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Antioxidant effects of the highly-substituted carbazole alkaloids and their related carbazoles

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ABSTRACT

Antioxidant activities of 3-oxygenated and 3,4-dioxygenated carbazole alkaloids and their related carbazoles were comprehensively evaluated. In all assay systems, the 3,8-dihydroxycarbazoles carbazomadurin A (**2**) and B (**3**), and their synthetic precursors **2a** and **3a** exhibited higher antioxidant activities than the 3-monohydroxycarbazoles carazostatin (**1**), and the synthetic precursors **4a** and **4b** of carquinostatin A (**4**). In particular, **2a** and **3a** exhibited strong scavenging activities due to the reducing ability of formyl group at the C-5 position of carbazoles. The results suggest that these compounds could serve as useful clues for designing and developing novel antioxidants.

High levels of free radicals and reactive oxygen species (ROS), including hydrogen peroxide, reactive hydroxyl, and several other free radicals produced by cells, play an important role in the initiation of various diseases such as carcinogenesis, drug-associated toxicity, inflammation, atherogenesis and aging in aerobic organisms.[1–3] Antioxidants are currently seeking as the drug candidates to treat these conditions. Due to their interesting biologic effects, carbazole alkaloids constitute an important class of natural products. In 1989, a new type of 3-hydroxycarbazole alkaloid carazostatin (**1**) was isolated from *Streptomycs chromofuscus* DC 118 as a new free radical- scavenging substance.[4] Carazostatin (**1**) exhibits strong inhibitory activity against free radical-induced lipid peroxidation and shows stronger antioxidant activity in liposomal membranes than α-tocopherol (VE).[5] In addition to carazostatin (**1**), 3-hydroxycarbazole alkaloids epocarbazolin A and B,[6] and carbazomadurins A (**2**) and B (**3**)[7] were isolated from *Streptomyces anulatus* T688-8 and *Actinomadura Madurae* 2808-SV-1, respectively. Closely related 3-hydroxycarbazole alkaloids lipocarbazole A1-A4 were recently found in *Tsukamurella pseudospumae* Acta 1857.[8] In addition, a new class of the 3,4-dioxo-functionalized carbazole, carquinostatin A (**4**)[9,10] were found in *Streptomyces exfoliates* 2419-SVT2. In addition to carquinostatin A (**4**), lavanduquinocin[11] and carbazoquinocins A–F[12] were isolated from *Streptomyces violaceus* 2448-SVT2 and *Streptomyces viridochromogenes*, respectively.

These 3-hydroxycarbazole alkaloids were initially independently evaluated in a bioassay system during isolation. Carazostatin (**1**) showed strong inhibitory activity toward lipid peroxidation-induced free radicals in rat brain homogenate.[4] The relative antioxidant activities, on the other hand, are opposite depending on the medium: that is, VE is a stronger antioxidant than carazostatin (**1**) in homogeneous solution, whereas carazostatin (**1**) is more active than VE in the membranes.[5] Epocarbazolines A and B are novel 5-lipoxygenase inhibitors.[6] The antioxidative activity of carbazomadurins A (**2**) and B (**3**) was evaluated by observing the inhibition of the L-glutamate toxicity in N18-RE-105 cells.[7] Furthermore, the inhibitory activity of the 3,4-dioxocarbazole (so-called carbazole-3.4-quinone) alkaloids carbazoquinocin A–F was evaluated against lipid peroxidation induced by free radicals in rat liver microsome preparations free from VE.[11] The antioxidative activity of carquinostatin A (**4**) was evaluated based on the inhibition of L-glutamate toxicity in N18-RE-105 cells.[9,10] To the best our knowledge, the antioxidative activity of naturally occurring 3-hydroxy- and 3,4-dioxocarbazole alkaloids has not yet been evaluated comprehensively. Here we describe a comprehensive evaluation of the in vitro antioxidant activities of carbazole alkaloids and their synthetic precursors.

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To evaluate the antioxidant activities of 3-hydroxycarbazole and 3,4-dioxocarbazole alkaloids, we prepared eight compounds including carazostatin (**1**),[13,14] carbazomadurins A (**2**) and its synthetic precursor (**2a**),[15a] and carbazomadurin B (**3**) and its precursor (**3a**),[15a,16a] and carquinostatin A (**4**) and its synthetic precursors (**4a**) and (**4b**),[17,18a] using our synthetic strategy based on the allene-mediated 6π-electrocyclic reaction involving the indole 2,3-bond.[19–21] The structures of the synthesized compounds (Fig. 1) were elucidated on the basis of ^{1}H and ^{13}C NMR spectra and high-resolution mass spectra. The structural data were consistent with previously reported data in all respects (see Supplementary information). The antioxidant activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH),[22] and 2,2′-azinobis- (3-ethylbenzthiazoline-6-sulfonate) cations ($ABTS^{+}$) were measured.[23] The inhibition concentrations at which 50% of the radicals were scavenged (IC_{50} values) were calculated to evaluate the antioxidant activity. A lower IC_{50} value indicates greater antioxidative activity. IC_{50} values of less than 10 mg/mL suggest effective antioxidative properties. Finally, antioxidant activity was measured using a test kit for potential antioxidant in oil solution (PAO-SO).[24]

Evaluation of DPPH radical scavenging activity is a rapid and convenient method of screening the antioxidant activities.[22] As shown in Table 1, in the DPPH assay, 3-hydroxycarbazoles **1**, **2**, **2a**, **3**, **3a**, **4a** and **4b** exhibited higher active radical-scavenging activities than VE (15.4) and MCI-186 (edaravon, 19.7).[25] Among them, **1**, **4a**, and **4b** possessed almost the same scavenging activities. 3,8-Dihydroxycarbazoles **2**, **2a**, **3**, and **3a** displayed the best radical scavenging activity in this assay. Furthermore, these four compounds had almost the same antioxidant activity as the potent antioxidant PG (6.1). The strong scavenging activities of **2a** and **3a** are likely due to the reducing ability of the formyl group at the 5-position of carbazole. No radical scavenging activity of 3,4-dioxocarbazole **4** was detected in this assay.

Table 1
Radical scavenging activities of carbazole alkaloids and related compounds

Compounds	Radical scavenging activities IC_{50} (μM)	
	DPPH radical	$ABTS^{+}$ radical
PG[a]	6.1 ± 0.2	187.5 ± 7.0
VE[b]	15.4 ± 0.3	211.1 ± 4.6
MCI-186[c]	19.7 ± 0.2	213.7 ± 4.0
1	12.0 ± 0.3	158.9 ± 4.6
2	9.9 ± 0.1	111.7 ± 3.5
2a	7.1 ± 0.1	52.6 ± 0.5
3	9.8 ± 0.4	115.9 ± 2.0
3a	7.6 ± 0.1	57.1 ± 0.5
4	>50	>500
4a	12.0 ± 0.1	160.6 ± 0.7
4b	12.0 ± 0.3	174.6 ± 2.7

Date are expressed as the mean ± SE of three experiments.
[a] PG = propyl gallate.
[b] VE = α-tocopherol.
[c] MCI-186 = edaravone.

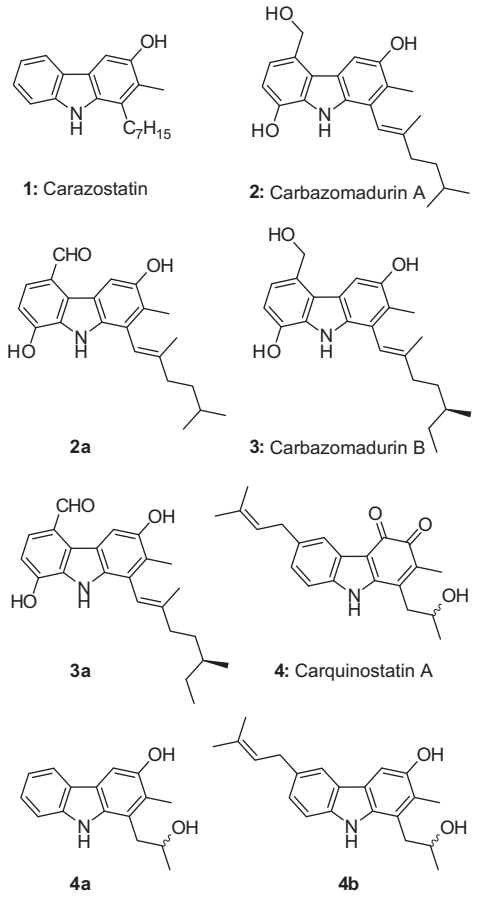

Figure 1. Oxygenated carbazole alkaloids and their related carbazoles.

The $ABTS^{+}$ radical assay is a conventional and excellent model for assessing the antioxidant activities of hydrogen-donating and chain-breaking antioxidants. In this assay, $ABTS^{+}$ radicals were produced by reacting ABTS with potassium persulfate in sodium phosphate buffer solution.[23] As shown in Table 1 that most compounds showed good inhibition of $ABTS^{+}$ radicals. All 3-hydroxycarbazoles **1**, **2**, **2a**, **3**, **3a**, **4a**, and **4b** exhibited stronger $ABTS^{+}$ radical scavenging activities than the standard antioxidants VE (213) and MCI-186 (214) with better IC_{50} values. In addition, the values of all compounds, except 3,4-dioxocarbazole **4**, indicated higher $ABTS^{+}$ radical scavenging activities than the potent antioxidant PG (185). Generally, 3-hydroxycarbazoles displayed strong $ABTS^{+}$ radical scavenging activity. Among them, 3,8-dihydroxycarbazoles **2**, **2a**, **3** and **3a** exhibited much higher antioxidant activities than 3-monohydroxycarbazoles **1**, **4a** and **4b**. 3,8-Dihydroxy-5-formylcarbazoles **2a** and **3a** showed the highest activity in this assay. The high activity of **2a** and **3a** are likely due to their reducing properties in the presence of a formyl group at the C5 position of the carbazole ring as well as to their DPPH radical scavenging activity. No $ABTS^{+}$ radical scavenging activity of 3,4-dioxocarbazole **4** was detected in this assay.

The antioxidant potential of carbazoles was measured by utilizing the reduction reaction of copper (from Cu^{++} to Cu^{+}) to calculate the total potential antioxidant capacity (PAO-SO method).[24] In the PAO-SO assay (Fig. 2), 3,8-dihydroxycarbazoles **2a** (4584) and **3a** (4580) had the best total potential antioxidant capacity compared with the other carbazoles (2411–3567), probably as a result of their reducing properties in the presence of a formyl group on the carbazole ring. 3,8-Dihydroxycarbazoles **2** (3567) and **3** (3413) showed better total potential antioxidant capacity than the standards PG (3096) and MCI-186 (3096). Carazostatin (**1**) (2803) exhibited almost the same total potential antioxidant capacity as **4a** (2881), but **4b** (2411) exhibited slightly lower activity than **1** (2803) and **4a** (2881). 3,4-Dioxocarbazole **4** (1182) exhibited the lowest total potential antioxidant capacity of all the compounds, including the standards.

The HCT-116 cell viability assay, which is based on the MTT method, was performed according to the method of Mosman.[26] Treatments of all tested carbazoles with 100 μM did not completely inhibit cell viability, although some of the chemicals, **4** and **4b**, slightly decreased cell viability.

In conclusions, the antioxidant effects of 3-oxygenated and 3,4-dioxygenated carbazole alkaloids and their related carbazoles