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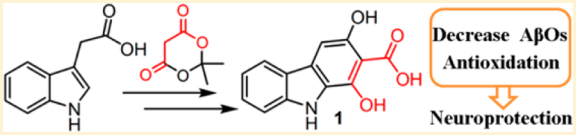
Highly Efficient Synthesis of 1,3-Dihydroxy-2-carboxycarbazole and Its Neuroprotective Effects

Kai Liu* and Shijun Zhang*

Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia 23298, United States

Supporting Information

ABSTRACT: Carbazoles represent a family of tricyclic compounds that widely appeared in nature. Numerous studies have revealed a diverse array of bioactivity associated with this scaffold. In the present study, a novel and highly efficient methodology for preparing 1,3-dihydroxy-2-carboxycarbazole from indole-3-acetic acid and Meldrum's acid was developed. Furthermore, biological characterization demonstrated that this multisubstituted carbazole analogue exhibited inhibitory activity on Aβ aggregation, antioxidative properties, and promising neuroprotective activities in a cellular model of Alzheimer's disease, thus further supporting the valuable application of this synthetic methodology in search for effective neuroprotectants.



KEYWORDS: *Carbazole derivative, synthesis, Meldrum's acid, neuroprotection, Aβ oligomers, antioxidant*

Alzheimer's disease (AD) is a progressive and neurodegenerative disorder and the most common cause of dementia.[1] Multiple pathogenic factors have been suggested to contribute to AD, and this includes amyloid-β (Aβ),[2−4] hyperphosphorylated tau protein, biometal dyshomeostasis, reactive oxygen species (ROS), and neuroinflammation, among others.[5−8] Considering the multifaceted nature of this disease, strategies aiming to develop small molecules with therapeutic polypharmacology have recently attracted extensive attention in overcoming the limitations of the traditional "one molecule, one target" approach in the development of effective AD treatments. Therefore, an efficient methodology to quickly provide novel chemical scaffolds to explore their therapeutic potential may represent a promising approach to overcome the paucity of disease-modifying agents in the pipeline of AD therapeutics.

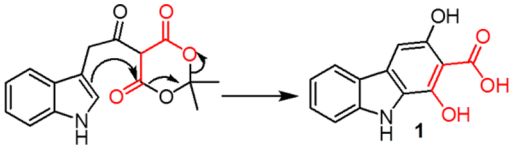
Carbazoles were first reported by Graebe and Glaser in 1872, and they represent a family of tricyclic scaffold that widely appeared in nature.[9] Since then, numerous studies have revealed a diverse array of bioactivity associated with this scaffold.[10−12] Therefore, chemical syntheses of substituted carbazoles have attracted considerable attention. As a result, a large number of methodologies have been developed to construct the skeleton of substituted carbazole analogues. These include Fischer−Borsche synthesis,[13] Graebe−Ullmann synthesis,[14] transition metal catalyzed cyclization of amino naphthalenes or diphenylamines,[15,16] and conversion of indole derivatives to carbazoles.[17] However, there are certain limitations associated with these methodologies, such as application of expensive metal catalysts, which are difficult to remove completely and may cause problems in biological studies.

Therefore, a concise and efficient methodology that can quickly produce carbazole analogues would represent an attractive avenue to explore the therapeutic potential of this scaffold.

Meldrum's acid was discovered by A. N. Meldrum more than 100 years ago and has been utilized extensively in organic synthesis as synthons for building diversified compounds.[18,19] It has been demonstrated by Fillion and co-workers that, under the catalysis of Sc(OTf)3, benzyl Meldrum's acids undergo intramolecular Friedel−Crafts acylation to give benzocyclic ketones.[20] It was further shown that intramolecular cyclization of 3,5-dimethoxyphenylacetyl Meldrum's acid in the presence of TFA efficiently yields 1,3-dihydroxy-6,8-dimethoxy-2-naphthoic acid analogues.[21]

Recently, phenolic compounds have been shown to exhibit promising biological activities, such as antioxidant and anti-inflammatory effects. Collectively, these studies inspired us to explore the cyclization reaction of indole-3-acetyl Meldrum's acid to produce the privileged scaffold 1,3-dihydroxy-2-carboxycarbazole (**1**, Scheme 1) and explore its therapeutic potential. Herein, we report, for the first time, a concise and efficient method to prepare 1,3-dihydroxy-2-carboxycarbazole starting from commercially available indole-3-acetic acid and Meldrum's acid. Biological characterization demonstrated that this multisubstituted carbazole analogue exhibited antioxidative

Scheme 1. Proposed Cyclization Reaction



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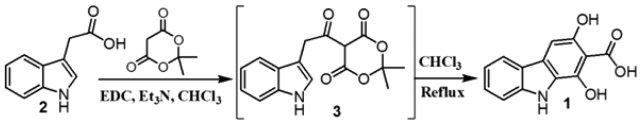
properties, inhibitory effects on Aβ aggregation, and promising neuroprotective activity in a cellular model of Alzheimer's disease.

The intermediate indole-3-acetyl Meldrum's acid was prepared by condensation of readily available indole-3-acetic acid 2 with Meldrum's acid in the presence of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) and triethylamine (TEA) (Scheme 2). After the indole-3-acetic acid was

Scheme 2. Route for 1,3-Dihydroxy-2-carboxycarbazole Preparation



fully consumed, as monitored by TLC, a simple acid wash work up removed the TEA, EDC, and 1-(3-(dimethylamino)propyl)-3-ethylurea (ECU). After removal of all solvents, the residue was subjected to cyclization without further purification. Different conditions including direct heating were explored for this cyclization reaction, and it was finally determined that refluxing the intermediate in a solution of chloroform resulted in the formation of 1,3-dihydroxy-2-carboxycarbazole as a brown solid with an overall yield of 39.4% over two steps (Scheme 2). The structure was confirmed by NMR and MS.

After the establishment of the synthesis of **1** following this method, we decided to test its biological activity in MC65 cells, a widely used cellular model of Alzheimer's disease.[22,23] MC65 cells are an immortalized line of human neuroblastoma cells that conditionally expresses C99, the C-terminus fragment of the amyloid precursor protein (APP) using tetracycline (TC) as transgene suppressor.[24] Upon removal of TC, these cells can produce intracellular Aβ aggregates including small AβOs that ultimately lead to oxidative stress and cell death. As shown in Figure 1, carbazole compound **1** significantly protected MC65 cells from TC removal-induced toxicity at concentrations as low as 0.3 μM and demonstrated full rescue of viability at 10 μM. To further validate the essential role of the carbazole scaffold on the neuroprotective activities, 2,6-dihydroxybenzoic acid, the phenolic benzoic acid portion of compound **1**, was

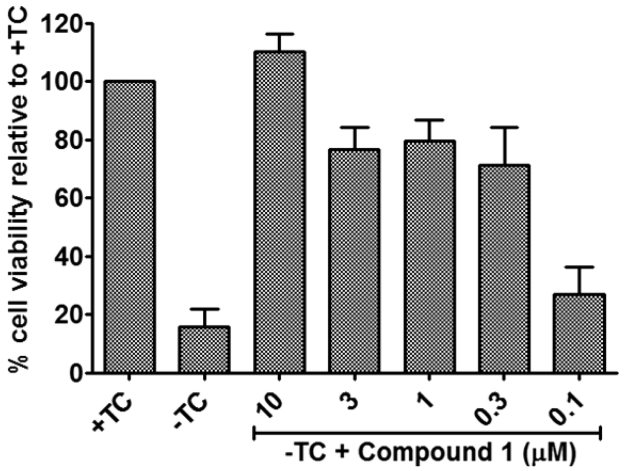


Figure 1. Neuroprotection of compound **1** in Mc65 cells. MC65 cells were treated with compound **1** at the indicated concentrations under −TC conditions for 72 h. Cell viability was assessed by MTT assay.

tested, and no protection was observed up to 100 μM in the same assay conditions (data not shown). To investigate whether compound **1** interferes with the production of Aβ, we next assessed the total concentration of Aβ by ELISA assay. The level of Aβ40 and Aβ42, as shown in Figure 2, remained

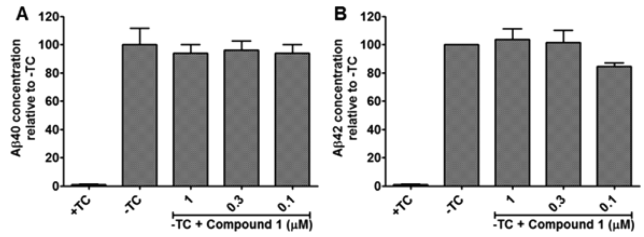


Figure 2. Compound **1** does not influence the production of Aβ. MC65 cells were treated with compound **1** at the indicated concentrations under −TC conditions for 48 h. Medium was collected, and the concentrations of (A) Aβ40 and (B) Aβ42 were determined by ELISA.

the same between the treated and nontreated groups under the −TC conditions, which suggests that compound **1** does not affect Aβ production. When the effects on the Aβ oligomerization were examined, as shown in Figure 3A,

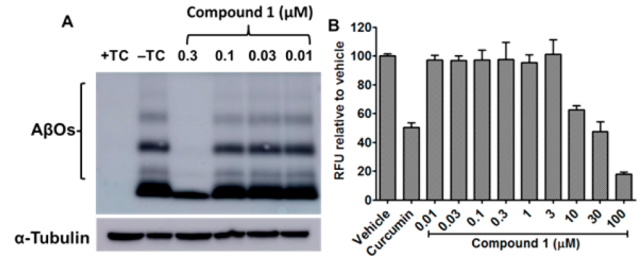


Figure 3. Compound **1** can inhibit Aβ aggregation. (A) MC65 cells were incubated with compound **1** at the indicated concentrations under −TC conditions for 48 h. Lysates from cultures were analyzed by Western blotting using a 6E10 antibody. (B) Compound **1** was incubated with Aβ42 (10 μM) at the indicated concentrations for 48 h. Aggregation levels were analyzed by ThT assay.

compound **1** was shown to significantly inhibit the formation of small AβOs at 0.3 μM, consistent with the neuroprotection results. This indicates that compound **1** may inhibit the aggregation process of Aβ. To further confirm this, thioflavin T assay was employed to examine the effects of compound **1** on aggregation of synthetic Aβ42 peptide. Curcumin, a known Aβ aggregation inhibitor,[25] was used as a positive control. As shown in Figure 3B, curcumin (10 μM) suppressed the aggregation by 50%, consistent with reported results.[25] Notably, compound **1** dose-dependently inhibited the aggregation of Aβ42 with an IC_{50} of 15 μM.

The production of ROS is another potential factor that has been suggested to contribute to the development of AD.[7] In addition, oxidative stress has been indicated as playing a role in neurotoxicity induced by the accumulation of intracellular AβOs in MC65 cells.[26] The presence of the polyphenol moiety in the structure of compound **1** may suggest antioxidative properties. Therefore, we further examined the radical quenching effect of compound **1** by DPPH assay. As shown in Figure 4, compound **1** exhibited comparable antioxidative capacity to trolox, a known antioxidant that is a widely used standard.[27]

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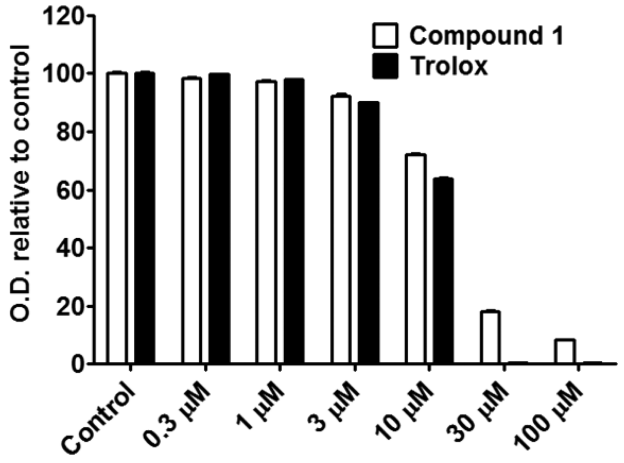

Figure 4. Compound **1** showed antioxidative ability. Compound **1** or trolox was incubated at the indicated concentrations with DPPH (50 μM) in MeOH at 37 °C for 30 min. Absorbance was recorded at 517 nm.

In conclusion, we have developed a novel and robust methodology to synthesize analogues of 1,3-dihydroxy-2-carboxycarbazole using Meldrum's acid. The reaction is mediated through an intramolecular electrophilic cyclization mechanism. Preliminary biological characterization demonstrated that the multisubstituted carbazole compound **1** showed neuroprotective effects in MC65 cells, possibly through multiple mechanisms such as inhibition of Aβ oligomerization and antioxidative activity. This further supports the value of this concise and efficient synthetic methodology in providing privileged templates to explore their therapeutic applications.

■ ASSOCIATED CONTENT

Supporting Information

Details for synthetic procedures, analytical data, and biological studies for compound **1**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00158.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: kliu4@vcu.edu.
*E-mail: szhang2@vcu.edu.

Author Contributions

Chemical synthesis, structural characterization, and biological studies were completed by K.L. Experiment design, data analysis, writing, and editing were completed by K.L. and S.Z.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

APP, amyloid precursor protein; Aβ, amyloid-β; AβOs, amyloid-β oligomers; AD, Alzheimer's disease; ROS, reactive oxygen species; TC, tetracycline; EDC, *N*-(3-(dimethylamino)-propyl)-*N*′-ethylcarbodiimide hydrochloride; ECU, 1-(3-(dimethylamino)propyl)-3-ethylurea; TEA, triethylamine; TFA, trifluoroacetic acid

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