JOURNAL OF NATURAL PRODUCTS

pubs.acs.org/jnp

Article

Lipid-Lowering Activities of Cucurbitacins Isolated from *Trichosanthes cucumeroides* and Their Synthetic Derivatives

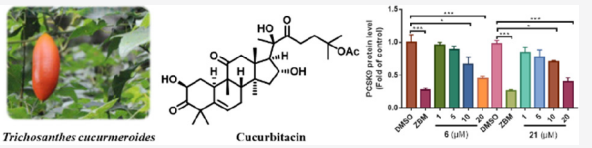
Xianjing Zhang, Huihui Li, Wenqiong Wang, Tong Chen, and Lijiang Xuan*

Cite This: https://dx.doi.org/10.1021/acs.jnatprod.0c00364

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: In the ongoing efforts to discover natural cholesterol-lowering compounds, dihydrocucurbitacin B, isolated from *Trichosanthes cucumeroides* roots, was found to promote LDL uptake by upregulating LDLR protein in a PCSK9-dependent process. In this study, an in-depth investigation of *T. cucumeroides* roots afforded 27 cucurbitacins (**1**−**27**), including seven new cucurbitacins (**1**−**7**), and their structures were elucidated by spectroscopic data analyses. In order to gain insight into their structure−activity relationship, cucurbitacin derivatives (**B1**−**11** and **DB1**−**11**) were synthesized. Evaluation of lipid-lowering activities of these cucurbitacins by an LDL uptake assay in HepG2 cells revealed that most of the compounds improved the LDL uptake rate, among which hexanorisocucurbitacin D (**6**) and isocucurbitacin D (**21**) exhibited the highest activities (rates of 2.53 and 2.47, respectively), which were comparable to that of the positive control, nagilactone B (rate of 2.07). According to a mechanistic study by Western blot analysis, compounds **6** and **21** dose-dependently increased LDLR protein levels and reduced PCSK9 protein levels, representing promising new lipid-lowering drug candidates.



Cardiovascular disease (CVD) is the leading cause of mortality worldwide.[1] Statins are the first-line drug therapy in the treatment of hyperlipidemia or CVD and act by lowering plasma low-density lipoprotein cholesterol (LDL-C). Despite the efficacy of statin therapy, some patients still face substantial residual risks associated with high levels of LDL-C.

One reason for the continued high LDL-C levels is that statins increase PCSK9 expression at higher drug doses, an effect believed to attenuate a further reduction in LDL-C levels.[2] Hence, inhibition of PCSK9 to enhance the effects of statins provides an alternative therapeutic option for patients who need additional lowering of LDL-C. For instance, PCSK9 antibodies have been shown to be clinically beneficial.[3] However, their drawbacks, such as high cost and inconvenient subcutaneous administration, lead to the need for small, orally available, and low-cost chemicals that regulate PCSK9.[3]

Recently, several compounds such as berberine and curcumin have been reported to inhibit PCSK9 expression, initiating a growing interest in discovering small molecules with PCSK9-modulating activity derived from natural products.[4,5]

Dihydrocucurbitacin B, isolated from the roots of *Trichosanthes cucumeroides*, was first reported by our group to elevate LDL uptake in HepG2 cells.[6] A mechanistic study indicated that dihydrocucurbitacin B upregulated LDLR expression by increasing SREBP2 protein levels and down-regulated PCSK9 expression by decreasing HNF1α protein levels.[6] These findings initiated our interest in the structure−activity relationships of cucurbitacins.

Therefore, a follow-up study of cucurbitacins from *T. cucumeroides* roots was conducted. Cucurbitacins (**1**−**27**), including seven new compounds (**1**−**7**), were identified, and cucurbitacin derivatives (**B1**−**11** and **DB1**−**11**) were synthesized. Most of these compounds were evaluated for lipid-lowering activity by the LDL uptake assay, and the effects of two active compounds on LDLR and PCSK9 protein levels were further tested by Western blot analysis.

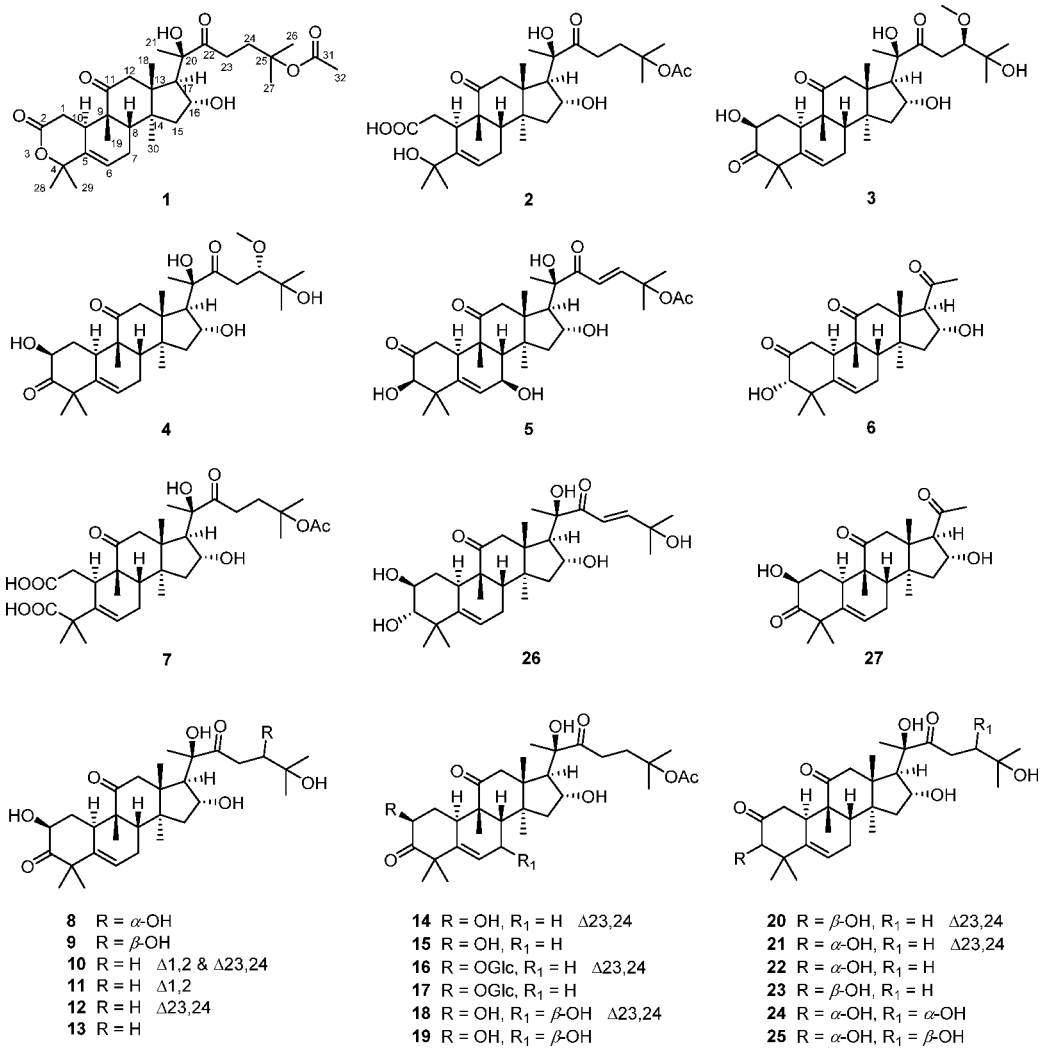
RESULTS AND DISCUSSION

Isolation and Structure Elucidation. Seven new cucurbitacins (**1**−**7**) and 20 known cucurbitacins (**8**−**27**) were obtained from the 95% EtOH extract of *T. cucumeroides* roots using various chromatographic techniques.

Compound **1** was obtained as a white amorphous powder. The molecular formula was determined as $C_{31}H_{46}O_8$ based on the ^{13}C NMR data and HRESIMS ion at m/z 569.3080 $[M + Na]^+$ (calcd for $C_{31}H_{46}O_8Na$, 569.3085). The ^1H NMR spectrum exhibited signals for nine methyl groups, a hydroxymethine (δ_H 4.32), and an olefinic proton (δ_H 5.75). The ^{13}C NMR spectrum displayed 31 carbon signals including nine methyls, six methylenes, four methines (one oxygenated), three quaternary carbons, three oxygenated tertiary carbons,

Received: April 7, 2020

Chart 1



two olefinic carbons (δ_C 120.7, 137.0), two ester carbonyl groups (δ_C 170.5, 172.1), and two ketocarbonyl groups (δ_C 211.8, 214.0). These data showed a signal pattern similar to that of 23,24-dihydrocucurbitacin B[7] except for the A-ring.

The partial structure of the A-ring was established by 2D NMR data. In addition to the $^1H-^1H$ COSY correlation between H_2-1 (δ_H 2.16, 2.50) and H-10 (δ_H 2.76), the HMBC correlations from H_2-1 to C-2 (δ_C 172.1) and C-5 (δ_C 136.9), as well as from H_3-28 and H_3-29 (δ_H 1.51 and 1.53, respectively) to oxygenated C-4 (δ_C 84.0), suggested a lactone-type structure in the A-ring. The NOESY correlations from H-10 to H_3-30, H-17, and H_3-21 suggested α-orientations of these protons and methyl groups, while those from H_3-19 to H-8, H_3-18, and H-16 revealed β-orientations.

The ECD spectrum (Figure S1.9, Supporting Information) of compound **1** showed Cotton effects at 300 nm ($\Delta\varepsilon$ +2.8) for the carbonyl n$-\pi^*$ transition and 200 nm ($\Delta\varepsilon$ +5.6) for the olefinic $\pi-\pi^*$ transition, confirming the (8*S*, 9*R*, 10*R*, 13*R*, and 14*S*) absolute configuration of compound **1**.[8] The NOESY cross-peaks of H_3-18/H-16 and H_3-30/H-17 indicated the (16*R*) and (17*R*) configuration. Because of the restricted rotation of the single bond between C-17 and C-20,[9] the NOE correlation of H_3-21/H-17 supported the (20*R*) configuration. Thus, the structure of neocucurbitacin E (**1**) was defined as shown. It represents the fifth example of a lactone-type norcucurbitacin.[10−12]

Compound **2** was assigned a molecular formula of $C_{31}H_{48}O_9$ deduced from the HRESIMS ion at *m*/*z* 587.3192 $[M + Na]^+$ (calcd for $C_{31}H_{48}O_9Na$, 587.3191). The ^1H and ^{13}C NMR data of **2** revealed a similar structure to that of **1**, with major differences in the A-ring. Compared to **1**, H_2-1a (δ_H 2.51) still showed a correlation with carboxylic carbon C-2 (δ_C 180.2) in the HMBC spectrum, which was consistent with its eight indices of hydrogen deficiency and the IR absorption band at 1694 cm^{-1}. Moreover, H_3-28 and H_3-29 (δ_H 1.35 and 1.62, respectively) were correlated to C-4 (δ_C 89.9). The ECD Cotton effects and NOE correlations of **2** were similar to those of **1**,[9] indicating that these two compounds had the same configuration (8*S*, 9*R*, 10*R*, 13*R*, 14*S*, 16*R*, 17*R*, and 20*R*). As the fifth example of a ring-A seco-cucurbitacin,[13] **2** was named colocynthenin E.

Compounds **3** and **4** had the same molecular formula of $C_{31}H_{48}O_8$, as indicated by their HRESIMS ions at *m*/*z* 571.3244 and 571.3245 $[M + Na]^+$ (calcd for $C_{31}H_{48}O_8Na$, 571.3241), respectively. The NMR spectra of **3** suggested that it was closely related to cucurbitacins H (**8**) and G (**9**). However, the hydroxy group at C-24 was replaced by a methoxy group, which was supported by the HMBC

https://dx.doi.org/10.1021/acs.jnatprod.0c00364
J. Nat. Prod. XXXX, XXX, XXX−XXX

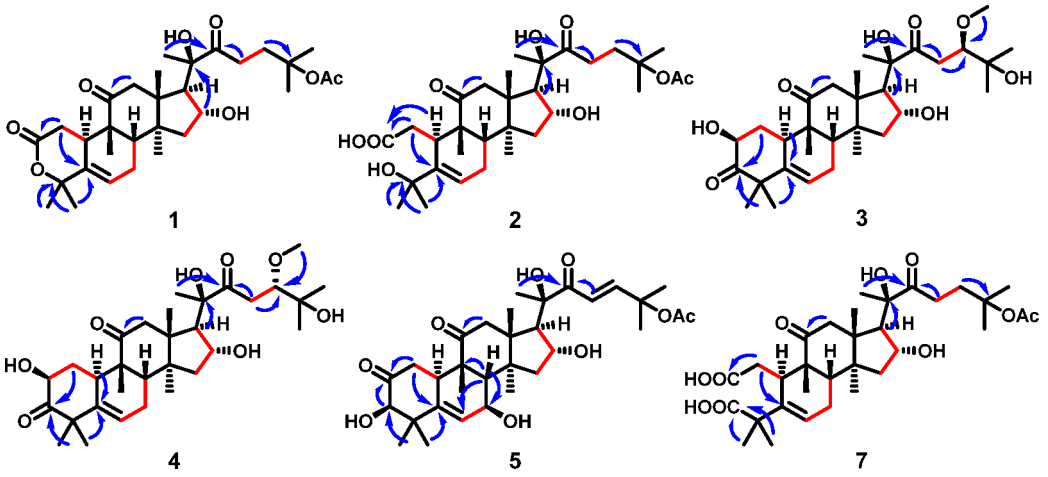


Figure 1. ^{1}H−^{1}H COSY (red) and select HMBC correlations (H → C, blue) of **1**−**5** and **7**.

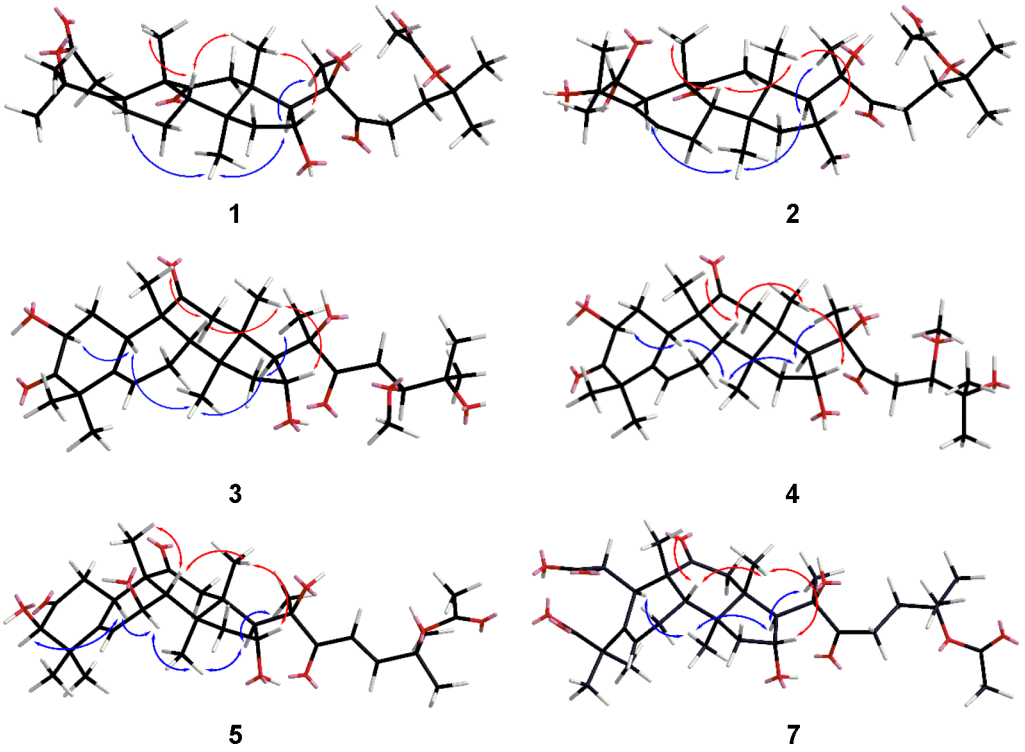


Figure 2. Key NOESY correlations of **1**−**5** and **7**.

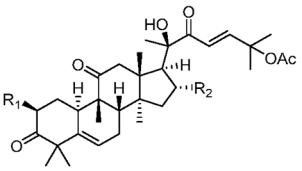
correlation from the methoxy protons (δ_H 3.43) to C-24 (δ_C 84.7). The NMR data of **4** resembled those of compound **3** but differed in the coupling constants of H_2-23 and H-24, implying that **4** was a C-24 epimer of **3**. Comparison of the ECD and NMR spectra of **3** and **4** with those of **2** confirmed that these cucurbitacins had the same configuration (8*S*, 9*R*, 10*R*, 13*R*, 14*S*, 16*R*, 17*R*, and 20*R*). According to the rule summarized by Gan Maolong,[9] the $J_{23a,24}$ and $J_{23b,24}$ values (H_2-23a is defined to be deshielded to a greater extent than H_2-23b) may be used to define the C-24 absolute configuration in cucurbitacins. The value of $J_{23a,24}$ (6.9 Hz) was larger than that of $J_{23b,24}$ (3.6 Hz) in **3**, confirming the (24*R*) configuration, while the value of $J_{23a,24}$ (2.6 Hz) was smaller than that of $J_{23b,24}$ (8.5 Hz) in **4**, establishing the (24*S*) configuration.

Compound **5** had a molecular formula of $C_{32}H_{46}O_9$ on the basis of its sodium adduct ion at *m*/*z* 597.3040 [M + Na]$^+$ (calcd for $C_{32}H_{46}O_9Na$, 597.3034). Comparison of its NMR data with those of 3-*epi*-isocucurbitacin B[14] revealed their structural similarity, except for an oxymethine (δ_H 4.13; δ_C 66.5) instead of a C-7 methylene (δ_C 24.8) in 3-*epi*-isocucurbitacin B and the deshielded resonance of C-8 (δ_C 53.8) in **5**. These data indicated a hydroxy group at C-7 in **5**, which was confirmed by the molecular formula as well as the ^{1}H−^{1}H COSY cross-peak between H-7 (δ_H 4.13) and H-6 (δ_H 6.04). The ^{1}H NMR signal for H-8 (δ_H 2.10) appeared as a broad singlet, suggesting a small coupling constant between H-7 and H-8, which corresponded to a dihedral angle of approximately 90° and indicated a β-OH group at C-7.[15] Therefore, the structure of compound **5** was defined as 7β-hydroxy-3-*epi*-isocucurbitacin B.

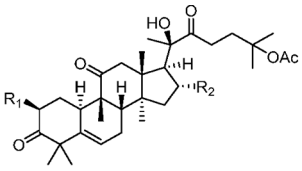
Compound **6** was assigned a molecular formula of $C_{24}H_{34}O_5$, as determined by its HRESIMS ion at *m*/*z*

https://dx.doi.org/10.1021/acs.jnatprod.0c00364
J. Nat. Prod. XXXX, XXX, XXX−XXX

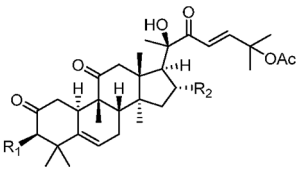
Chart 2



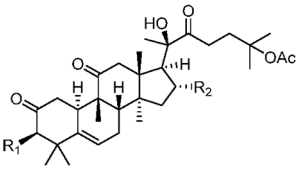
Comp	R_1	R_2
B1	=O	=O
B2	OH	=O
B3	$OCOCH_3$	OH
B4	OH	$OCOCH_3$
B5	$OCOCH_3$	$OCOCH_3$
B6	OCOPh	OCOPh
B7	OCOPh	OH
B11	AcO-2, AcO-16, 7=O	



Comp	R_1	R_2
DB1	=O	=O
DB2	OH	=O
DB3	$OCOCH_3$	$OCOCH_3$
DB4	$OCOC_2H_5$	$OCOC_2H_5$
DB5	$OCO(CH_2)_2CO_2H$	OH
DB6	OCOPh	OCOPh
DB7	OCOPh	OH
DB11	AcO-2, AcO-16, 7=O	



Comp	R_1	R_2
B8	$OCOCH_3$	OH
B9	OH	$OCOCH_3$
B10	$OCOCH_3$	$OCOCH_3$



Comp	R_1	R_2
DB8	$OCOCH_3$	OH
DB9	OH	$OCOCH_3$
DB10	$OCOCH_3$	$OCOCH_3$

425.2296 $[M + Na]^+$ (calcd for $C_{24}H_{34}O_5Na$, 425.2298). Its spectroscopic data were comparable to those of hexanorcucurbitacin D,[15] with the difference being a sharp singlet (δ_H 3.91) assigned to an α-OH group at C-**3**.[16] Therefore, the structure of compound **6** was defined as hexanorisocucurbitacin D.

Compound **7** had a molecular formula of $C_{32}H_{48}O_{10}$ based on its HRESIMS ion at m/z 615.3137 $[M + Na]^+$ (calcd for $C_{32}H_{48}O_{10}Na$, 615.3140). Its NMR data showed similarity to those of dihydrocucurbitacin B with major differences in the A-ring. The HMBC correlations from H_2-1 (δ_H 2.46, 2.75) to the carboxylic C-2 (δ_C 180.2), as well as from H_3-28 and H_3-29 (δ_H 1.32 and 1.44, respectively) to the carboxylic C-3 (δ_C 184.2), suggested a ring-A seco-cucurbitacin, which was consistent with its nine indices of hydrogen deficiency. The NOESY correlations of H-10/H_3-30/H-17/H_3-21 and of H_3-19/H-8/H_3-18/H-16, as well as the ECD Cotton effects at 300 nm ($\Delta\varepsilon$ +3.4) and 202 nm ($\Delta\varepsilon$ +17.4), indicated its (8*S*, 9*R*, 10*R*, 13*R*, 14*S*, 16*R*, 17*R*, 20*R*) absolute configuration, which was different from the compound reported in a patent[17] [same 2D structure with an (8*R*, 9*R*, 10*S*, 13*R*, 14*S*, 16*R*, 17*R*, 20*R*) absolute configuration]. As the sixth example of a ring-A seco-cucurbitacin,[12] **7** was named colocynthenin F.

By comparison of the spectroscopic data with reported data, known compounds **8**−**27** were identified as cucurbitacin H (**8**),[13] cucurbitacin G (**9**),[13] cucurbitacin I (**10**),[18] cucurbitacin L (**11**),[18] cucurbitacin D (**12**),[18] dihydrocucurbitacin D (**13**),[18] cucurbitacin B (**14**),[18] 23,24-dihydrocucurbitacin B (**15**),[7] arvenin I (**16**),[19] arvenin II (**17**),[19] 7β-hydroxycucurbitacin B (**18**),[15] 7β-hydroxydihydrocucurbitacin B (**19**),[15] 3-*epi*-isocucurbitacin D (**20**),[20] isocucurbitacin D (**21**),[19] 23,24-dihydroisocucurbitacin D (**22**),[21] 23,24-dihydro-3-*epi*-isocucurbitacin D (**23**),[21] 24α-hydroxyisocucurbitacin D (**24**),[13] 24β-hydroxyisocucurbitacin D (**25**),[13] cucurbitacin F (**26**),[22] and hexanorcucurbitacin D (**27**).[15]

Synthesis of Cucurbitacin Derivatives. Since the separation of the *T. cucumeroides* extract led to the isolation of cucurbitacin B (**14**, **B**, 3 g) and dihydrocucurbitacin B (**15**, **DB**, 2 g) in large amounts, semisynthetic derivatives were prepared to examine the structure−activity relationships (SARs) of cucurbitacins.

In order to study the influence of the hydroxy groups at C-2 and C-16 on the biological activity, we introduced modifications at C-2 and C-16. The reaction of **B** or **DB** with Dess-Martin periodinane yielded **B1**,**2** or **DB1**,**2**.[23] Compounds **B** or **DB** were treated with acetic, propionic, succinic, or benzoic anhydrides to yield esters **B3**−**7** or **DB3**−**7**.[24] To analyze the importance of HO-3 in cucurbitacin, compounds with a 2-oxo-3-hydroxy A-ring were explored. 3-*epi*-Isocucurbitacin B (obtained from the interconversion of **B** with silica gel in CH_2Cl_2) was treated with Ac_2O to yield **B8**−**10**, and 3-*epi*-iso-dihydrocucurbitacin B (obtained from the interconversion of **DB**) was treated with Ac_2O to yield **DB8**−**10**.[24,25] To explore the role of the C-7 allylic position, **B11** and **DB11** were synthesized from **B5** and **DB3**, respectively, by oxidation with CrO_3 and pyridine in CH_2Cl_2.[24]

LDL Uptake Activity. An LDL uptake assay was first conducted to evaluate the effects of 47 cucurbitacins (5 μM) on LDL uptake in HepG2 cells. The results in Figure 3 show that most of the compounds improved the LDL uptake rates,

https://dx.doi.org/10.1021/acs.jnatprod.0c00364
J. Nat. Prod. XXXX, XXX, XXX−XXX

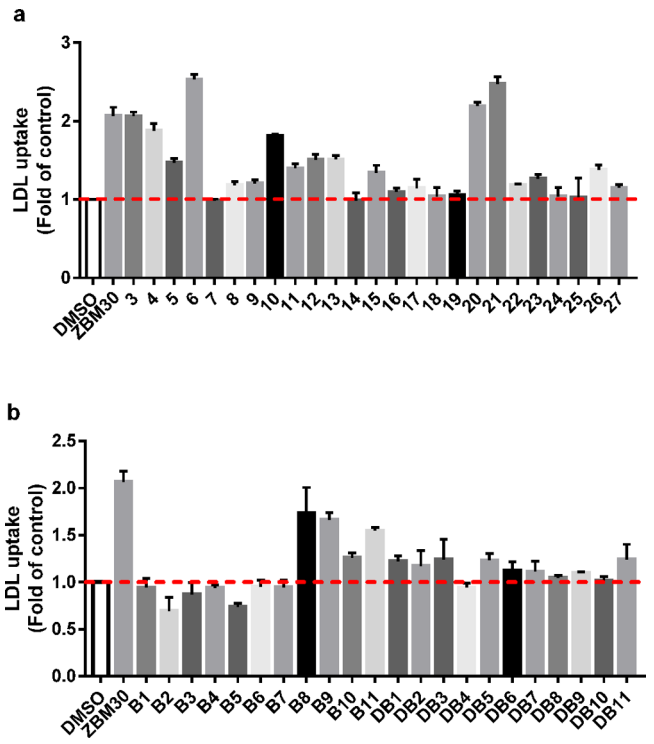


Figure 3. Effects of cucurbitacins on LDL uptake rates in HepG2 cells (n = 3). (a) Effects of cucurbitacins isolated from *T. cucumeroides* roots on LDL uptake rates. (b) Effects of cucurbitacin derivatives on LDL uptake rates. Nagilactone B (ZBM30, 5 μM) was used as a positive control. The red dotted line indicates an uptake rate of 1.0. The results are presented as the means ± SEM, n = 3.

especially those of hexanorisocucurbitacin D (**6**, rate of 2.53) and isocucurbitacin D (**21**, rate of 2.47), which were comparable to that of the positive control, nagilactone B (designated ZBM30, rate of 2.07).[26]

According to the observed lipid uptake rates, the preliminary SARs were summarized as shown in Figure 3. The 2-oxo-3α-hydroxy A-ring was found to be essential for activity: the compounds with a 2-oxo-3α-hydroxy A-ring, such as **6** and **21**, had a distinct advantage over those with a 2β-hydroxy-3-oxo A-ring, such as **12** and **13**. Similar activities were recorded for **B** with **B1**−**10** and **DB** with **DB1**−**10**, indicating that either substitution or oxidation at C-2, C-3, or C-16 was not significant for activity. Comparing the LDL uptake rate of **B** with **B11**, and of **DB** with **DB11**, suggested that the C-7 carbonyl group may promote activity. A 24-methoxy moiety could enhance activity, as shown by comparison of the three groups **3**/**4**, **8**/**9**, and **12**/**13**.

Influence on LDLR and PCSK9 Protein Levels. Since LDLR protein is a key cell surface receptor for cholesterol internalization, Western blot analysis was carried out to examine whether these compounds impact LDLR protein levels in HepG2 cells. Compounds **6** and **21**, which strongly promoted LDL uptake, were chosen for this study. It is clear that LDLR protein levels increased dose-dependently in response to these two cucurbitacins, which may explain why these compounds could improve LDL uptake.

We further assessed whether these two cucurbitacins regulate cholesterol metabolism in a PCSK9-dependent process. The results show that they dose-dependently reduced PCSK9 protein levels, suggesting that PCSK9 is involved in the LDLR uptake elevation induced by compounds **6** and **21**.

To study the lipid-lowering activities of cucurbitacins, a small library of this class of compounds was prepared. Among compounds **1**−**27** isolated from *T. cucumeroides* roots, **1** was a novel lactone-type norcucurbitacin, while **2** and **7** were rare ring-A seco-cucurbitacins. Modifications of the groups contributing to activity of cucurbitacins yielded 22 derivatives.

Most of natural cucurbitacins and their derivatives were first screened for lipid-lowering activities. The results revealed that most of the compounds could improve the LDL uptake rate, among which hexanorisocucurbitacin D (**6**) and isocucurbitacin D (**21**) displayed the strongest effects compared to the positive control, nagilactone B. In addition, some general trends were observed that may be useful for the discovery of more active compounds: a 2-oxo-3α-hydroxy A-ring constitution is essential for activity, while modifications of the hydroxy groups at C-2, C-3, or C-16 make no difference; additionally, 7-carbonyl and 24-methoxy groups may be favorable in certain types of compounds.

Further assays indicated that compounds **6** and **21** dose-dependently increased LDLR protein levels and reduced PCSK9 protein levels, suggesting that **6** and **21** are potent PCSK9 modulators for the treatment of hyperlipidemia. Nevertheless, additional studies to determine such information as the intact mechanism, effects in animals, in vivo pharmacokinetics, and drug safety should be carried out on cucurbitacins in the future.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Rudolph Autopol VI automatic polarimeter. IR

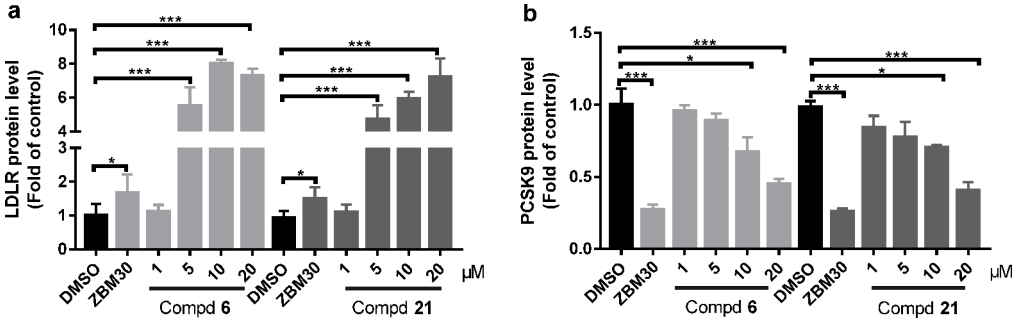


Figure 4. Effects of compounds **6** and **21** on LDLR and PCSK9 protein levels in HepG2 cells. (a) Compounds **6** and **21** dose-dependently upregulated LDLR protein levels. (b) Compounds **6** and **21** dose-dependently downregulated PCSK9 protein levels. The results are presented as the means ± SEM, $n \geq 5$. *P < 0.05, **P < 0.01, ***P < 0.001 vs vehicle control by one-way ANOVA (Dunnett's multiple comparisons test).

https://dx.doi.org/10.1021/acs.jnatprod.0c00364
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. ^{1}H NMR Data of Compounds 1−4 (δ in ppm, J in Hz)

	1[a]	2[b]	3[c]	4[c]
1	2.50, m	2.51, m	2.10, ddd	2.11, ddd
	2.16, dd	2.46, m	(12.3, 5.8, 3.6)	(12.3, 5.8, 3.6)
	(16.2, 13.5)		1.20, m	1.41, m
2			4.58, m	4.58, dd (13.0, 5.9)
6	5.75, dd (5.2, 2.7)	6.06, dd (5.1, 2.8)	5.82, dt (6.0, 2.2)	5.83, dd (6.1, 2.3)
7	2.40, ddd	2.43, m	2.42, dd (19.7, 8.2)	2.43, ddd
	(20.2, 8.2, 3.9)	2.03, m	2.03, dd (19.7, 5.6)	(19.3, 8.4, 2.8)
	2.04, m			2.04, m
8	2.02, d (8.3)	2.00, d (8.0)	1.98, d (8.1)	2.00, d (8.1)
10	2.76, d (13.5)	3.26, m	2.96, m	3.01, br d (12.4)
12	3.12, d (14.8)	3.35, d (14.7)	3.43, d (15.4)	3.47, d (14.7)
	2.68, d (14.8)	2.56, d (14.7)	2.61, d (15.4)	2.63, d (14.7)
15	1.84, dd (13.4, 8.7)	1.81, dd (13.3, 9.0)	1.89, dd (13.2, 9.0)	1.87, dd (13.4, 8.8)
	1.40, m	1.40, m	1.40, m	1.40, m
16	4.32, t (8.3)	4.45, t (7.9)	4.57, t (8.2)	4.46, t (7.7)
17	2.49, d (6.6)	2.53, d (7.2)	2.60, d (6.5)	2.59, d (7.3)
18	0.96, s	0.90, s	0.93, s	0.96, s
19	1.05, s	1.13, s	1.05, s	1.06, s
21	1.40, s	1.37, s	1.39, s	1.42, s
23	2.81, m	2.83, ddd	3.05, dd (18.5, 6.9)	3.08, dd (17.7, 2.6)
	2.52, m	(18.0, 10.3, 5.5)	2.97, dd (18.5, 3.6)	2.84, dd (17.7, 8.5)
		2.70, ddd		
		(18.0, 10.3, 5.5)		
24	2.06, dd (9.9, 5.3)	1.98, m	3.57, dd (6.9, 3.6)	3.63, dd (8.5, 2.6)
26	1.43, s	1.44, s	1.19, s	1.17, s
27	1.46, s	1.44, s	1.14, s	1.20, s
28	1.51, s	1.35, s	1.29, s	1.30, s
29	1.53, s	1.62, s	1.30, s	1.31, s
30	1.33, s	1.32, s	1.40, s	1.40, s
32	1.96, s	1.95, s		
OCH_3			3.43, s	3.43, s

[a]Recorded at 500 MHz, in $CDCl_3$. [b]Recorded at 400 MHz, in methanol-d_4. [c]Recorded at 600 MHz, in methanol-d_4.

Table 2. ^{1}H NMR Data of Compounds 5−7 (δ in ppm, J in Hz)

	5[a]	6[b]	7[c]
1	2.21, m	2.25, m	2.75, dd (20.0, 3.0)
	2.03, m	2.13, m	2.46, dd (20.0, 5.4)
3	4.12, s	3.91, s	
6	6.04, dd (5.0, 2.4)	5.94, d (5.6)	5.84, dt (5.3, 2.5)
7	4.13, dd (5.0, 2.1)	2.47, ddd	2.35, ddd
		(19.1, 5.1, 2.6)	(19.2, 8.4, 3.1)
		2.04, dd (19.1, 6.6)	2.05, m
8	2.10, br s	2.01, d (7.6)	2.05, d (7.9)
10	3.12, m	2.83, br d (13.1)	3.70, dd (5.4, 3.0)
12	3.21, d (14.6)	3.31, d (14.8)	3.25, d (15.1)
	2.52, d (14.6)	2.33, d (14.8)	2.60, d (15.1)
15	2.08, m	1.91, dd (13.0, 9.0)	1.83, dd (13.3, 8.8)
	1.54, m	1.45, d (13.0)	1.43, m
16	4.61, t (7.5)	4.79, t (7.8)	4.30, t (7.8)
17	2.51, d (7.3)	3.15, d (6.7)	2.52, d (7.0)
18	0.89, s	0.66, s	0.99, s
19	1.16, s	0.82, s	1.06, s
21	1.40, s	2.12, s	1.40, s
22		1.24, s	
23	6.84, d (15.8)	1.25, s	2.86, ddd
			(17.5, 10.0, 5.6)
			2.58, ddd
			(17.5, 11.0, 6.5)
24	6.97, d (15.8)	1.11, s	2.05, m
26	1.54, s		1.45, s
27	1.57, s		1.47, s
28	1.27, s		1.32, s
29	0.98, s		1.44, s
30	1.29, s		1.37, s
32	2.00, s		1.96, s

[a]Recorded at 600 MHz, in methanol-d_4. [b]Recorded at 400 MHz, in methanol-d_4. [c]Recorded at 600 MHz, in $CDCl_3$.

spectra were recorded on a Thermo Scientific Thermo IS5 spectrometer. HRESIMS data were collected using an Agilent 1290 Infinity HPLC and an Agilent 6224 TOF mass spectrometer. NMR spectra were recorded on Bruker AM-400, AVANCE III 500, and AVANCE III 600 spectrometers. Semipreparative HPLC was performed on an Agilent 1100 series HPLC system equipped with a YMC-Pack ODS-A column (250 × 10 mm, 5 μm). Silica gel (200−300 mesh, Qingdao Haiyang Chemical Co., Ltd., People's Republic of China) and ODS (20−45 μm, Fuji Silysia Chemical Ltd., Japan) were used for column chromatography (CC). Silica gel GF254 and RP-18 F254S TLC plates were obtained from Qingdao Haiyang Chemical Co. Ltd. and Merck (Darmstadt, Germany), respectively. All solvents were purchased from Sinopharm Chemical Reagent Co., Ltd.

Plant Material. Roots of *Trichosanthes cucumeroides* were collected in Yunnan Province, China, in July 2014 and identified by Prof. Heming Yang. A voucher specimen (no. SIMM239) was deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China.

Extraction and Isolation. Dried roots of *T. cucumeroides* (10.0 kg) were crushed into a coarse powder and cold-soaked in 95% EtOH. The solvent was evaporated to produce a crude extract (500 g). The extract was suspended in water (2 L) and extracted with EtOAc to afford the EtOAc-soluble fraction (120 g). The EtOAc layer was divided into fractions A−H by silica gel CC using gradients of increasing polarity from petroleum ether/acetone (10:1) to acetone.

Fraction C (5 g) was applied to an ODS column (CH_3OH/H_2O, 50:50 to 100:0) to give subfractions Fr. C1−C7. Fr. C2 was purified first by silica gel CC (CH_2Cl_2/EtOAc, 6:1 to 1:1) and with an RP-C_{18} column (CH_3OH/H_2O, 65:35) to obtain **1** (3 mg) and **7** (6 mg). Fr. C5 was subjected to silica gel CC (petroleum ether/EtOAc, 4:1 to 2:1) followed by semipreparative HPLC (CH_3CN/H_2O, 68:32) to yield **2** (7 mg).

Fraction D (60 g) was separated by silica gel CC (CH_2Cl_2/CH_3OH, 50:1 to 5:1) to give Fr. D1−D5. Fr. D3 was subjected to silica gel CC (petroleum ether/acetone, 5:1 to 3:1, with 0.1% formic acid) to produce Fr. D31−D35. Fr. D31 and Fr. D33 were purified by an ODS column (CH_3CN/H_2O, 25:65 to 45:55) and HPLC (CH_3CN/H_2O, 43:57); Fr. D31 produced **8** (8 mg) and **9** (8 mg), while Fr. D33 produced **20** (10 mg) and **27** (7 mg).

Fraction E (20 g) was subjected to silica gel CC (CH_2Cl_2/CH_3OH, 100:1 to 1:1) to give Fr. E1−E6. Fr. E2 was separated by silica gel CC (petroleum ether/acetone, 5:1 to 3:1, with 0.1% formic acid) to produce six fractions. Fr. E23 was purified by HPLC (CH_3CN/H_2O, 55:45) to yield **6** (2 mg), **14** (3 g), and **15** (2 g). Fr. E24 was separated by silica gel CC (petroleum ether/EtOAc, 6:1 to 1:1, with 0.1% formic acid) and HPLC (CH_3OH/H_2O, 65:35) to obtain **3** (1 mg), **4** (5 mg), **10** (6 mg), **11** (5 mg), **12** (600 mg), **21** (12 mg), **22** (4 mg), and **23** (7 mg).

Fr. E3 was separated into Fr. E31−E34 by an ODS column (CH_3OH/H_2O, 60:40 to 70:30). Fr. E33 and E34 were subjected to

https://dx.doi.org/10.1021/acs.jnatprod.0c00364
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. ^{13}C NMR (125 MHz) Data of Compounds 1−7 (δ in ppm)

	1[a]	2[b]	3[b]	4[b]	5[b]	6[b]	7[a]
1	30.4, CH_2	31.2, CH_2	37.1, CH_2	37.1, CH_2	37.8, CH_2	40.2, CH_2	32.1, CH_2
2	172.1, C	180.2, C	72.8, CH	72.8, CH	212.4, C	211.8, C	180.2, C
3			214.0, C	214.0, C	80.7, CH	81.4, CH	184.2, C
4	84.0, C	89.9, C	49.0, C	49.2, C	49.5, C	49.6, C	47.2, C
5	136.9, C	138.4, C	142.7, C	140.0, C	145.3, C	140.1, C	140.6, C
6	120.7, CH	128.2, CH	121.2, CH	121.2, CH	122.7, CH	122.5, CH	122.9, CH
7	23.9, CH_2	24.6, CH_2	24.8, CH_2	24.8, CH_2	66.5, CH	24.9, CH_2	24.7, CH_2
8	42.2, CH	44.1, CH	44.1, CH	44.1, CH	53.8, CH	44.6, CH	42.7, CH
9	47.8, C	49.4, C	49.9, C	49.8, C	48.1, C	49.7, C	48.7, C
10	33.4, CH	33.6, CH	34.9, CH	34.8, CH	34.4, CH	37.3, CH	34.9, CH
11	211.8, C	215.8, C	215.9, C	215.7, C	215.7, C	213.6, C	214.4, C
12	48.9, CH_2	49.9, CH_2	49.9, CH_2	49.9, CH_2	49.8, CH_2	48.1, CH_2	49.9, CH_2
13	50.5, C	51.9, C	51.87, C	51.9, C	50.8, C	51.2, C	50.6, C
14	48.4, C	52.8, C	51.82, C	51.8, C	48.4, C	50.1, C	48.7, C
15	45.8, CH_2	46.4, CH_2	46.6, CH_2	46.6, CH_2	46.5, CH_2	46.1, CH_2	45.9, CH_2
16	71.1, CH	71.4, CH	71.6, CH	71.5, CH	71.8, CH	72.5, CH	71.1, CH
17	57.9, CH	59.5, CH	59.9, CH	58.9, CH	60.1, CH	67.9, CH	58.6, CH
18	20.0, CH_3	20.5, CH_3	20.5, CH_3	20.6, CH_3	20.8, CH_3	20.2, CH_3	20.8, CH_3
19	18.6, CH_3	20.2, CH_3	20.2, CH_3	20.2, CH_3	20.4, CH_3	20.2, CH_3	20.4, CH_3
20	79.0, C	80.8, C	81.1, C	80.8, C	80.2, C	210.6, C	79.2, C
21	24.6, CH_3	25.8, CH_3	25.0, CH_3	26.4, CH_3	25.4, CH_3	31.8, CH_3	24.6, CH_3
22	214.0, C	216.6, C	216.4, C	215.2, C	205.3, C	24.6, CH_3	214.6, C
23	30.8, CH_2	32.9, CH_2	40.0, CH_2	40.2, CH_2	124.8, CH	21.7, CH_3	30.9, CH_2
24	34.9, CH_2	35.8, CH_2	84.7, CH	85.3, CH	151.6, CH	19.5, CH_3	35.1, CH_2
25	81.4, C	83.1, C	73.8, C	73.8, C	81.1, C		81.5, C
26	26.0, CH_3	26.2, CH_3	25.7, CH_3	25.2, CH_3	26.4, CH_3		26.0, CH_3
27	26.3, CH_3	26.3, CH_3	26.5, CH_3	25.3, CH_3	26.9, CH_3		26.3, CH_3
28	31.5, CH_3	25.5, CH_3	29.8, CH_3	29.8, CH_3	27.8, CH_3		28.1, CH_3
29	30.7, CH_3	25.6, CH_3	21.9, CH_3	21.9, CH_3	21.9, CH_3		27.8, CH_3
30	18.3, CH_3	18.8, CH_3	19.5, CH_3	19.5, CH_3	19.5, CH_3		18.8, CH_3
OCH_3			59.7, CH_3	60.8, CH_3			
31	170.5, C	172.4, C			171.9, C		170.6, C
32	22.6, CH_3	22.4, CH_3			24.2, CH_3		22.6, CH_3

[a]Recorded in $CDCl_3$. [b]Recorded in methanol-d_4.

silica gel CC (petroleum ether/EtOAc, 3:1 to 1:2, with 0.1% formic acid) to afford Fr. E331−334 and Fr. E340−343. Fr. E334 was purified by an ODS column (CH_3CN/H_2O, 55:45) to yield **13** (700 mg). Compounds **5** (2 mg), **18** (3 mg), and **19** (2 mg) were obtained from Fr. E340 by HPLC (CH_3OH/H_2O, 53:47); **24** (3 mg) and **25** (3 mg) were obtained from Fr. E341 by HPLC (CH_3CN/H_2O, 37:63). Fr. E4 was purified by silica gel CC (petroleum ether/acetone, 4:1 to 1:1, with 0.1% formic acid) followed by an ODS column (CH_3OH/H_2O, 45:55 to 65:35) to give **26** (3 mg). Fraction F was separated by silica gel CC (CH_2Cl_2/CH_3OH, 20:1 to 5:1) and HPLC (CH_3CN/H_2O, 50:50) to yield **16** (700 mg) and **17** (800 mg).

*Neocucurbitacin E (**1**):* white amorphous powder; $[\alpha]_D^{20}$ −9 (*c* 0.1, $CHCl_3$); ECD (CH_3OH) 300 nm ($\Delta\varepsilon$ +2.8), 200 nm ($\Delta\varepsilon$ +5.6); IR (KBr) ν_{max} 3439, 2924, 1734, 1697, 1654, 1369, 1219, 772 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 569.3080 [M + Na]$^+$ (calcd for $C_{31}H_{46}O_8Na$, 569.3085).

*Colocynthenin E (**2**):* white amorphous powder; $[\alpha]_D^{20}$ +32 (c 0.1, $CHCl_3$); ECD (CH_3OH) 300 nm ($\Delta\varepsilon$ +4.6), 202 nm ($\Delta\varepsilon$ +16.6); IR (KBr) ν_{max} 3443, 2921, 1770, 1695, 1369, 1255, 1128, 1023 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 587.3192 [M + Na]$^+$ (calcd for $C_{31}H_{48}O_9Na$, 587.3191).

*24β-Methoxydihydrocucurbitacin D (**3**):* white amorphous powder; $[\alpha]_D^{20}$ +60 (c 0.1, CH_3OH); ECD (CH_3OH) 301 nm ($\Delta\varepsilon$ +3.2), 203 nm ($\Delta\varepsilon$ +13.0); IR (KBr) ν_{max} 3446, 2971, 2394, 2923, 1504, 1032, 1013 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 571.3244 [M + Na]$^+$ (calcd for $C_{31}H_{48}O_8Na$, 571.3241).

*24α-Methoxydihydrocucurbitacin D (**4**):* white amorphous powder; $[\alpha]_D^{20}$ +63 (c 0.1, CH_3OH); ECD (CH_3OH) 304 nm ($\Delta\varepsilon$ +3.6), 202 nm ($\Delta\varepsilon$ +15.8); IR (KBr) ν_{max} 3437, 2976, 2930, 1688, 1654, 1384, 1091, 1047 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 571.3245 [M + Na]$^+$ (calcd for $C_{31}H_{48}O_8Na$, 571.3241).

*7β-Hydroxy-3-epi-isocucurbitacin B (**5**):* white amorphous powder; $[\alpha]_D^{20}$ +6 (*c* 0.1, $CHCl_3$); ECD (CH_3OH) 300 nm ($\Delta\varepsilon$ +1.1), 201 nm ($\Delta\varepsilon$ +5.0); IR (KBr) ν_{max} 3437, 2956, 2923, 2852, 1718, 1689, 1626, 1459, 1377, 1259, 1125 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m*/*z* 597.3040 [M + Na]$^+$ (calcd for $C_{32}H_{46}O_9Na$, 597.3034).

*Hexanorisocucurbitacin D (**6**):* white amorphous powder; $[\alpha]_D^{20}$ +109 (*c* 0.1, $CHCl_3$); ECD (CH_3OH) 295 nm ($\Delta\varepsilon$ +4.0), 200 nm ($\Delta\varepsilon$ +9.6); IR (KBr) ν_{max} 3446, 2958, 2923, 2853, 1697, 1655, 1458, 1380, 1101 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m*/*z* 425.2296 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_5Na$, 425.2298).

*Colocynthenin F (**7**):* white amorphous powder; $[\alpha]_D^{20}$ +18 (*c* 0.1, CH_3OH); ECD (CH_3OH) 300 nm ($\Delta\varepsilon$ +3.4), 202 nm ($\Delta\varepsilon$ +3.6); IR (KBr) ν_{max} 3444, 2974, 2925, 1705, 1385, 1370, 1270, 1209, 1129, 1023 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m*/*z* 615.3137 [M + Na]$^+$ (calcd for $C_{31}H_{48}O_9Na$, 615.3140).

Cell Culture. HepG2 cells (ATCC HB-8065) were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS, v/v) and incubated under a humidified atmosphere of 5% CO_2 and 95% O_2 at 37 °C. Cells from passages 4 to 11 were used; subcultures were performed once every 2 days.

https://dx.doi.org/10.1021/acs.jnatprod.0c00364
J. Nat. Prod. XXXX, XXX, XXX−XXX

Lipoprotein Isolation and DiI-LDL Preparation. Human plasma was obtained from Shanghai Xuhui Central Hospital, China, after informed consent and approval from the Ethics Committee. The procedures were performed under the principles of the Declaration of Helsinki.[27] LDL and lipoprotein-deficient serum (LPDS) were isolated from the pooled plasma of healthy volunteers by ultracentrifugation and dialysis against dialysis buffer and PBS. The obtained LDL was labeled with the fluorescent probe 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI, Biotium, CA, USA) as previously described, with minor modifications.[28]

In short, DiI dissolved in DMSO (15 mg/mL) was added to the LDL/LPDS mixture (v/v, 1:2) to a final concentration of 300 mg DiI/mg LDL protein. After incubation at 37 °C for 18 h, the DiI-labeled LDL (DiI-LDL) was separated by ultracentrifugation and dialysis against buffer and PBS. After sterilization using 0.45 μm filters (Millipore, MA, USA), DiI-LDL was stored at 4 °C.

DiI-LDL Uptake Assay. DiI-LDL uptake assays were conducted as described previously, with slight modifications.[28] HepG2 cells seeded in 24-well plates, after treatment with DMSO or compounds, were removed and placed in DiI-LDL DMEM (20 μg/mL) at 37 °C for 3 h in the dark. The cells were rinsed twice with ice-cold PBS containing 0.4% albumin (Sigma-Aldrich, St. Louis, MO, USA) and washed twice with PBS. After that, 500 μL of isopropanol was added to each well following a 20 min incubation at room temperature under constant shaking in the dark. Finally, 200 μL aliquots were used for fluorescence detection with a SpectraMax M2e microplate reader (520−578 nm, Molecular Devices, San Jose, CA, USA). Nagilactone B (ZBM30, 5 μM), a natural product isolated from *Podocarpus nagi*, significantly improved LDL uptake in a PCSK9-dependent process[26] and thus was used as a positive control in this research.

Western Blot Analysis. HepG2 cells were cultured in six-well plates for 12 h. The medium was incubated for an additional 24 h, after the addition of 2% LPDS. After treatment with various concentrations of compounds for the indicated times, cells were washed three times with PBS. Total cellular proteins were extracted with 100 μL of lysis buffer containing a protease and phosphatase inhibitor cocktail (catalogue number: 539134, Calbiochem, Merck Millipore, Germany) and centrifuged at 12000*g* and 4 °C for 10 min. Cell nuclear and cytoplasmic proteins were extracted using a nuclear and cytoplasmic protein extraction kit (catalogue number: P0013B, Beyotime Biotechnology) according to the manufacturer's instructions. Protein concentrations were determined with a BCA protein assay kit (catalogue number: P0010, Beyotime Biotechnology).

Protein (30 μg) was loaded in each well for 8% SDS PAGE and transferred to PVDF membranes (catalogue number: 1620177, Bio Rad, Hercules, CA, USA). The membranes were blocked with 5% skim milk for 2 h at room temperature and incubated with primary antibodies overnight at 4 °C. After three washes with TBST solution, the membranes were incubated with secondary antibodies (catalog numbers: 1706515 and 1706516, Bio-Rad) for 2 h. Finally, the bands were visualized by Clarity Western ECL blotting substrates (catalogue number: 1705061, Bio Rad), and the values were normalized to that of a housekeeping protein, either lamin B1 or β-actin.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00364.

General procedures for the synthesis and spectroscopic data of cucurbitacin derivatives; copies of NMR, HRESIMS, IR, and CD spectra of compounds **1**−**7**; copies of the ^{1}H and ^{13}C NMR spectra of cucurbitacin derivatives (DOCX)

■ AUTHOR INFORMATION

Corresponding Author

Lijiang Xuan − *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China; University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China;* orcid.org/0000-0002-0593-3921; Phone: 86-21-20231968; Email: ljxuan@simm.ac.cn

Authors

Xianjing Zhang − *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China; University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China*

Huihui Li − *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China; University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China*

Wenqiong Wang − *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China*

Tong Chen − *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.0c00364

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge grants from the "Personalized Medicines Molecular Signature based Drug Discovery and Development", Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA12040335), the National Natural Science Foundation of China (No. 81773863), the Youth Innovation Promotion Association, and the National Science and Technology Major Project (Nos. 2019ZX09201004-003-041 and 2019ZX09201004-003-042).

■ REFERENCES

(1) Roth, G. A.; Johnson, C.; Abajobir, A.; Abd-Allah, F.; Abera, S. F.; Abyu, G.; Ahmed, M.; Aksut, B.; Alam, T.; Alam, K.; Alla, F.; Alvis-Guzman, N.; Amrock, S.; Ansari, H.; Ärnlöv, J.; Asayesh, H.; Atey, T. M.; Avila-Burgos, L.; Awasthi, A.; Banerjee, A.; et al. *J. Am. Coll. Cardiol.* **2017**, *70*, 1−25.

(2) Gu, L.; Gong, Y.; Zhao, C.; Wang, Y.; Tian, Q.; Lei, G.; Liang, Y.; Zhao, W.; Tan, S. *Molecules* **2019**, *24*, 4140.

(3) Nishikido, T.; Ray, K. K. *Front. Cardiovasc. Med.* **2019**, *5*, 199.

(4) Li, H.; Dong, B.; Park, S. W.; Lee, H. S.; Chen, W.; Liu, J. *J. Biol. Chem.* **2009**, *284*, 28885−28895.

(5) Tai, M. H.; Chen, P. K.; Chen, P. Y.; Wu, M. J.; Ho, C. T.; Yen, J. H. *Mol. Nutr. Food Res.* **2014**, *58*, 2133−2145.

(6) Li, H. H.; Li, J.; Zhang, X. J.; Li, J. M.; Xi, C.; Wang, W. Q.; Lu, Y. L.; Xuan, L. J. *Acta Pharmacol. Sin.* **2020**, *41*, 327−335.

(7) Farias, M. R.; Schenkel, E. P.; Mayer, R.; Rücker, G. *Planta Med.* **1993**, *59*, 272−275.

(8) Zhu, N.; Sun, Z.; Hu, M.; Li, Y.; Zhang, D.; Wu, H.; Tian, Y.; Li, P.; Yang, J.; Ma, G.; Xu, X. *Phytochemistry* **2018**, *147*, 49−56.

(9) Gan, M.; Liu, M.; Liu, B.; Lin, S.; Zhang, Y.; Zi, J.; Song, W.; Ye, F.; Chen, X.; Shi, J. *J. Nat. Prod.* **2011**, *74*, 2431−2437.

(10) Kawahara, N.; Kurata, A.; Hakamatsuka, T.; Sekita, S.; Satake, M. *Chem. Pharm. Bull.* **2001**, *49*, 1377−1379.
(11) Sahranavard, S.; Naghibi, F.; Siems, K.; Jenett-Siems, K. *Planta Med.* **2010**, *76*, 1014−1017.
(12) Jiang, H. Z.; Hu, S.; Tan, R. X.; Tan, R.; Jiao, R. H. *Nat. Prod. Res.* **2018**, 1−7.
(13) Liu, Y.; Chen, G.; Chen, X.; Chen, S. X.; Gan, L. S.; Yuan, T. *J. Nat. Prod.* **2018**, *81*, 2115−2119.
(14) Monte, F. J. Q.; Kintzinger, J. P.; Braz-Filho, R. *Magn. Reson. Chem.* **1997**, *35*, 802−805.
(15) Chen, C.; Qiang, S.; Lou, L.; Zhao, W. *J. Nat. Prod.* **2009**, *72*, 824−829.
(16) Sallam, A. A.; Hitotsuyanagi, Y.; Mansour, E. S. S.; Ahmed, A. F.; Gedara, S.; Fukaya, H.; Takeya, K. *Phytochem. Lett.* **2010**, *3*, 117−121.
(17) Ray, A.; Boyle, S. M. Natural Sweetener Compositions. US20180132514, 2018.
(18) Oh, H.; Mun, Y. J.; Im, S. J.; Lee, S. Y.; Song, H. J.; Lee, H. S.; Woo, W. H. *Planta Med.* **2002**, *68*, 832−833.
(19) Yamada, Y.; Hagiwara, K.; Iguchi, K.; Suzuki, S.; Hsu, H. Y. *Chem. Pharm. Bull.* **1978**, *26*, 3107−3112.
(20) Kupchan, S. M.; Meshulam, H.; Sneden, A. T. *Phytochemistry* **1978**, *17*, 767−769.
(21) Cattel, L.; Caputo, O.; Delprino, L.; Biglino, G. *Gazz. Chim. Ital.* **1978**, *108*.
(22) Fang, X.; Phoebe, C. H., Jr.; Pezzuto, J. M.; Fong, H. H.; Farnsworth, N. R.; Yellin, B.; Hecht, S. M. *J. Nat. Prod.* **1984**, *47*, 988−993.
(23) Rosales, A.; Muñoz-Bascón, J.; Manuel Morales-Alcázar, V.; Castilla-Alcalá, J. A.; Enrique Oltra, J. *RSC Adv.* **2012**, *2*, 12922.
(24) Lang, K. L.; Silva, I. T.; Zimmermann, L. A.; Machado, V. R.; Teixeira, M. R.; Lapuh, M. I.; Galetti, M. A.; Palermo, J. A.; Cabrera, G. M.; Bernardes, L. S.; Simoes, C. M.; Schenkel, E. P.; Caro, M. S.; Duran, F. J. *Bioorg. Med. Chem.* **2012**, *20*, 3016−3030.
(25) Hall, J. A.; Seedarala, S.; Rice, N.; Kopel, L.; Halaweish, F.; Blagg, B. S. J. *J. Nat. Prod.* **2015**, *78*, 873−879.
(26) Ma, Y. L. Chinese Academy of Sciences, 2012 (in Chinese).
(27) World Medical, A.. *JAMA* **2013**, *310*, 2191−2194.
(28) Stephan, Z. F.; Yurachek, E. C. *J. Lipid. Res.* **1993**, *34*, 325−330.

https://dx.doi.org/10.1021/acs.jnatprod.0c00364
J. Nat. Prod. XXXX, XXX, XXX−XXX