 (s, 3H), 3.98 (s, 3H), 6.38 (s, 1H), 6.73 (s, 1H), 6.93 (ddd, 2H, J = 8.8, 2.8, 2.0 Hz), 7.29 (ddd, 2H, J = 8.8, 2.8, 2.0 Hz), 12.59 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 55.3, 56.2, 60.9, 61.1, 94.4, 108.6, 112.6, 120.0, 129.0, 133.2, 136.8, 138.7, 151.0, 152.5, 156.6, 159.0, 163.7. IR (KBr) ν_{max} 2961, 1654, 1613, 1360, 1244, and 1118 cm^{-1}; HRESITOFMS *m*/*z* 342.1377 (M^++H; calcd for $C_{19}H_{20}NO_5$, 342.1342).

6-Methoxy-4-(*p*-fluorophenyl)-2(1*H*)-quinolinone 4e
Cyclization of **3e** gave quinolinone **4e** in 67% yield; mp 289 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 3.64 (s, 3H), 6.39 (s, 1H), 6.78 (d, 1H, J = 2.4 Hz), 7.22 (dd, 1H, J = 8.8, 2.4 Hz), 7.34 (d, 1H, J = 8.8 Hz), 7.36 (dd, 2H, J = 9.2, 8.8 Hz), 7.53 (dd, 2H, J = 8.8, 5.0 Hz), 11.80 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 55.5, 108.2, 115.9 (d, J = 86.0 Hz), 117.3, 119.0, 119.5, 122.2, 131.1 (d, J = 32.8 Hz), 133.3 (d, J = 12.4 Hz), 134.0, 150.1, 154.3, 161.0, 161.3, 163.8. IR (KBr) ν_{max} 2837, 1655, 1498, 1422 and 1226 cm^{-1}; HRESITOFMS m/z 270.0929 (M^++H; calcd for $C_{16}H_{13}FNO_2$, 270.0930).

4-(*p*-Fluorophenyl)- 5,6,7-trimethoxy-2(1*H*)-quinolinone 4f
Cyclization of **3f** gave quinolinone **4f** in 79% yield; mp 282-283 °C (EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 3.26 (s, 3H), 3.78 (s, 1H), 3.95 (s, 3H), 6.37 (s, 1H), 6.81 (s, 1H), 7.07 (s, 2H), 7.30 (s, 2H), 13.22 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 56.3, 60.7, 61.0, 94.6, 108.5, 114.2 (d, J = 86.0 Hz), 119.5, 129.3 (d, J = 31.6 Hz), 136.7 (d, J = 12.4 Hz), 136.8, 138.8, 150.7, 151.8, 156.8, 160.9, 163.4, 163.6. IR (KBr) ν_{max} 3432, 2931, and 1667 cm^{-1}; HRESITOFMS m/z 330.1137 (M^++H; calcd for $C_{18}H_{17}FNO_4$, 330.1142).

6-Chloro-4-(*p*-methoxyphenyl)-7-methoxy-2(1*H*)-quinolinone 4g
Cyclization of **3g** gave quinolinone **4g** in 90%; mp > 300 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 3.83 (s, 3H), 3.90 (s, 3H), 6.24 (s, 1H), 7.04 (s, 1H), 7.10 (d, 2H, J = 8.8 Hz), 7.34 (s, 1H), 7.40 (d, 2H, J = 8.8), 11.82 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 55.5, 56.5, 98.9, 113.0, 114.5, 115.8, 119.1, 126.8, 128.7, 130.1, 140.1, 150.4, 156.1, 160.0, 161.7. IR (KBr) ν_{max} 3446, 2955, 1666, 1609, and 1252 cm^{-1}; HRESITOFMS m/z 316.0781 (M^++H; calcd for $C_{17}H_{15}ClNO_3$, 316.0741).

Demethylation of aryl methyl ethers 4b-g
Method A (Heating in a microwave oven)
A mixture of 2(1*H*)-quinolinone (**4b**, **4c** and **4f**; 100 mg) and pyridine hydrochloride (1.0 g) was heated in a microwave at 210 °C for 20 min. The mixture was poured into water. The precipitates were filtered to afford a brown crude product. The solid was recrystallized from EtOH.
Method B (Heating in an oil bath)
A mixture of 2(1*H*)-quinolinone (**4d**, **4e** and **4g**; 100 mg) and pyridine hydrochloride (1.0 g) was heated in an oil bath at 225 °C for 15－50 min. The same workup as Method A was carried out.

4-*p*-Hydroxyphenyl-2(1*H*)-quinolinone 5b
98% yield; mp > 300 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.31 (s, 1H), 6.90 (d, 2H, J = 8.4 Hz), 7.13 (dd, 1H, J = 7.6, 7.4 Hz), 7.29 (d, 2H, J = 8.4 Hz), 7.36 (d, 1H, J = 7.8 Hz), 7.47 (d, 1H, J = 8.4 Hz), 7.50 (1H, dd, J = 7.8, 7.6 Hz), 11.79 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 115.7, 116.0, 118.8, 120.8, 121.9, 126.5, 127.4, 130.3, 130.6, 139.5, 151.8, 158.3, 161.6. IR (KBr) ν_{max} 3010 and 1658 cm^{-1}; HRESITOFMS m/z 238.0837 (M^++H; calcd for $C_{15}H_{12}NO_2$, 238.0868).

6-Hydroxy-4-phenyl-2(1*H*)-quinolinone 5c
92% yield; mp 273 °C (decomp.) (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.32 (s, 1H), 6.75 (d, 1H, J = 2.8 Hz), 7.01 (dd, 1H, J = 8.8, 2.8 Hz), 7.24 (d, 1H, J = 8.8 Hz), 7.43-7.56 (m, 5H), 9.33 (s, 1H), 11.68 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 110.1, 117.1, 119.4, 120.2, 121.6, 128.8, 128.8, 128.9, 132.8, 137.3, 151.1, 152.3, 161.0. IR (KBr) ν_{max} 3453, 3000, 1652, 1399, and 1275 cm^{-1}; HRESITOFMS m/z 238.0849 (M^++H; calcd for $C_{15}H_{12}NO_2$, 238.0868).

5,6,7-Trihydroxy-4-(*p*-hydroxyphenyl)-2(1*H*)-quinolinone 5d
84% yield; mp > 300 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 5.78 (s, 1H), 6.39 (s, 1H), 6.71 (d, 2H, J = 8.2 Hz), 7.08 (d, 2H, J = 8.2 Hz), 8.32 (s, 1H), 9.42 (s, 1H), 10.06 (s, 1H), 11.35 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 92.5, 102.6, 114.2, 118.1, 128.0, 129.3, 132.5, 134.8, 144.2, 150.3, 151.8, 156.9, 161.5. IR (KBr) ν_{max} 3368, 3148, and 1648 cm^{-1}; HRESITOFMS *m*/*z* 286.0710 (M^++H; calcd for $C_{15}H_{12}NO_5$, 286.0716).

6-Hydroxy-4-(*p*-fluorophenyl)-2(1*H*)-quinolinone 5e

87% yield; mp > 300 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.33 (s, 1H), 6.72 (s, 1H), 7.02 (d, 1H, *J* = 8.6 Hz), 7.25 (d, 1H, *J* = 8.6 Hz), 7.37 (dd, 2H, *J* = 8.8, 8.0 Hz), 7.50 (dd, 2H, *J* = 8.0, 5.6 Hz), 11.69 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 110.0, 115.8 (d, *J* = 86.0 Hz), 117.1, 119.4, 120.3, 121.8, 131.0 (d, *J* = 33.2 Hz), 132.8, 133.6 (d, *J* = 11.6 Hz), 150.0, 152.3, 161.0, 161.3, 163.7. IR (KBr) ν_{max} 3043, 1656, and 1505 cm^{-1}; HRESITOFMS *m*/*z* 256.0774 (M^++H; calcd for $C_{15}H_{11}FNO_2$, 256.0774).

4-(*p*-Fluorophenyl)- 5,6,7-trihydroxy-2(1*H*)-quinolinone 5f

78% yield; mp > 300 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 5.78 (s, 1H), 6.39 (s, 1H), 7.14 (dddd, 2H, *J* = 9.2, 8.8, 2.8, 2.4 Hz), 7.29 (dddd, 2H, *J* = 8.8, 5.6, 2.8, 2.4 Hz), 8.20 (brs, 1H), 8.42 (s, 1H), 10.08 (s, 1H), 11.38 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 92.5, 102.3, 114.1 (d, *J* = 84.8 Hz), 118.4, 128.1, 130.0 (d, *J* = 32.4 Hz), 134.7, 138.2 (d, *J* = 13.2 Hz), 143.9, 150.4, 150.5, 160.4, 161.1, 162.8. IR (KBr) ν_{max} 3306, 2935, 1648, 1507, 1412, and 1312 cm^{-1}; HRESITOFMS *m*/*z* 310.0491 (M^++H; calcd for $C_{15}H_{10}FNO_4Na$, 310.0492).

6-Chloro-4-(*p*-hydroxyphenyl)-7-hydroxy-2(1*H*)-quinolinone 5g

92% yield; mp > 300 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.12 (s, 1H), 6.90 (ddd, 2H, *J* = 8.4, 2.8, 1.8 Hz), 6.98 (s, 1H), 7.26 (ddd, 2H, *J* = 8.4, 2.8, 1.8 Hz), 7.30 (s, 1H), 9.82 (s, 1H), 11.03 (s, 1H), 11.71 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 101.9, 112.8, 115.5, 115.8, 118.0, 127.2, 127.4, 130.2, 140.0, 151.1, 155.2, 158.4, 162.1. IR (KBr) ν_{max} 3323, 3125, 1657, 1610, 1400, 1256, and 1245 cm^{-1}; HRESITOFMS *m*/*z* 288.0435 (M^++H; calcd for $C_{15}H_{11}ClNO_3$, 288.0428).

Biological assay

Inhibition of Aβ$_{1-42}$ fibrillogenesis by synthetic compounds 4a-g and 5b-g

Aβ$_{1-42}$ (Peptide Institute, Japan) 0.5 mg was dissolved in HIPF (0.5 mL). This solution was treated by the supersonic wave for 10 min and concentrated at 60 °C in vacuo. The Aβ$_{1-42}$ (0.5 mg) was dissolved in DMSO (0.5 mL) and this solution was stocked at -20 °C. Aβ$_{1-42}$ (5 μM) was incubated (37 °C, 24 h) with quinolinone (20 μM) in Tris–HCl (pH 7.6, 50 mM). Control was incubated without quinolinone under the same conditions. Incubations were run in quintuplicate.

Thioflavin T (ThT) fluorescence assay

To perform ThT fluorescence assay on our samples, we followed the well-established protocol of H. Levine III.[7] The solution of ThT (1 μM) used for fluorimetric measures was prepared in water. For each measurement, ThT solution (0.2 μmol) was added. Fluorescent measurements were made using JASCO FP-6500 spectrofluorimeter at 25 °C. The measurement condition was the emission wavelength: 480 nm and the excitation wavelength: 450 nm.

Observation of Transmission Electron Microscope (TEM)

Samples of inhibition of Aβ$_{1-42}$ aggregation and fibril formation of Aβ$_{1-42}$ fibril were observed by a JEOL JEM-1400F transmission electron microscope at an accelerating voltage of 80 kV. The negative staining used the 2% ytterbium triacetate solution on carbon-coated copper grids (400 mesh).

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