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Synthesis of dihydrofuran-fused perhydrophenanthrenes having a phenolic hydroxyl group as a novel anti-Alzheimer's disease agent

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ABSTRACT

As a part of our research program on developing novel anti-Alzheimer's disease medicines, several dihydrofuran-fused perhydrophenanthrenes (**DFs**) possessing a phenolic hydroxyl group were found to exhibit potent dendritic and axonal regeneration activities. Introduction of a methoxy group into the perhydrophenanthrene skeleton was successfully achieved via a $PhI(OAc)_2$-mediated phenolic oxidation of a benzocyclobutene nucleus and subsequent tandem intramolecular electrocyclic reactions based on *o*-quinodimethane chemistry. We could reveal that a new methoxy derivative having a phenolic hydroxyl group exerted the most significant effects on the dendritic and axonal extensions in the damaged neurons, among **DFs** examined in this study.

Alzheimer's disease, which is caused by the neuronal damages in the brain,[1] is one of the most serious medical problems and requires a novel drug for the fundamental cure in place of the symptomatic treatment with cholinesterase inhibitors such as Aricept (Eisai). In the last decade, several strategies for lowering amyloid β (Aβ) have been studied in basic research and clinical trials, instead of enhancing cholinergic function, because the Aβ targeting is an etiology-reducing approach for Alzheimer's disease. However, a decrease in the level of Aβ does not reduce advanced memory disorder.[2] If the brain function can be improved by reactivating and re-establishing a nervous network, it may be possible to return to a normal memory even in the state of neuronal degeneration. For the purpose of exploring novel anti-Alzheimer's disease medicines with neuro-regeneration, we recently focused on an Indian folk medicine Ashwagandha, which was taken to promote learning and memory,[3] and finally revealed that withanoside IV and its active metabolite sominone induced axonal and dendritic regeneration and synaptic reconstruction.[4] Furthermore, we found that denosomin, the structurally simplified derivative of sominone, demonstrated a significant axonal extension effect in Aβ-damaged neurons.[5] In this context, we turned our attention into dihydrofuran-fused perhydrophenanthrenes (**DFs**) for further extension of the foregoing researches, because this series of compounds have been proved to have high potential as a various drug lead with a variety of bioactivities. For example, we had previously reported that **DF-1**, **DF-2**, and **DF-6** exhibited potent antivirus activities against HVJ[6a] or various influenza viruses,[6b–d] and that **DF-2** also significantly induced an apoptotic tumor cell death under the hyperthermia conditions.[6e] These **DF** derivatives commonly possess a hydrophobic ethereal substituent on the aromatic ring, such as trifluoromethyl, trialkylsilyl, and benzyl-type appendages (Fig. 1).

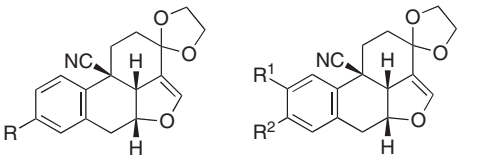

Figure 1. Bioactive dihydrofuran-fused compounds (**DFs**).

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On the other hand, new **DF** congeners possessing a phenolic hydroxyl group (a hydrophilic substituent) have been found to exhibit a potent anti-Alzheimer effect as a dendritic and axonal regenerator in this research. We will report herein the efficient synthesis and biological assessment of novel phenol-type **DF** derivatives, utilizing hypervalent iodine oxidation[7] and original *o*-quinodimethane chemistry of benzocyclobutenes.[8,9]

At first, we surveyed the known **DF** derivatives (**DF-1** to **DF-7**), which have been synthesized previously,[6a,b,d] on the dendritic extension activity in Aβ-damaged neurons (Fig. 2). The compounds **DF-1 to DF-7** (1 μM) were administered to rat cortical neurons after 4 days treatment with Aβ, and dendrite lengths were quantified after further 4 days. In the cells treated with 10 μM Aβ followed by vehicle (0.1% DMSO instead of **DFs**), the length of the dendrite was obviously shorter than that in control cells (without Aβ treatment). However, **DF-3**, which was equipped with a phenolic hydroxyl group, was revealed to exhibit the significant dendrite re-extension activity, while the other **DFs** did not show any activities as shown in Figure 2. This behavior markedly contrasts with the previous biological profile of **DFs** requiring a hydrophobic substituent on the aromatic ring.[6]

These results prompted us to investigate the other **DF** derivatives bearing a phenolic hydroxyl group, and we prepared 10-hydroxyperhydrophenathrene derivative **DF-8**,[10] regioisomer of **DF-3**, by simple deprotection of formerly reported **DF-2′**[6b] as shown in Scheme 1.

For the purpose of getting further information concerning effects of oxygen functionalities on the bioactivity, we examined the syntheses of novel mono-protected catechol derivatives (**DF-9** and **DF-10**). Since the **DF** tetracyclic framework containing an enol ether substructure is rather susceptible to harsh reaction conditions, an oxidative introduction of the oxygen functions on advanced perhydrophenanthrenes is out of our choice. In addition, a chemoselective functionalization of the two hydroxyl groups on a catechol moiety in the early stage of the synthesis seemed to be difficult. Consequently, we planned to set up them from the corresponding

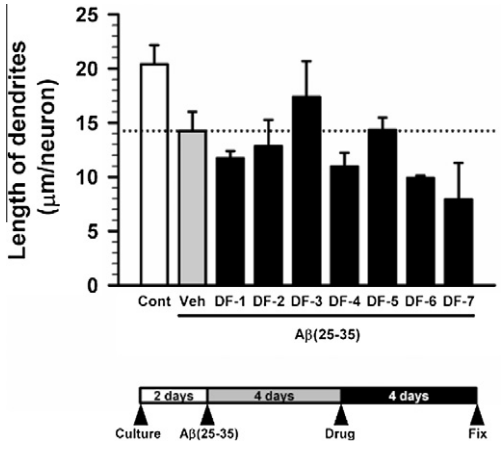
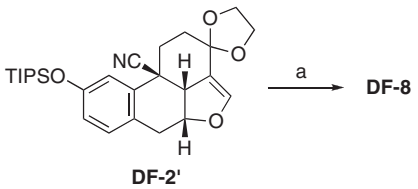
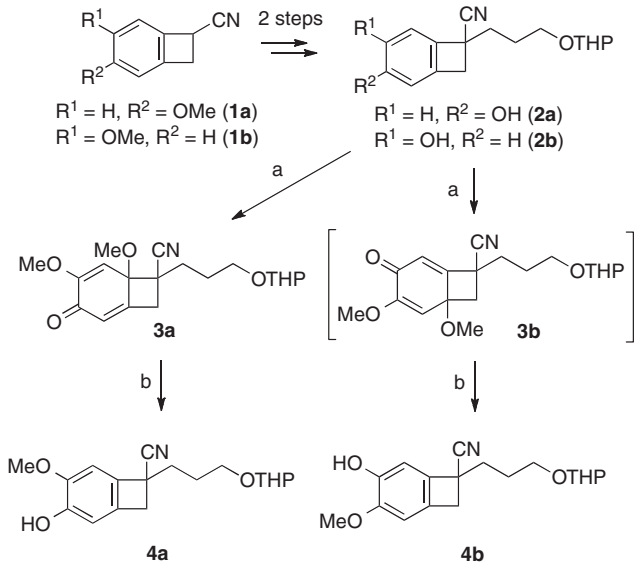


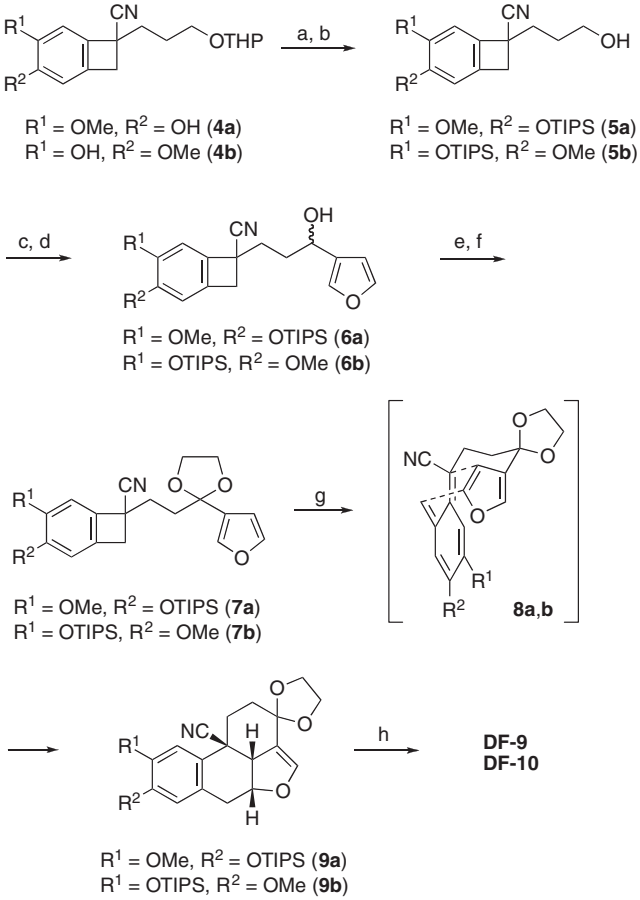
Figure 2. Effect of **DF-1** to **DF-7** on the dendritic extension in Aβ-damaged neurons.



Scheme 1. Synthesis of **DF-8**. Reagents and conditions: (a) TBAF, THF, rt, 95%.



Scheme 2. Hypervalent iodine oxidation of benzocyclobutenes **2a** and **2b**. Reagents and conditions: (a) $PhI(OAc)_2$ (2.2 equiv), MeOH, rt, 51% (**3a**); (b) Zn, THF/H_2O, rt, 85% (**4a**) and 13% (**4b**, 2 steps).



Scheme 3. Synthesis of **DF-9** and **DF-10**. Reagents and conditions: (a) TIPSOTf, NEt_3, CH_2Cl_2, rt; (b) PPTS, EtOH, reflux, 77% (**5a**, 2 steps) and 71% (**5b**, 2 steps); (c) SO_3·Py, NEt_3, DMSO, rt; (d) 3-lithiofuran, Et_2O, −78 °C to 0 °C, 30% (**6a**, 2 steps) and 38% (**6b**, 2 steps); (e) SO_3·Py, NEt_3, DMSO, rt; (f) ethylene glycol, TsOH, benzene, reflux, 62% (**7a**, 2 steps) and 66% (**7b**, 2 steps); (g) *o*-dichlorobenzene, reflux, 65% (**9a**) and 65% (**9b**); h) TBAF, THF, rt, 84% (**DF-9**) and quant. (**DF-10**).

monohydroxy benzocyclobutenes via the phenolic oxidation (Scheme 2).[7] The benzocyclobutene derivative **2a**, which was prepared in the established manner from **1a**,[6a] was once oxidized by 2 equiv of $PhI(OAc)_2$ in the presence of MeOH to afford the desired methoxyquinone derivative **3a** with satisfactory yield. Successive reductive rearomatization smoothly proceeded to give the requisite methoxybenzocyclobutene **4a** within 85% yield. In the same manner, the regioisomeric product **4b** could be obtained from **2b**,[6b] albeit rather in low yield because of the instability of the intermediate **3b**, which could not be isolated.

With these methoxybenzocyclobutenes in hand, we then transferred these compounds into the corresponding perhydrophenanthrenes by means of *o*-quinodimethane chemistry (Scheme 3). After protection of the phenolic hydroxyl group on **4a** as TIPS ether, acidic hydrolysis of THP ether gave the alcohol **5a**. Parikh-Döring oxidation and subsequent nucleophilic addition of lithiofuran to the resulting aldehyde furnished the alcohol **6a**. The compound **7a** as a precursor of the *o*-quinodimethane **8a** was prepared via oxidation followed by acetalization of the resultant ketone. By the thermal treatment of **7a** in refluxing *o*-dichlorobenzene, intramolecular cycloaddition via *o*-quinodimethane intermediate proceeded uneventfully to furnish the desired perhydrophenanthrene **9a** as a single diastereomer through an exclusive *endo* transition state **8a**. The stereochemistry of **9a** was determined as an all-*cis* configuration by the comparison of the ^1H NMR spectra of **9a** with that of another perhydrophenanthrene derivative whose structure was unambiguously confirmed by the X-ray crystallographic analysis.[6a] Finally, desilylation of **9a** by the treatment with TBAF quantitatively accomplished **DF-9**.[11] In accordance with the preparation of **DF-9**, regioisomer **DF-10**[12] was also obtained satisfactorily.

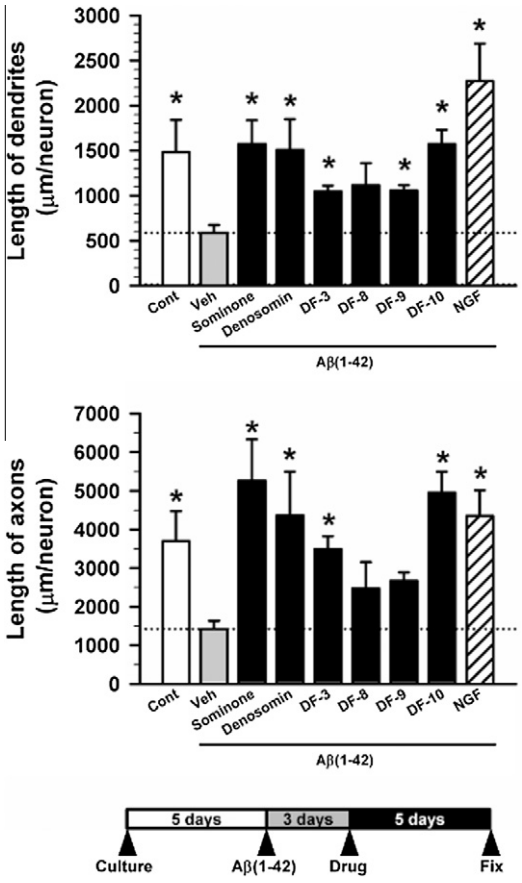


Figure 3. Effect of **DF-8** to **DF-10** on the dendritic and axonal extension in Aβ-damaged neurons.

Having prepared several phenolic **DFs**, we further estimated the efficacy of them on dendrite and axon extensions in Aβ-damaged neurons (Figure 3). Sominone, denosomin, **DF-3**, **DF-8 to DF-10** (1 μM each), and NGF (positive control: 100 ng/mL) were administered to rat cortical neurons on the third day after the treatment with Aβ, and the dendrite and axon lengths were gauged after further incubation for 5 days. As shown in Figure 3, the dendrite extension was evidently observed in the Aβ-exposed cells treated with novel **DFs**. Among them, **DF-10** displayed the most significant effects on dendrite extensions to realize the comparable level with those of previously reported, potent anti-Alzheimer's disease candidates sominone[4e] and denosomin.[5] Furthermore, the axon lengths in the damaged cells were apparently re-extended by the **DF-8 to DF-10**. Especially, it was remarkable that **DF-10** expressed more powerful axonal extension activity than the positive control NGF.

In conclusion, we accomplished the synthesis of novel mono-protected catechol derivatives of perhydrophenanthrenes via phenolic oxidation with $PhI(OAc)_2$ on the basis of the *o*-quinodimethane chemistry. To the best of our knowledge, this is the first successful application of the phenolic oxidation on functionalized benzocyclobutenes. Furthermore, we disclosed the promising pharmacophore with unprecedented structural characteristic for the development of anti-Alzheimer's disease drugs. It is an important note that the requisite property of the substituent for exerting the neuronal effects is orthogonal to those for the other bioactivities.[6] Taking advantage of the synthetic viability of dihydrofuran-fused perhydrophenanthrenes, the structure-activity-relationship study and the elucidation on the mechanism of action are ongoing in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.127.

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7. It was previously reported that *p*-quinone dimethylketals were produced by the treatment of 4-unsubstituted phenols with 2 equiv of $PhI(OAc)_2$ in MeOH. In our case, since the substrates **2a** and **2b** were equipped with the benzocyclobutene substituent on 4-positions, they afforded the 2,4-dimethoxy cyclohexadienones **3a** and **3b** respectively. (a) Pelter, A.; Elgendy, S. *Tetrahedron Lett.* **1988**, *29*, 677; For recent accounts and reviews on hypervalent iodine-mediated phenol oxidation, see (b) Quideau, S.; Pouységu, L.; Deffieux, D. *Synlett* **2008**, 467; (c) Ciufolini, M. A.; Braun, N. A.; Canesi, S.; Ousmer, M.; Chang, J.; Chai, D. *Synthesis* **2007**, 3759; (d) Rodriguez, S.; Wipf, P. *Synthesis* **2004**, 2767; applications in natural product synthesis, see (e) Pouységu, L.; Deffieux, D.; Quideau, S. *Tetrahedron* **2010**, *66*, 2235.
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10. *Spectral data for* **DF-8**: ^{1}H NMR (300 MHz, $CDCl_3$): *d* 7.12 (1H, δ, *J* = 8.2 Hz), 6.86 (1H, d, *J* = 2.2 Hz), 6.74 (1H, dd, *J* = 8.2, 2.5 Hz), 6.03 (1H, d, *J* = 1.9 Hz), 5.29 (1H, dt, *J* = 10.7, 2.8 Hz), 4.04–3.98 (1H, m), 3.96–3.87 (4H, m), 3.18 (1H, dd, *J* = 15.9, 3.3 Hz), 3.11 (1H, dd, *J* = 15.9, 2.2 Hz), 2.80 (1H, dt, *J* = 14.8, 3.3 Hz), 2.58–2.47 (1H, m), 1.98–1.82 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.0, 143.0, 132.0, 131.3, 127.0, 121.8, 115.5, 113.1, 110.2, 104.6, 79.8, 65.3, 63.7, 51.0, 35.8, 32.9, 31.5, 29.7; IR (KBr): 3396, 2232 cm^{-1}; MS (EI); *m*/*z* 311 (M^+); HRMS (EI): calcd for $C_{18}H_{17}NO_4$: 311.1158 (M^+), found 311.1152.
11. *Spectral data for* ***DF-9***: ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.85 (1H, s), 6.77 (1H, s), 6.04 (1H, d, *J* = 2.2 Hz), 5.66 (1H, s), 5.28 (1H, ddd, *J* = 10.4, 3.6, 2.2 Hz), 4.06–3.97 (1H, m), 3.95–3.82 (4H, m), 3.88 (3H, s), 3.19 (1H, dd, *J* = 15.9, 3.6 Hz), 3.05 (1H, dd, *J* = 15.9, 2.2 Hz), 2.84 (1H, ddd, *J* = 14.8, 14.8, 3.3 Hz), 2.55 (1H, ddd, *J* = 14.8, 12.4, 5.5 Hz), 1.97–1.84 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.71, 145.65, 143.2, 128.6, 124.4, 121.9, 116.6, 110.1, 108.4, 104.6, 79.8, 65.4, 63.7, 56.2, 51.1, 35.5, 33.3, 31.6, 30.0; IR (neat): 3536, 2229 cm^{-1}; MS (EI): *m*/*z* 341 (M^+). HRMS (EI): calcd for $C_{19}H_{19}NO_5$: 341.1263 (M^+), found: 341.1297.
12. *Spectral data for* ***DF-10***: ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.90 (1H, s), 6.76 (1H, s), 6.04 (1H, d, *J* = 2.2 Hz), 5.62 (1H, s), 5.28 (1H, ddd, *J* = 10.4, 3.3, 2.2 Hz), 4.13–3.95 (1H, m), 3.94–3.82 (4H, m), 3.88 (3H, s), 3.22 (1H, dd, *J* = 15.9, 3.3 Hz), 3.06 (1H, dd, *J* = 15.9, 2.2 Hz), 2.80 (1H, ddd, *J* = 14.6, 3.3, 3.3 Hz), 2.50 (1H, ddd, *J* = 14.6, 14.6, 4.4 Hz), 1.98–1.80 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.3, 144.6, 142.8, 126.9, 123.0, 121.9, 112.4, 112.1, 110.3, 104.5, 79.7, 65.2, 63.6, 55.9, 50.9, 35.3, 33.4, 31.3, 29.7; IR (neat): 3444 cm^{-1}; MS (EI): *m*/*z* 341 (M^+); HRMS (EI): calcd for $C_{19}H_{19}NO_5$: 341.1263 (M^+), found: 341.1286.