Bioorg. Med. Chem. Lett. 31 (2021) 127674

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological activity of (±)-7,3′,4′-trihydroxyhomoisoflavan and its analogs

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ARTICLE INFO

Keywords:
Dragon's blood
Homoisoflavan
AChE inhibitory activity
Neurite outgrowth promoting activity
Dracaena cambodiana

ABSTRACT

Acetylcholinesterase (AChE) inhibitors and neurite outgrowth promoters are thought to alleviate the symptoms of degenerative brain disorders, such as Alzheimer's disease. We designed and synthesized a series of homoisoflavonoids based on the structure of natural homoisoflavan isolated from *Dracaena cambodiana* dragon's blood. The homoisoflavonoids were then evaluated as AChE inhibitors and neurite outgrowth promoters. The catechol structure of the homoisoflavan B rings was important for AChE inhibition, and some of the homoisoflavonoids significantly promoted neurite outgrowth induced by nerve growth factor (NGF).

The aging of Japanese society has been accompanied by an increase in the incidence of dementia caused by Alzheimer's disease (AD). According to the New Orange Plan to promote measures against dementia, over 4,000,000 Japanese individuals had dementia in 2012.[1] The incidence of dementia has since increased, so the search for new drugs and natural compounds that can alleviate the symptoms of dementia is becoming increasingly important. Acetylcholinesterase (AChE) inhibitors are promising therapeutic agents for dementia. Several AChE inhibitors, including donepezil,[2] galantamine,[3] and rivastigmine,[4] are currently used to treat mild and moderate AD symptoms. Neurodegenerative diseases, including AD, are characterized by dysfunction of the nervous system due to the progressive disintegration of neuronal networks.[5] Nerve growth factor (NGF) promotes the growth and proliferation of neurons,[6] so increasing NGF activity with a pharmacological agent could relieve the symptoms of AD and other types of dementia. Compounds that can promote neurite outgrowth are thus of great interest.[7]

In 2017, Li et al. reported isolating the flavonoids (*R*)-7,4′-dihydroxy-8-methylflavan (**1**), (*R*)-7,4′-dihydroxy-6-methylflavan (**2**), and (*R*)-7,3′,4′-trihydroxyhomoisoflavan (**3**) from *Dracaena cambodiana* dragon's blood (Fig. 1). Compound **3** had particularly strong AChE inhibiting activity,[8] which made it an appropriate lead compound for the development of therapeutic drugs to treat dementia. In this study, we synthesized the natural AChE inhibitor **3** and several analogs. The neurite outgrowth promoting activity of each compound was then evaluated by performing an AChE bioassay.

The synthetic route for 7,3′,4′-trihydroxyhomoisoflavan (**3**) is shown in Scheme 1. The homoisoflavone skeleton was synthesized using Vilsmeier reagent, which was obtained by mixing methanesulfonyl chloride with dihydrochalcone derived from 4-benzyloxy-2-hydroxy acetophenone[9] and 3,4-dimethoxybenzaldehyde according to the method reported by Gan et al.[10] The homoisoflavone was then subjected to catalytic hydrogenation using 10% Pd on carbon (Pd/C) as a catalyst. No homoisoflavanone was obtained after this reduction step, and we were unable to identify reduction conditions that would generate the homoisoflavanone. Demethylation was performed by treating the intermediate with BBr_3. Thus, the desired homoisoflavan **3** afforded in five steps, and the overall yield is 48%. The spectral profile of synthetic compound **3** was consistent with that of **3** from natural sources. Although we obtained a racemate, this was the first complete synthesis reported for 7,3′,4′-trihydroxyhomoisoflavan (**3**). We then synthesized analogs **3a**–**3f** using the same synthetic route (Scheme 2). The chemical structures of all the synthetic compounds were determined by performing ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry. Only the catechol-type homoisoflavans with two phenolic hydroxy groups on their B rings exhibited potent AChE inhibition. We

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https://doi.org/10.1016/j.bmcl.2020.127674
Received 14 September 2020; Received in revised form 26 October 2020; Accepted 1 November 2020
Available online 6 November 2020
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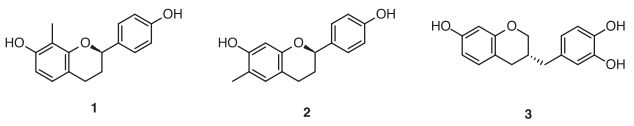
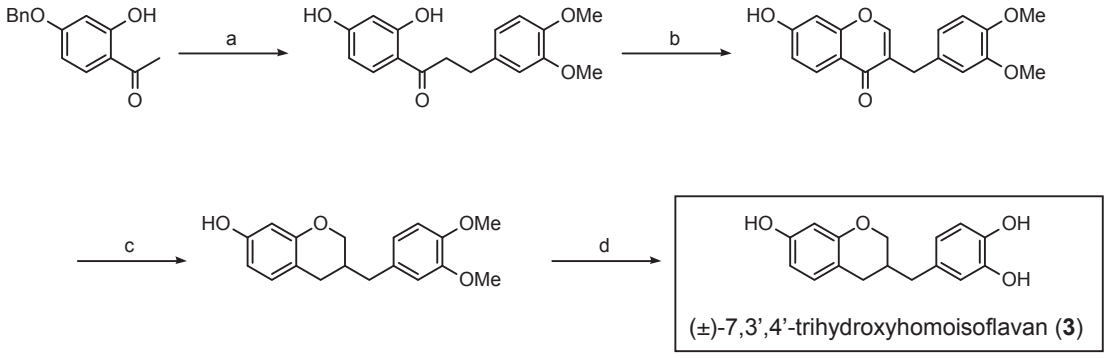


Fig. 1. Flavonoids isolated from *Dracaena cambodiana* dragon's blood.[8]

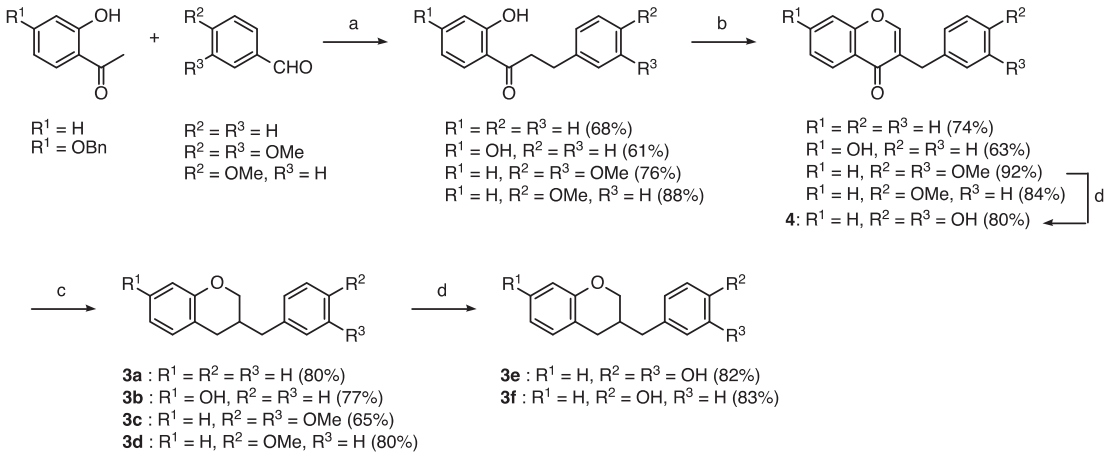
thus examined the inhibitory activities of homoisoflavans **3**, **3e**, **3f** and homoisoflavone **4** in detail. We also tested caffeic acid to investigate the influence of the homoisoflavonoid A and C rings. Homoisoflavans **3** and **3e** exhibited potent AChE inhibiting activities that were comparable to that of neostigmine, which served as a positive control (Fig. 2). Homoisoflavone **4** and caffeic acid exhibited inhibitory activity, but the activities of **3** and **3e** were stronger. The homoisoflavan with one phenolic hydroxy group on its B ring (**3f**) had no notable inhibitory activity. These results suggested that three factors affected inhibitory activity. Strong inhibitory activity was observed only when the B ring had a catechol structure. In addition, AChE inhibitory activity decreased when the homoisoflavan skeleton was converted to a homoisoflavone skeleton. Finally, the presence of the flavonoid A and C rings was necessary.

Among the compounds exhibited remarkable activity in the neurite outgrowth promotion assay, compounds **3**, **3e** and **4** were exhibited remarkable inhibitory activity in the AChE bioassay. A compound having both AChE inhibiting and neurite outgrowth promoting activity could serve as a lead compound for the development of drugs to improve brain function. The effect of each compound on neurite outgrowth induced by NGF is shown in Figs. 3 and 4. None of the tested compounds increased the neurite outgrowth activity of PC12 cells in the absence of NGF. However, each compound promoted neurite outgrowth by PC12 cells in the presence of NGF. The activities of the compounds followed the order **3e** > **4** » **3** ≈ **3f**. The effect of compound **3e** was particularly notable (Fig. 3). NGF-mediated neurite growth on PC12 cells was significant in all cases, but the effects of compounds **3e** and **4** were remarkable (Fig. 4). These compounds had similar effects on elongation, while the ratios of positive cells exposed to compounds **3** and **3f** were nearly identical to those exposed to the control.

Since only four compounds were evaluated in the bioassay, it was not



Scheme 1. Synthesis of (±)-7,3′,4′-trihydroxyhomoisoflavan (**3**). Reagents and conditions: (a) i: 3,4-dimethoxy benzaldehyde, NaH, DMF, rt., 89%; ii: 10% Pd/C, H_2, THF, rt., 67%; (b) BF_3•Et_2O, $MeSO_2Cl$, DMF, 80℃, 87%; (c) 10% Pd/C, H_2, MeOH,rt., 94%; (d) BBr_3, CH_2Cl_2, room temperature, 99%.



Scheme 2. Syntheses of analogs (**3a**-**3f**, and **4**). Reagents and conditions: (a) i, NaH, DMF, rt.; ii, 10% Pd/C, H_2, THF, rt.; (b) BF_3•Et_2O, $MeSO_2Cl$, DMF, 80℃; (c) 10% Pd/C, H_2, MeOH, 55℃; (d) BBr_3, CH_2Cl_2, rt.

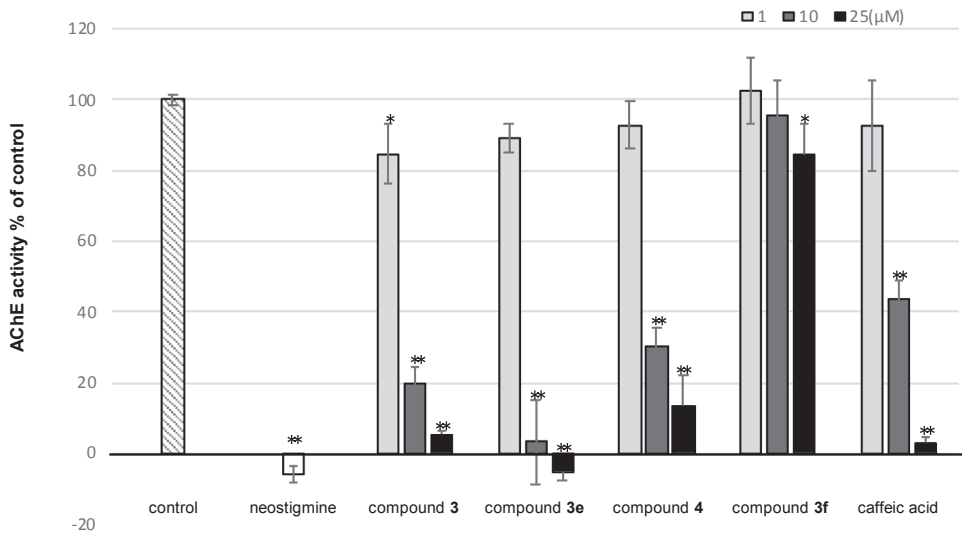


Fig. 2. AChE inhibiting activities of compounds **3**, **3e**, **3f**, **4**, and control compounds. Each result is reported as the mean ± the standard deviation (SD). The bars reflect the SD ranges, and significance is indicated by $*p < 0.05$ or $**p < 0.01$ based on Dunnett's tests.

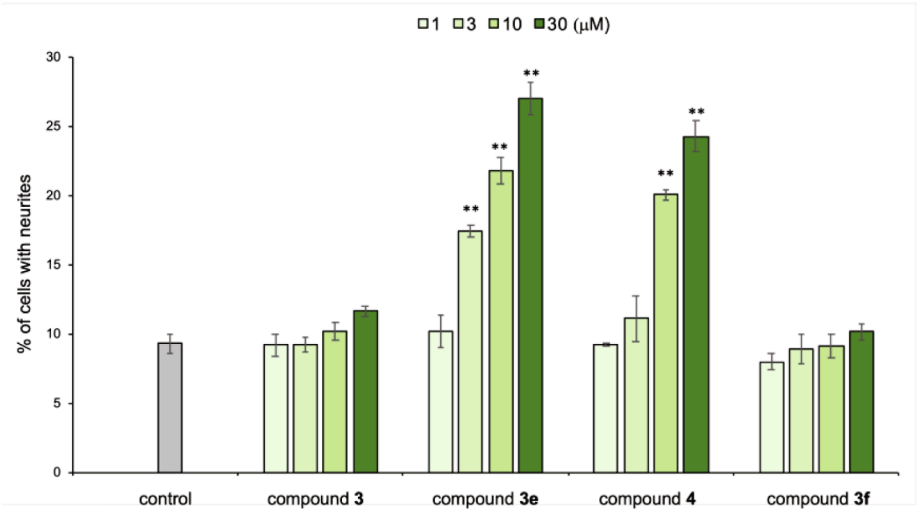


Fig. 3. Effects of the flavonoids on NGF-induced neurite outgrowth on PC12 cells. Each result is reported as the mean ± SD with significance indicated by $**p < 0.01$ based on Dunnett's tests.

possible to determine the relationship between structure and activity. However, we have identified potential lead compounds for the development of therapeutic drugs for dementia, and we are the first to report on homoisoflavonoids that inhibit AChE and promote neurite outgrowth.

We designed and synthesized a series of homoisoflavonoids with structures based on that of natural homoisoflavan isolated from *Dracaena cambodiana* dragon's blood. The synthesized compounds were evaluated as AChE inhibitors and neurite outgrowth promoters. We found that homoisoflavan B rings with a catechol structure were important for AChE inhibition. Several of the synthesized compounds had a significant effect on promoting NGF-induced neurite outgrowth. Homoisoflavan **3e** was a potential lead compound for dementia drugs, because it exhibited both significant acetylcholinesterase inhibiting activity and neurite outgrowth promoting activity. As differences between the activities of enantiomers are of interest, research on the synthesis of optically active homoisoflavans is ongoing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

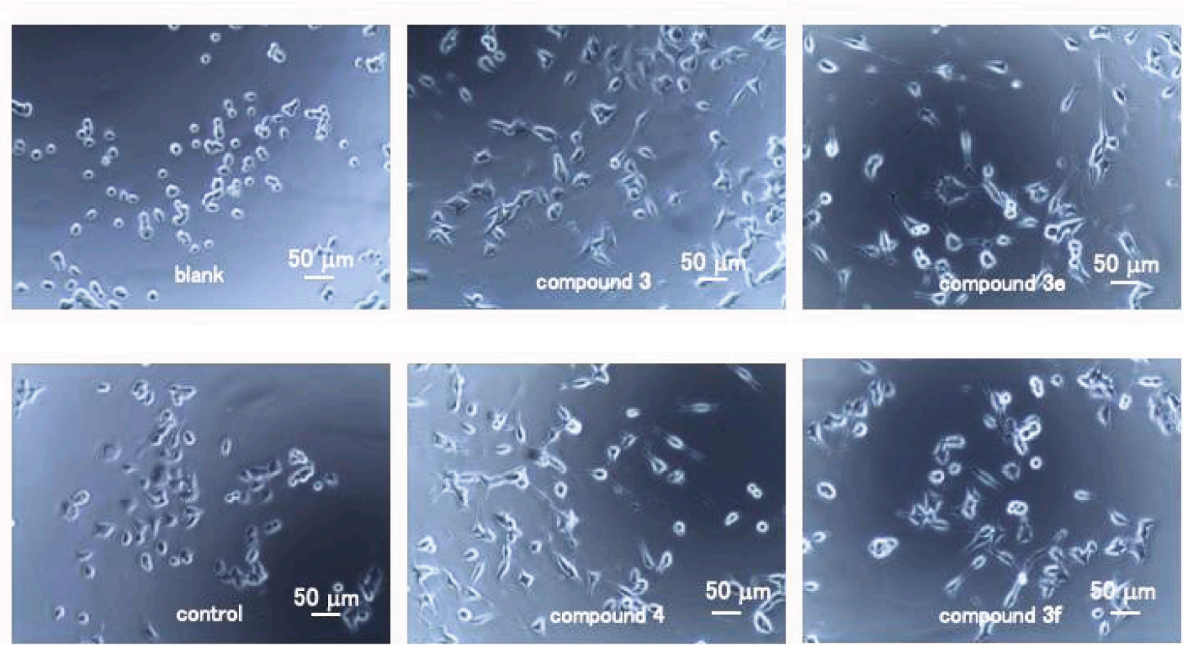


Fig. 4. Effects of the flavonoids on neurite outgrowth induced by nerve growth factor (NGF). The PC12 cells were incubated for 48 h without NGF (Blank) or with NGF and compound **3**, **3e**, **4**, **3f**, or NGF only (Control). The final NGF concentration was 10 ng/mL, and the final concentration of each synthesized compound was 30 μM. Scale bar = 50 μm.

Acknowledgments

The authors would like to thank Enago (www.enago.jp) for the English language review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127674.

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