JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

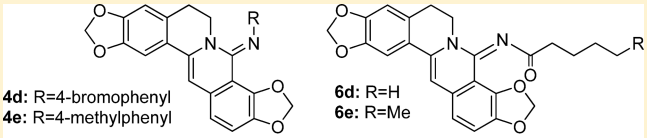
Synthesis and Structure–Activity Relationships of *N*-Dihydrocoptisine-8-ylidene Aromatic Amines and *N*-Dihydrocoptisine-8-ylidene Aliphatic Amides as Antiulcerative Colitis Agents Targeting XBP1

Meng Xie, Hai-Jing Zhang, An-Jun Deng, Lian-Qiu Wu,* Zhi-Hui Zhang, Zhi-Hong Li, Wen-Jie Wang, and Hai-Lin Qin*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

Supporting Information

ABSTRACT: In this study, natural quaternary coptisine was used as a lead compound to design and synthesize structurally stable and actively potent coptisine analogues. Of the synthesized library, 13 *N*-dihydrocoptisine-8-ylidene amines/amides were found not only to be noncytotoxic toward intestinal epithelial cells (IECs), but they were also able to activate the transcription of X-box-binding protein 1 (XBP1) targets to varying extents in vitro. Antiulcerative colitis (UC) activity levels were assessed at the in vitro molecular level as well as in vivo in animals using multiple biomarkers as indices. In an in vitro XBP1 transcriptional activity assay, four compounds demonstrated good dose–effect relationships with EC_{50} values of 0.0708–0.0132 μM. Moreover, two compounds were confirmed to be more potent in vivo than a positive control, demonstrating a curative effect for UC in experimental animals. Thus, the findings of this study suggest that these coptisine analogues are promising candidates for the development of anti-UC drugs.



EC_{50}: (**4d**) 0.0489 μM; (**4e**) 0.0132 μM; (**6d**) 0.0325 μM; (**6e**) 0.0708 μM.

Ulcerative colitis (UC), a type of inflammatory bowel disease (IBD), is a recurrent and progressive inflammatory intestinal disorder, characterized by superficial mucosal lesions that extends through the rectum and progresses upstream.[1] Common clinical symptoms of UC include bloody diarrhea, colon ulcers, and weight loss.[2] Approximately 0.5–1% of patients with long-term UC have an annual increased risk of colorectal cancer.[3] There is a growing body of evidence suggesting that the occurrence of UC is increasing.[4] Although the pathogenesis of UC is currently not fully clarified, it is generally accepted that multiple factors contribute to this disease and that host genetic and environmental factors and unregulated immune responses are involved.[5] Depending on the degree of severity, different therapies are developed against UC, but none of them are directed at curing the disease. Instead, they focus on attenuating its symptoms. As a consequence, these treatments lead to limited remission, often with major side effects and refractory patients arising. Thus, there is an urgent need to develop effective anti-UC drugs.

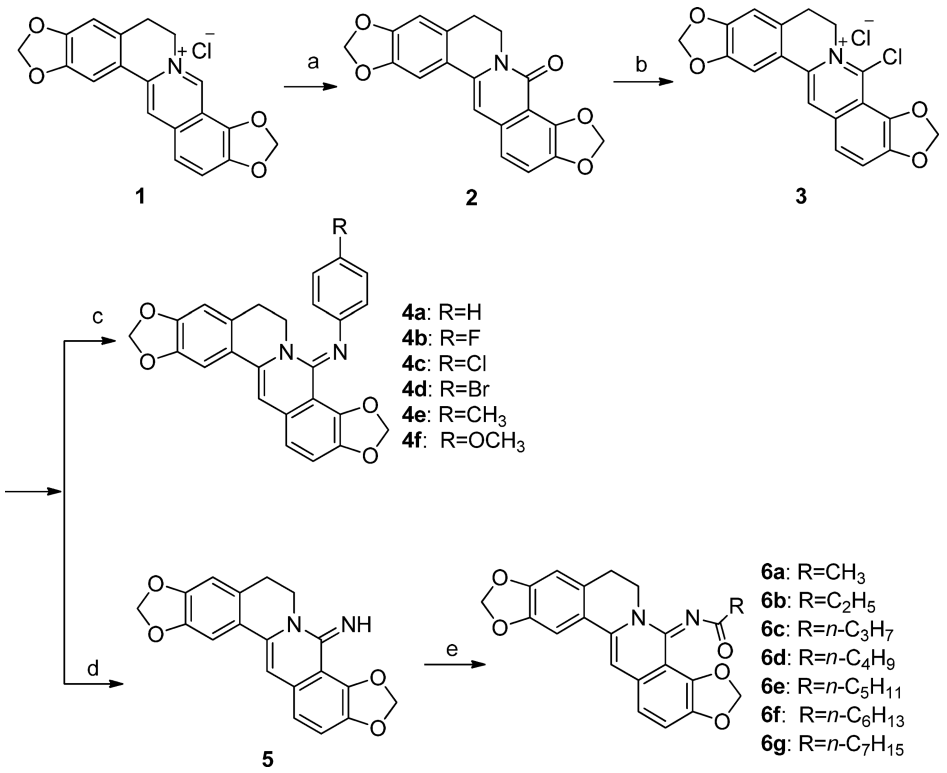
Recent scientific data from studies in animal models showed that gut inflammation might be related to an inability to manage the unfolded protein response (UPR) in the epithelial barrier.[6] Other studies showed that the transcription factor (TF) X-box binding protein-1 (XBP1) could activate UPR target genes.[7] Decreased or absent XBP1 function in intestinal epithelial cells (IECs) resulted in the dysfunction of Paneth cells and apoptotic depletion.[6] XBP1 in the intestinal epithelium not only regulates local inflammation but also, at the same time, determines the propensity of the epithelium to develop tumors.[8] Therefore, it was speculated that XBP1 might be a potential new drug target for treating UC. Nevertheless, there has been no claim thus far regarding the development of anti-UC drugs targeting XBP1 except for the work from our laboratory.[9]

Quaternary coptisine (**1**) is a typical natural benzyltetrahydroisoquinoline-type alkaloid isolated from certain *Coptis* and *Corydalis* species belonging to the Ranunculaceae and Papaveraceae families, respectively. The bioactivities of **1** have been reported to include antidiabetic, antimicrobial, antiviral, antihepatoma, and antileukemia effects and a cardioprotective function.[10] Our laboratory previously reported the cytotoxicities of coptisine analogues, mainly quaternary 13-substituted coptisine derivatives.[11] In view of the anti-UC effects of the extract from the rhizome of *Coptis chinensis* Franch, which is an important source plant of quaternary coptisine, and based on the ongoing need to improve the chemical stability and liposolubility of coptisine, a series of coptisine derivatives was recently designed and synthesized in this study. Another goal of this study was to obtain more bioactive compounds indicated by multiple biomarkers, especially the activity of an XBP1-

Received: September 8, 2015

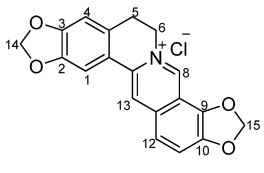
DOI: 10.1021/acs.jnatprod.5b00807
J. Nat. Prod. XXXX, XXX, XXX–XXX

Scheme 1. Preparation of Compounds 2, 3, 4a−4f, 5, and 6a−6g[‡]

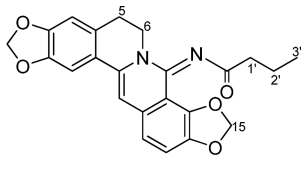


[‡]Reagents and conditions: (a) $K_3[Fe(CN)_6]$, aq NaOH or KOH, reflux; (b) $POCl_3$, reflux; (c) *p*-substituted anilines, toluene, rt; then HCl (g); (d) NH_3 (g); toluene, rt; (e) RCOCl, Et_3N, $CHCl_3$, rt.

activating, dose−effect relationship, and an in vivo curative effect, to develop anti-UC agents targeting XBP1, a TF associated with the occurrence, exacerbation, and potential treatment of UC. Of the synthesized library, some compounds were found to exhibit XBP1-activating activity and a more significant anti-UC effect than the positive controls, 8-oxodihydrocoptisine (8-ODC, **2**) and salazosulfapyridine (SASP), when evaluated at both the in vitro molecular level and in vivo in animals. This paper describes the design and synthesis of active compounds, analysis of the structure−activity relationship (SAR), the evaluation of efficacy, and additional in vitro and in vivo data of the targeted compounds for the treatment of UC.



1



6c

RESULTS AND DISCUSSION

To obtain structurally stable and actively potent coptisine analogues, a series of coptisine derivatives, including 8,8-dihomosubstituted dihydrocoptisines, 8-alkylene substituted dihydrocoptisines, and classes of *N*-dihydrocoptisine-8-ylidene amides and amines, among others, was designed and synthesized using natural quaternary coptisine as a lead compound. Of the synthesized library, 13 derivatives of *N*-dihydrocoptisine-8-ylidene aromatic amines and aliphatic amides were found to activate the transcription of XBP1 in vitro. An SAR analysis and a curative effect evaluation conducted at the in vitro molecular level and in vivo in animals confirmed the promising role of these compounds for the development of anti-UC drugs.

(*Z*)-*N*-Dihydrocoptisine-8-ylidene aromatic amines **4a−4f** were readily synthesized and structurally characterized. First, the synthesis of 8-ODC (**2**) as an intermediate was modeled after previous work.[9a] Treating **2** with $POCl_3$ at 110 °C for 2 h afforded quaternary 8-chlorocoptisine chloride (**3**) in a 85% yield from **2**. Substituted anilines were reacted with **3** via a nucleophilic addition−elimination reaction to generate the target compounds **4a−4f** in 63−71% yields (Scheme 1). The structures and the shared 8*Z* configuration of **4a−4f** were ascertained by IR, ^1H and ^{13}C NMR, HRESIMS, and NOE spectra. No correlation was found between the protons of the aniline moiety and the dihydrocoptisine moiety in the NOE experiments, which means that the protons of the benzene rings were remote from not only the C-5 and C-6 methylenes but also the C-15 methylenedioxy moiety of the dihydrocoptisine core. However, only the H-1, H-6, and H_2-14 resonances were shielded compared with their counterparts in the synthetic dihydrocoptisine-8-imine (**5**) due to the shielding effect of the aniline moiety. For example, in the case of compound **4d**, significant upfield shifts of Δ_δ −0.13, −0.23, and −0.74 for H-1, H-6, and H_2-14, respectively, were observed in the ^1H NMR spectrum compared with **5** (Figure S32, Supporting Information).

Dihydrocoptisine-8-imine (**5**) was synthesized as an intermediate and obtained a 91.0% yield in one step via treating **3** with anhydrous ammonia gas in toluene at room temperature. The synthesis of (*E*)-*N*-dihydrocoptisine-8-ylidene aliphatic amides **6a−6g** was achieved with yields

DOI: 10.1021/acs.jnatprod.5b00807
J. Nat. Prod. XXXX, XXX, XXX−XXX

between 43.5% and 80.1% in one step from **5** via reaction with different aliphatic acyl chlorides in the presence of trimethylamine or pyridine (Scheme 1). The structures and the 8*E* configuration of **6a**–**6g** were elucidated by IR, ^{1}H and ^{13}C NMR, HRESIMS, and NOE spectra. Irradiating the H-1′ or H-2′ signals of the acyl moiety resulted in the NOE enhancement of the H_2-15 signal, but no NOE enhancement of the H_2-5 and H_2-6 signals was observed (**6c** for example, Figures 1 and S33, Supporting Information). Detailed characterizations of all the target compounds are provided in the Experimental Section and the Supporting Information.

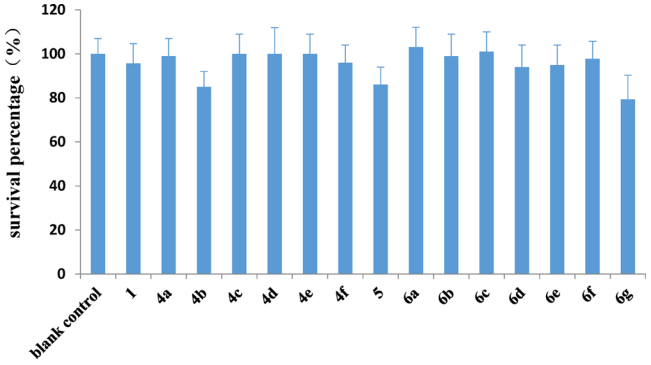


Figure 1. Toxicity test results of compounds **1**, **4a**–**4f**, **5**, and **6a**–**6g** on IEC-6 cells determined by MTT assay.

To assess the biological activity, the target compounds were first investigated for their cytotoxicity toward IEC-6 cells in vitro using the MTT assay at a constant concentration of 10 μM. None of the compounds were found to exhibit cytotoxicity, with the survival percentages of IEC-6 cells ranging from 79 to 103% (Figure 1). The cytotoxicity evaluation suggested that all of the compounds were suitable for studying anti-UC activity.

Next, the XBP1-activating abilities of all of the target compounds were evaluated using an in vitro XBP1 transcriptional activity assay and dual luciferase reporter detection. Test compounds at 10 μM concentration were added into each well containing IEC-6 cells in the growth phase at a density of 5×10^4 cells/well. The cells were subjected to 4 h of plasmid transfection in a humidified incubator filled with 5% CO_2 at 37 °C, and coincubated for an additional 48 h. Luciferase activity was detected using the dual luciferase reporter gene detection kit. The activation effects of all the target compounds are shown in Figure 2, in which con 1 was used for background contrast and con 2 was the pGL3-basic vector control. The XBP1 activation abilities of all of the active compounds were further evaluated by determining the in vitro dose–effect relationship, and the EC_{50} values are given in Table 1. Considering that 8-ODC (**2**) has been shown to activate XBP1 transcription, it was selected as the positive control in this study.[9]

As shown in Figure 2, based on the activation times relative to con 2, the starting quaternary coptisine (**1**), the intermediate dihydrocoptisine-8-imine (**5**), the (*Z*)-*N*-(dihydrocoptisine-8-ylidene)-4-methoxyaniline (**4f**), and the (*E*)-*N*-dihydrocoptisine-8-ylidene aliphatic amides **6f** and **6g** exhibited low activity, with relative activation ratios of 0.78–1.07 times, while all of the other compounds activated the transcription of XBP1 to a larger degree. The activation ability of the (*Z*)-*N*-dihydrocoptisine-8-ylidene aromatic amines ranged from 1.41 to 3.18 times

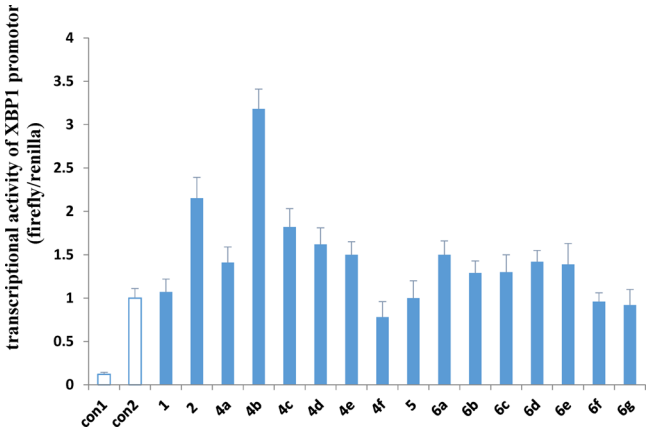


Figure 2. Effects of compounds **1**, **2**, **4a**–**4f**, **5**, and **6a**–**6g** on activating XBP1 transcription.

Table 1. EC_{50} Values of the Target Compounds

compounds	R	EC_{50} (μM)	compounds	R	EC_{50} (μM)
1		-	**5**		-
2 (positive control)		0.0798	**6a**	Me	0.283
4a	H	0.113	**6b**	Et	0.289
4b	F	0.346	**6c**	*n*-propyl	0.119
4c	Cl	0.174	**6d**	*n*-butyl	0.0325
4d	Br	0.0489	**6e**	*n*-pentyl	0.0708
4e	Me	0.0132	**6f**	*n*-hexyl	-
4f	MeO	0.184	**6g**	*n*-heptyl	-

that of con 2, and the (*E*)-*N*-dihydrocoptisine-8-ylidene aliphatic amides ranged from 1.29 to 1.50 times. Based on the EC_{50} values, the behavior was similar to that described above, except for compound **4f**, which also showed better dose–effect relationships in vitro with an EC_{50} value of 0.184 μM. Compounds **1**, **5**, **6f**, and **6g** showed no activity, and their EC_{50} values were not determined, while all of the other compounds exhibited activity to some or even a significant extents. The EC_{50} values of the most active compounds, **4d**, **4e**, **6d**, and **6e**, were 0.0489, 0.0132, 0.0325, and 0.0708 μM, respectively (Figures 3–6). From the SAR analysis, several general conclusions emerged. Collectively, the results of the in vitro XBP1 transcriptional activity assay resulted in the hypothesis that the synthetic (*Z*)-*N*-dihydrocoptisine-8-ylidene aromatic amines and (*E*)-*N*-dihydrocoptisine-8-ylidene aliphatic amides activated the transcription of XBP1 targets.

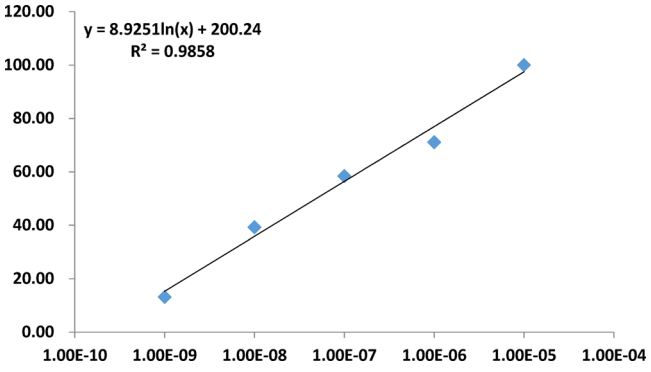


Figure 3. EC_{50} value of **4d** (EC_{50}: 0.0489 μM).

DOI: 10.1021/acs.jnatprod.5b00807
J. Nat. Prod. XXXX, XXX, XXX–XXX

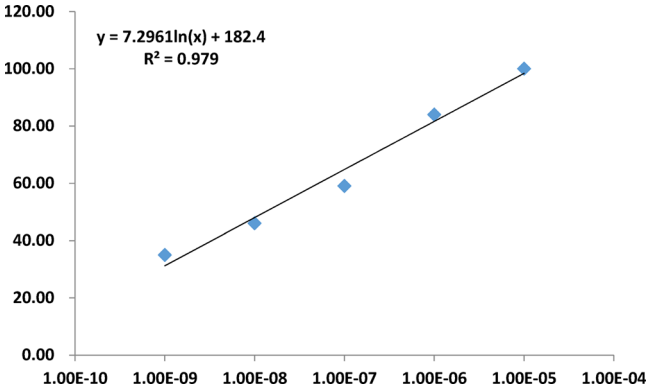


Figure 4. EC_{50} value of **4e** (EC_{50}: 0.0132 μM).

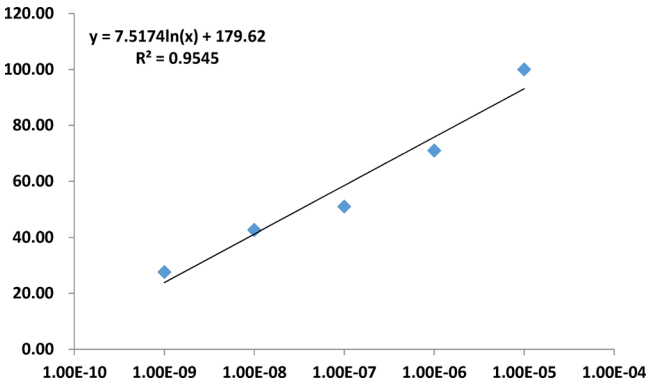


Figure 5. EC_{50} value of **6d** (EC_{50}: 0.0325 μM).

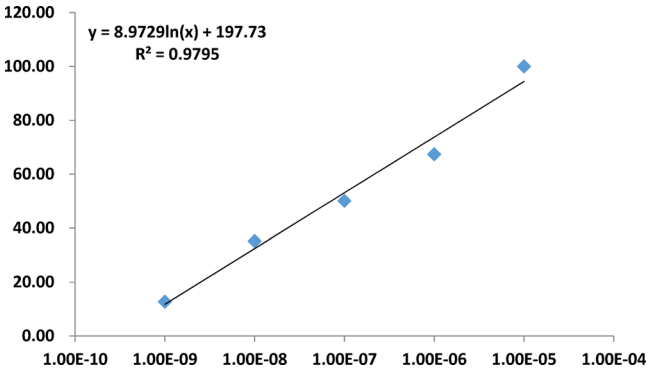


Figure 6. EC_{50} value of **6e** (EC_{50}: 0.0708 μM).

Indeed, testing the dose−effect relationships was more critical for showing whether or not the test compounds were active. In this regard, quaternary coptisine (**1**), the starting material, showed low activity. Among the synthesized compounds **4a**−**4f**, introducing the electron-withdrawing Br-substituent (**4d**) and the weak electron-donating methyl group (**4e**) made some difference for improving the activity. Among the compounds **6a**−**6g**, the activity was significantly enhanced as the length of the aliphatic chain increased from one up to five carbon atoms (i.e., from acetyl to *n*-hexanoyl). As soon as the aliphatic chain grew beyond five carbon atoms, the activity decreased rapidly. Although the (*Z*)-*N*-dihydrocoptisine-8-ylidene aromatic amines were slightly more active than the (*E*)-*N*-dihydrocoptisine-8-ylidene aliphatic amides at 10 μM in the preliminary in vitro XBP1 transcriptional activity assay, the EC_{50} values were not significantly different between these two series of compounds.

After the target compounds **4d**, **4e**, **6d**, and **6e** were found to exhibit impressive pharmacodynamics in vitro when evaluating EC_{50} values, in vivo efficacy was next assessed, and **4e** and **6d** were utilized as test compounds. Animal experiments using an acute UC animal model induced by dextran sodium sulfate (DSS) in C57bl/6j mice were carried out according to a published procedure.[9b,12] Compounds **4e** and **6d** were administered orally at a dose of 200 mg/kg. Animals treated with the well-known anti-UC drug, SASP, at a dose of 300 mg/kg as well as normal control animals were used as a positive control and a reference group, respectively. The effects of **4e** and **6d** on weight changes of the UC model animals were first assessed. As shown in Table 2, the body weight of the normal control group increased, whereas the body weights of the other groups decreased to different extents through the end of the trial over a period of 7 days. The body weight of the model group decreased by 18% compared with the initial weight. When treated with SASP and the target compounds **4e** and **6d**, body weight loss in the experimental animals was all significantly ameliorated. The curative effects of **4e** and **6d** were almost the same as that of SASP, with the body weight loss rates of **4e**, **6d**, and SASP being −11, −10, and −9%, respectively, compared with initial values.

The colon contractures of the experimental animals were taken as another biomarker to evaluate the curative effects. Measurements at the end of the trial showed that the colon length of the normal control group was as long as 7.16 cm, and that of the DSS model group was only 4.48 cm, which is 37% shorter than that of the normal control group. The SASP positive control group and the groups where compounds **4e** and **6d** were administered all exerted significant therapeutic effects on the experimental UC animals by improving colon contractures. Compound **4e** possessed the best efficacy, with an 9% contracture rate, and compound **6d** also exhibited better efficacy than SASP, with contracture rates of 15 versus 24%, respectively; the administered dosages for SASP, **4e**, and **6d** were 300, 200, and 200 mg/kg, respectively (Table 3).

As per standard procedure, the anti-UC efficacies of the target compounds in the experimental animals were evaluated

Table 2. Therapeutic Effects of 4e and 6d in Vivo Evaluated by the Body Weight Change on C57bl/6j Mice with DSS-Induced Acute UC

groups	*n* (start/end)	body weight (g) start	$x \pm$ SD end	percentage (%)
normal control group	8/8	21.65 ± 1.12	23.50 ± 0.71	+9
model group (DSS)	8/8	22.20 ± 1.09	18.20 ± 1.15	−18**
SASP group (300 mg/kg)	8/8	21.5 ± 0.41	19.50 ± 1.87	−9$^{\#\#}$
4e (200 mg/kg)	8/8	20.42 ± 1.20	18.25 ± 1.37	−11$^{\#\#}$
6d (200 mg/kg)	8/8	20.50 ± 7.87	18.42 ± 4.37	−10$^{\#\#}$

$^{**}p < 0.01$ when compared with the normal control group. $^{\#\#}p < 0.01$ when compared with the model group.

DOI: 10.1021/acs.jnatprod.5b00807
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. Effects of 4e and 6d on Improving the Colon Contracture of C57bl/6j Mice with DSS-Induced Acute UC Evaluated by Contracture Percentage (%)

groups	*n* (start/end)	colon length (cm)	percentage (%)
normal control group	8/8	7.16 ± 0.21	0
model group (DSS)	8/8	4.48 ± 0.13**	37
SASP group (300 mg/kg)	8/8	5.43 ± 0.50$^{\#\#}$	24
4e (200 mg/kg)	8/8	6.55 ± 0.51$^{\#\#}$	9
6d (200 mg/kg)	8/8	6.06 ± 0.58$^{\#\#}$	15

$^{**}p < 0.01$ when compared with the normal control group. $^{\#\#}p < 0.01$ when compared with the model group.

using the disease activity index (DAI)[13] and colon macroscopic damage index (CMDI)[14] scores. The DAI score was obtained on the basis of tests for weight loss, stool features, and hematochezia, whereas the CMDI score was computed on the basis of the hyperemia of the intestinal mucosa, the edema of the bowel wall, and the ulcer size, among other factors. Lower DAI and CMDI scores in the experimental animals imply that the treated animals are closer to the normal physiological state. As shown in Table 4, the DAI and CMDI scores of the DSS model group were as high as 3.33 and 4.63 ± 0.52, respectively. The positive-control anti-UC drug SASP showed clear curative effect for UC symptoms, with DAI and CMDI scores of 2.73 ± 0.50 and 3.17 ± 0.98, respectively, at a dose of 300 mg/kg. Compared with SASP, compounds **4e** and **6d**, at a dose of 200 mg/kg, both showed more obvious curative effects on UC symptoms. The DAI and CMDI scores of **4e** were as low as 2.44 ± 0.62 and 2.63 ± 0.74, respectively. In the case of **6d**, the scores were 2.22 ± 0.54 and 2.63 ± 0.74, respectively.

Table 4. Effects of 4e and 6d on Treatment of UC Evaluated by DAI and CMDI Scorings of Colon Tissue in C57bl/6j Mice with DSS-Induced Acute UC

groups	*n* (start/end)	DAI (IR[a] %)	CMDI
normal control group	8/8	0.00 ± 0.00	0.00 ± 0.00
model group (DSS)	8/8	3.33 ± 0.00**	4.63** ± 0.52
SASP group (300 mg/kg)	8/8	2.73 ± 0.50 (18%)$^{\#}$	3.17 ± 0.98$^{\#}$
4e (200 mg/kg)	8/8	2.44 ± 0.62 (27%)$^{\#}$	2.63$^{\#\#}$ ± 0.74
6d (200 mg/kg)	8/8	2.22 ± 0.54 (33%)$^{\#\#}$	2.63$^{\#\#}$ ± 0.74

[a]IR: inhibition radio. $^{**}p < 0.01$ when compared with the normal control group. $^{\#}p < 0.05$. $^{\#\#}p < 0.01$ when compared with the model group.

At the end of the animal experiment, the influence of **4e** and **6d** on the pathological changes of the colon tissue of the experimental animals was inspected by a microscopic examination. The normal control group exhibited a typical colon mucosal histological structure (Figure 7A). In the DSS model group, compared with the normal control group, the basic structure of the IEC cells was completely lost, and inflammatory edema, mucosal exfoliation with serious congestion and hemorrhage, the infiltration of inflammatory cells into the muscular layer, and the destroyed structure of the muscular layer were obvious (Figure 7B). These findings indicated that the use of the animal model was successful. In contrast, the positive control compound SASP and the target compounds **4e** and **6d** all improved the colon conditions in UC for the better. SASP exerted a definite curative effect on UC, with the partial disappearance of inflammatory edema (Figure 7C). Compound **4e** exhibited the most significant curative effect, with regular arrangement of IEC cells, and the polar arrangement of IEC cells even recovered to the normal physiological state (Figure 7D). Compound **6d** was also clearly better than SASP as judged by the improvement of the colon lesions in the C57bl/6j mice with DSS-induced acute UC (Figure 7E). Thus, compounds **4e** and **6d** were both superior to SASP for treating UC.

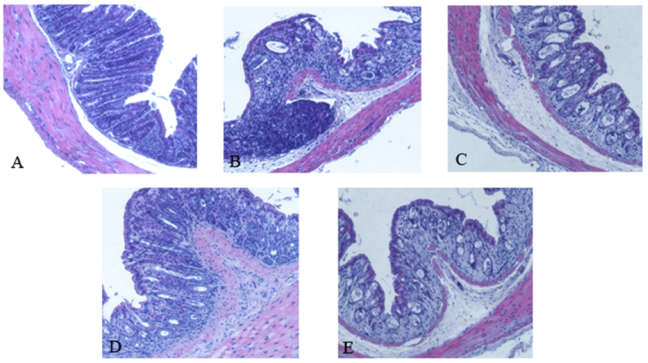


Figure 7. Effect of **4e** and **6d** on the pathological change of colon tissue of C57bl/6j mice getting acute UC induced by DSS (HE × 200). A: normal control group; B: DSS model group; C: positive SASP group (300 mg/kg); D: **4e** group (200 mg/kg); E: **6d** group (200 mg/kg).

In conclusion, this article described the synthesis of 13 coptisine derivatives classified into (*Z*)-*N*-dihydrocoptisine-8-ylidene aromatic amines and (*E*)-*N*-dihydrocoptisine-8-ylidene aliphatic amides. The anti-UC activities of the synthesized compounds were evaluated at the in vitro molecular level and in vivo in animals using multiple biomarkers as indices. The major findings were as follows. First, all the coptisine derivatives demonstrated low cytotoxicity toward IEC-6 cells and thus were suitable for studying anti-UC activity. Second, the results of the in vitro bioactivity assay suggested that the synthesized (*Z*)-*N*-dihydrocoptisine-8-ylidene aromatic amines and (*E*)-*N*-dihydrocoptisine-8-ylidene aliphatic amides were able to activate the transcription of XBP1 targets. Third, testing the dose–effect relationships for all of the target compounds showed that several compounds, such as **4e** and **6d**, exhibited significant activity, with EC_{50} values of 0.0132 and 0.0325 μM, respectively. Fourth, based on body weight changes, DAI and CMDI scores, colon contracture rates, and pathological changes in the colon tissue of experimental UC animals, compounds **4e** and **6d** both exhibited more significant anti-UC activity than the positive control, the anti-UC drug SASP. Thus, this study suggests that (*Z*)-*N*-dihydrocoptisine-8-ylidene aromatic amides and (*E*)-*N*-dihydrocoptisine-8-ylidene aliphatic amides are promising coptisine derivatives for the development of anti-UC drugs.

EXPERIMENTAL SECTION

General Experimental Procedures. IR spectra were recorded on a Thermo Nicolet 5700 FT-IR spectrophotometer. NMR spectra were acquired on either a Varian Inova 600 NMR or a Bruker Avance III 400 NMR spectrometer, and chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. DMSO-d_6 was used as both a solvent

DOI: 10.1021/acs.jnatprod.5b00807
J. Nat. Prod. XXXX, XXX, XXX−XXX

and an internal reference at δ_H 2.49 and δ_C 39.5. HRMS data were collected on a Hexin API-TOFMS 10000 instrument with an ESI source. All reagents and solvents were of reagent grade or were purified by standard procedures before use. The reaction progress was monitored by TLC on glass plates precoated with silica gel GF_{254} (Qingdao Haiyang Chemical, Qingdao, China). CC was carried out on silica gel (200−300 mesh size; Qindao Haiyang Chemical, Qingdao, China). The quaternary coptisine starting material was isolated and purified from natural sources, with the structure confirmed by chemical and spectroscopic data (data not shown) and the purity determined to be >99% by HPLC.

Procedures for Synthesizing Intermediates and Target Compounds. *8-ODC (**2**).* The synthesis of 8-ODC (**2**) was modeled on a published procedure.[9a] ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.85 (t, J = 6.0 Hz, 2H, Ar$\underline{CH_2}CH_2$N), 4.08 (t, J = 6.0 Hz, 2H, ArCH$_2$ $\underline{CH_2}$N), 6.06 (s, 2H, OCH$_2$O), 6.18 (s, 2H, OCH$_2$O), 6.91 (s, 1H, ArH), 7.11 (s, 1H, ArH), 7.14 (d, J = 8.4 Hz, 1H, ArH), 7.33 (d, J = 8.4 Hz, 1H, ArH), 7.46 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 27.55, 38.57, 101.27, 101.53, 102.02, 104.64, 107.82, 109.74, 113.92, 119.30, 123.07, 129.78, 131.52, 134.76, 145.59, 146.00, 146.85, 147.90, 158.11; HRESIMS m/z 336.0859 [M + H]$^+$ (calcd for $C_{19}H_{14}NO_5$, 336.0872).

*8-Chlorocoptisine Chloride (**3**).* A suspension of **2** (2.0 g, 5.96 mmol) in $POCl_3$ (20 mL) was refluxed for 2 h. The mixture was cooled to room temperature and filtered. The precipitate was washed, successively, with $CHCl_3$ and Et_2O to provide crude compound **3** (1.98 g, 85%), which was used for the next step without further purification. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.85 (t, J = 6.0 Hz, 2H, Ar$\underline{CH_2}CH_2$N), 4.07 (t, J = 6.0 Hz, 2H, ArCH$_2\underline{CH_2}$N), 6.06 (s, 2H, OCH$_2$O), 6.19 (s, 2H, OCH$_2$O), 6.92 (s, 1H, ArH), 7.12 (s, 1H, ArH), 7.14 (d, 1H, J = 8.4 Hz, ArH), 7.34 (d, 1H, J = 8.4 Hz, ArH), 7.47 (s, 1H, ArH); HRESIMS m/z 354.0524 [M + H]$^+$ (calcd for $C_{19}H_{13}ClNO_4$, 354.0528).

*General Procedure for Synthesizing Compounds **4a**−**4f**.* All of the target compounds **4a**−**4f** were prepared using the same method. The appropriate substituted anilines were added in excess (2.0 equiv) into a stirred suspension of **3** (1.0 equiv) in anhydrous toluene (20 mL), and the mixture was stirred at room temperature for 2 h. After dilution with Et_2O, dry HCl gas was passed through the reaction mixture until precipitation was complete. The precipitated product was obtained via filtering and washing successively with 1% aqueous $NaHCO_3$ solution and H_2O. All of the target compounds **4a**−**4f** were purified by flash CC over silica gel using a gradient of $CHCl_3$/MeOH (40:1 to 20:1) as an eluent.

*(Z)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)aniline (**4a**).* Yellow amorphous solid; yield: 71.2%; IR (neat) ν_{max} 3009, 2892, 1611, 1586, 1479, 1269, 1228, 822 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.82 (t, J = 5.6 Hz, 2H, Ar$\underline{CH_2}CH_2$N), 3.89 (t, J = 5.6 Hz, 2H, ArCH$_2\underline{CH_2}$N), 5.42 (s, 2H, O$\underline{CH_2}$O), 6.06 (s, 2H, O$\underline{CH_2}$O), 6.60 (d, J = 8.0 Hz, 2H, ArH), 6.79 (t, J = 8.0 Hz, 1H, ArH), 6.84 (s, 1H, ArH), 6.87 (s, 1H, ArH), 7.01 (d, J = 8.0 Hz, 1H, ArH), 7.14 (m, 3H, ArH), 7.45 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 28.01, 43.22, 100.13, 101.01, 101.26, 104.49, 107.11, 107.72, 112.49, 119.02, 119.42(2C), 120.47, 123.28, 128.28(2C), 129.57, 131.31, 135.42, 143.52, 145.39, 146.79, 147.46, 147.73, 151.32; HRESIMS m/z 411.1348 [M + H]$^+$ (calcd for $C_{25}H_{19}N_2O_4$, 411.1345).

*(Z)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)-4-fluoroaniline (**4b**).* Yellow amorphous solid; yield: 65.7%; IR (neat) ν_{max} 3035, 2868, 1609, 1502, 1478, 1276, 1225, 832 cm^{-1} ; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.84 (t, J = 5.2 Hz, 2H, Ar$\underline{CH_2}$ CH_2N), 3.92 (t, J = 5.2 Hz, 2H, ArCH$_2$ $\underline{CH_2}$N), 5.48 (s, 2H, OCH$_2$O), 6.06 (s, 2H, OCH$_2$O), 6.60 (m, 2H, ArH), 6.85 (s, 1H, ArH), 6.88 (s, 1H, ArH), 6.95 (m, 2H, ArH), 7.01 (d, J = 8.0 Hz, 1H, ArH), 7.15 (d, J = 8.0 Hz, 1H, ArH), 7.45 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 27.99, 43.09, 100.07, 101.00, 101.27, 104.47, 106.70, 107.73, 112.63, 114.74 (d, J = 21.9 Hz, 2C), 119.15, 120.47 (d, J = 7.65 Hz, 2C), 123.22, 129.58, 131.46, 135.39, 143.32, 145.34, 146.80, 147.75, 147.78, 147.93, 157.05 (d, J = 234.9 Hz); HRESIMS m/z 429.1252 [M + H]$^+$ (calcd for $C_{25}H_{18}FN_2O_4$, 429.1251).

*(Z)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)-4-chloroaniline (**4c**).* Yellow amorphous solid; yield: 63.3%; IR (neat) ν_{max} 3047, 2891, 1609, 1577, 1481, 1389, 1271, 832 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.84 (t, J = 6.0 Hz, 2H, Ar$\underline{CH_2}CH_2$N), 3.93 (t, J = 6.0 Hz, 2H, ArCH$_2\underline{CH_2}$N), 5.49 (s, 2H, OCH$_2$O), 6.06 (s, 2H, OCH$_2$O), 6.60 (d, J = 8.4 Hz, 2H, ArH), 6.88 (s, 1H, ArH), 6.89 (s, 1H, ArH), 7.03 (d, J = 8.4 Hz, 1H, ArH), 7.15 (d, J = 8.4 Hz, 2H, ArH), 7.18 (d, J = 8.4 Hz, 1H, ArH), 7.45 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 27.93, 43.15, 100.50, 100.97, 101.29, 104.53, 106.70, 107.73, 112.86, 119.29, 120.99 (2C), 123.17, 123.79, 128.10 (2C), 129.62, 131.42, 135.30, 143.37, 145.42, 146.82, 147.79, 148.03, 150.42; HRESIMS m/z 445.0940 [M + H]$^+$ (calcd for $C_{25}H_{18}ClN_2O_4$, 445.0955).

*(Z)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)-4-bromoaniline (**4d**).* Yellow amorphous solid; yield: 68.3%; IR (neat) ν_{max} 3008, 2876, 1574, 1475, 1274, 1226, 834 cm^{-1} ; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.84 (t, J = 6.0 Hz, 2H, Ar$\underline{CH_2}CH_2$N), 3.93 (t, J = 6.0 Hz, 2H, ArCH$_2\underline{CH_2}$N), 5.50 (s, 2H, OCH$_2$O), 6.06 (s, 2H, OCH$_2$O), 6.55 (d, J = 8.4 Hz, 2H, ArH), 6.88 (s,1H, ArH), 6.89 (s,1H, ArH), 7.04 (d, J = 8.0 Hz, 1H, ArH), 7.18 (d, J = 8.0 Hz, 1H, ArH), 7.27 (d, J = 8.4 Hz, 2H, ArH), 7.45 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 27.92, 43.16, 100.56, 100.97, 101.29, 104.53, 106.70, 107.73, 111.60, 112.88, 119.31, 121.51 (2C), 123.17, 129.62, 130.98 (2C), 131.40, 135.29, 143.38, 145.43, 146.82, 147.80, 147.99, 150.83. HRESIMS m/z 489.0444 [M + H]$^+$ (calcd for $C_{25}H_{18}{}^{79}BrN_2O_4$, 489.0450).

*(Z)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)-4-methylaniline (**4e**).* Yellow amorphous solid; yield: 70.9%; IR (neat) ν_{max} 3073, 2868, 1587, 1478, 1272, 1044, 828 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.22 (s, 3H, ArCH$_3$), 2.82 (t, J = 5.6 Hz, 2H, Ar$\underline{CH_2}CH_2$N), 3.85 (t, J = 5.6 Hz, 2H, ArCH$_2\underline{CH_2}$N), 5.48 (s, 2H, OCH$_2$O), 6.06 (s, 2H, OCH$_2$O), 6.52 (d, J = 8.0 Hz, 2H, ArH), 6.82 (s, 1H, ArH), 6.87 (s, 1H, ArH), 6.96 (d, J = 8.0 Hz, 2H, ArH), 7.00 (d, J = 8.4 Hz, 1H, ArH), 7.14 (d, J = 8.4 Hz, 1H, ArH), 7.44 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 20.03, 27.95, 43.33, 99.92, 100.92, 101.18, 104.40, 107.42, 107.64, 112.23, 118.82, 119.28(2C), 123.27, 128.70(2C), 129.08, 129.46, 131.16, 135.43, 143.48, 145.31, 146.70, 147.25, 147.62, 148.59; HRESIMS m/z 425.1476 [M + H]$^+$ (calcd for $C_{26}H_{21}N_2O_4$, 425.1501).

*(Z)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)-4-methoxyaniline (**4f**).* Yellow amorphous solid; yield: 63.9%; IR (neat)

DOI: 10.1021/acs.jnatprod.5b00807
J. Nat. Prod. XXXX, XXX, XXX−XXX

ν_{max} 3043, 2871, 1608, 1589, 1477, 1270, 1225, 829 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.81 (t, J = 5.2 Hz, 2H, $ArCH_2CH_2N$), 3.67 (s, 3H, $ArOCH_3$), 3.85 (t, J = 5.2 Hz, 2H, $ArCH_2CH_2N$), 5.47 (s, 2H, OCH_2O), 6.05 (s, 2H, OCH_2O), 6.55 (d, J = 8.4 Hz, 2H, ArH), 6.73 (s, 1H, ArH), 6.76 (d, J = 8.4 Hz, 2H, ArH), 6.86 (s, 1H, ArH), 6.97 (d, J = 8.0 Hz, 1H, ArH), 7.11 (d, J = 8.0 Hz, 1H, ArH), 7.43 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 28.06, 43.27, 55.15, 99.72, 101.06, 101.24, 104.43, 107.72(2C), 112.21, 113.75(2C), 118.86, 120.21(2C), 123.34, 129.52, 131.34, 135.57, 143.42, 144.48, 145.32, 146.76, 147.32, 147.67, 153.79; (+)-HRESIMS m/z 441.1451 $[M + H]^+$ (calcd for $C_{26}H_{21}N_2O_5$, 441.1450).

*6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino[3,2-a]-[1,3]dioxolo[4,5-g]isoquinolin-4-imine (**5**).* Anhydrous NH_3 gas was bubbled through a slurry of **3** (1 g, 2.99 mmol) in toluene (20 mL) at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was washed with H_2O (2 × 30 mL) and partitioned between $CHCl_3$ and aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous $MgSO_4$, filtered, and the remaining solvent was evaporated under reduced pressure to afford **5** (0.78 g, 91%), which was reprecipitated from $CHCl_3$ as amorphous yellow solid. IR (neat) ν_{max} 3324, 3038, 2917, 1654, 1492, 1291, 1237, 1060, 902 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.84 (t, J = 5.6 Hz, 2H, $ArCH_2CH_2N$), 4.08 (t, J = 5.6 Hz, 2H, $ArCH_2CH_2N$), 6.06 (s, 2H, OCH_2O), 6.22 (s, 2H, OCH_2O), 6.69 (s, 1H, ArH), 6.89 (s, 1H, ArH), 6.99 (d, J = 8.4 Hz, 1H, ArH), 7.19 (d, J = 8.4 Hz, 1H, ArH), 7.39 (s, 1H, ArH), 7.88 (C = NH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 26.75, 42.56, 101.46, 103.16, 104.99, 105.48, 107.61, 115.31, 115.40, 120.36, 122.29, 129.28, 129.53, 134.30, 144.19, 145.81, 147.00, 148.33, 152.98; HRESIMS m/z 335.1022 $[M + H]^+$ (calcd for $C_{19}H_{15}N_2O_4$, 335.1032).

*(E)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g] isoquinolin-4-ylidene)acetamide (**6a**).* A slurry of **5** (200 mg, 0.59 mmol) in pyridine (5 mL) and Ac_2O (0.55 mL, 5.9 mmol) was stirred overnight. After the solvent was removed under reduced pressure, the residue was resolved in an aqueous 1% solution of HCl (15 mL) and extracted with $CHCl_3$ (2 × 20 mL). The combined organic solution was dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give the crude product, which was purified by flash CC over silica gel using isocratic $CHCl_3$ /MeOH (50:1) as an eluent to afford pure **6a** (180.3 mg, 80.1%) as a pale amorphous yellow solid. IR (neat) ν_{max} 3004, 2888, 1604, 1543, 1500, 1480, 1285, 1033, 929, 831 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.07 (s, 3H, $NCOCH_3$), 2.90 (t, J = 6.0 Hz, 2H, $ArCH_2CH_2N$), 4.22 (m, 2H, $ArCH_2CH_2N$), 6.09 (s, 2H, OCH_2O), 6.16 (s, 2H, OCH_2O), 6.95 (s, 1H, ArH), 7.28 (d, J = 8.4 Hz, 1H, ArH), 7.45 (s, 1H, ArH), 7.46 (d, J = 8.0 Hz, 1H, ArH), 7.53 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 26.36, 27.20, 42.39, 101.39, 101.84, 104.85, 105.63, 107.70, 108.46, 114.86, 120.04, 122.56, 129.88, 130.81, 134.35, 144.93, 145.85, 146.97, 148.20, 152.74, 174.83; HRESIMS m/z 399.0950 $[M + Na]^+$ (calcd for $C_{21}H_{16}N_2NaO_5$, 399.0957).

*General Procedure for Synthesizing Compounds **6b**−**6g**.* All of the target compounds **6b**−**6g** were prepared using the following uniform procedure. To a solution containing **5** (1.0 equiv) and Et_3N (2.0 equiv) in $CHCl_3$ (15 mL) at 0 °C was added a solution of aliphatic acyl chlorides (1.18 mmol) in $CHCl_3$ (5 mL) dropwise. After a few minutes, the ice-bath was removed, and the mixture was allowed to warm to room temperature and was stirred for 5 h. The mixture was poured into an aqueous 1% solution of HCl (20 mL). The aqueous layer was extracted twice with $CHCl_3$ (2 × 20 mL). The combined organic solution was dried over anhydrous $MgSO_4$. After filtration, the solution was concentrated under reduced pressure to give the crude product, which was purified by flash CC over silica gel using isocratic $CHCl_3$ /MeOH (50:1) as an eluent to afford pure **6b**−**6g**.

*(E)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)-propionamide (**6b**).* Yellow amorphous solid; yield: 56%; IR (neat) ν_{max} 3043, 2904, 1609, 1522, 1483, 1278, 1227, 1041, 889 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.08 (t, J = 7.6 Hz, 3H, $COCH_2CH_3$), 2.38 (q, J = 7.6 Hz), 2H, $COCH_2CH_3$), 2.89 (t, J = 6.0 Hz, 2H, $ArCH_2CH_2N$), 4.20 (t, J = 6.0 Hz, 2H, $ArCH_2CH_2N$), 6.09 (s, 2H, OCH_2O), 6.13 (s, 2H, OCH_2O), 6.94 (s, 1H, ArH), 7.26 (d, J = 8.4 Hz, 1H, ArH), 7.41 (s, 1H, ArH), 7.43 (d, J = 8.4 Hz, 1H, ArH), 7.52 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 9.99, 27.23, 31.74, 42.25, 101.38, 101.76, 104.82, 105.32, 107.69, 108.35, 114.71, 120.01, 122.60, 129.86, 130.79, 134.37, 144.85, 145.81, 146.95, 148.17, 152.66, 178.45; HRESIMS m/z 391.1272 $[M + H]^+$ (calcd for $C_{22}H_{19}N_2O_5$, 391.1294).

*(E)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)butyramide (**6c**).* Yellow amorphous solid; yield: 54.1%; IR (neat) ν_{max} 3051, 2961, 2905, 1618, 1542, 1484, 1270, 1034, 937 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.96 (t, J = 7.6 Hz, 3H, $COCH_2CH_2CH_3$), 1.62 (m, 2H, $COCH_2$ CH_2 CH_3), 2.34 (t, J = 7.6 Hz, 2H, $COCH_2CH_2CH_3$), 2.90 (t, J = 6.0 Hz, 2H, $ArCH_2CH_2N$), 4.20 (t, J = 6.0 Hz, 2H, $ArCH_2CH_2N$), 6.09 (s, 2H, OCH_2O), 6.13 (s, 2H, OCH_2O), 6.94 (s, 1H, ArH), 7.27 (d, J = 8.4 Hz, 1H, ArH), 7.44 (s, 1H, ArH), 7.45 (d, J = 8.4 Hz, 1H, ArH), 7.53 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 14.12, 18.73, 27.22, 40.93, 42.34, 101.38, 101.78, 104.84, 105.52, 107.69, 108.50, 114.77, 120.00, 122.59, 129.87, 130.79, 134.39, 144.91, 145.84, 146.96, 148.18, 152.77, 177.37; HRESIMS m/z 405.1439 $[M + H]^+$ (calcd for $C_{23}H_{21}N_2O_5$, 405.1450).

*(E)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)pentanamide (**6d**).* Yellow amorphous solid; yield: 59.9%; IR (neat) ν_{max} 3004, 2956, 1640, 1580, 1520, 1459, 1281, 1040, 937, 855 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.91 (t, J = 7.6 Hz, 3H, $COCH_2CH_2CH_2CH_3$), 1.37 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.60 (m, 2H, $COCH_2CH_2CH_2CH_3$), 2.36 (t, J = 7.2 Hz, 2H, $COCH_2CH_2CH_2$ CH_3), 2.90 (t, J = 6.0 Hz, 2H, $ArCH_2CH_2N$), 4.19 (t, J = 6.0 Hz, 2H, $ArCH_2CH_2N$), 6.08 (s, 2H, OCH_2O), 6.12 (s, 2H, OCH_2O), 6.93 (s, 1H, ArH), 7.26 (d, J = 8.4 Hz, 1H, ArH), 7.39 (s, 1H, ArH), 7.42 (d, J = 8.4 Hz, 1H, ArH), 7.51 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.89, 22.11, 27.22, 27.59, 38.53, 42.33, 101.38, 101.77, 104.83, 105.52, 107.69, 108.50, 114.76, 120.00, 122.59, 129.86, 130.79, 134.38, 144.90, 145.84, 146.96, 148.18, 152.73, 177.55; HRESIMS m/z 419.1595 $[M + H]^+$ (calcd for $C_{24}H_{23}N_2O_5$, 419.1607).

*(E)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)hexanamide (**6e**).* Yellow amorphous solid; yield: 56.5%; IR (neat) ν_{max} 2951 1609 1527 1500 1277 1042 941 848 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.89 (t, J = 6.4 Hz, 3H, $COCH_2CH_2CH_2CH_2CH_3$), 1.33 (m, 4H, $COCH_2CH_2$ $CH_2CH_2CH_3$), 1.61 (m, 2H, $COCH_2CH_2CH_2CH_2CH_3$), 2.35

DOI: 10.1021/acs.jnatprod.5b00807
J. Nat. Prod. XXXX, XXX, XXX−XXX

(t, J = 7.6 Hz, 2H, CO$\underline{CH_2}CH_2CH_2CH_2$ CH_3), 2.89 (t, J = 6.0 Hz, 2H, Ar$\underline{CH_2}CH_2N$), 4.19 (t, J = 6.0 Hz, 2H, Ar$CH_2\underline{CH_2}N$), 6.09 (s, 2H, OCH_2O), 6.13 (s, 2H, OCH_2O), 6.94 (s, 1H, ArH), 7.26 (d, J = 8.0 Hz, 1H, ArH), 7.42 (s, 1H, ArH), 7.45 (d, J = 8.0 Hz, 1H, ArH), 7.52 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.90, 22.01, 25.08, 27.21, 31.28, 38.80, 42.34, 101.38, 101.76, 104.83, 105.49, 107.68, 108.49, 114.74, 119.99, 122.59, 129.85, 130.78, 134.37, 144.91, 145.83, 146.95, 148.17, 152.74, 177.55; HRESIMS m/z 433.1757 $[M + H]^+$ (calcd for $C_{25}H_{25}N_2O_5$, 433.1763).

*(E)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)heptanamide (**6f**).* Yellow amorphous solid; yield: 43.5%; IR (neat) ν_{max} 2925, 1619, 1566, 1503, 1484, 1079, 1041, 940, 855 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.88 (t, J = 6.4 Hz, 3H, $COCH_2CH_2CH_2CH_2CH_2\underline{CH_3}$), 1.33 (m, 6H, $COCH_2CH_2\underline{CH_2CH_2CH_2}CH_3$), 1.61 (m, 2H, $COCH_2\underline{CH_2}$-$CH_2CH_2CH_2CH_3$), 2.35 (t, J = 7.6 Hz, 2H, $CO\underline{CH_2}CH_2CH_2CH_2CH_2CH_3$), 2.89 (t, J = 6.0 Hz, 2H, Ar$\underline{CH_2}CH_2N$), 4.19 (t, J = 6.0 Hz, 2H, Ar$CH_2\underline{CH_2}N$), 6.08 (s, 2H, OCH_2O), 6.12 (s, 2H, OCH_2O), 6.93 (s, 1H, ArH), 7.27 (d, J = 8.4 Hz, 1H, ArH), 7.40 (s, 1H, ArH), 7.42 (d, J = 8.4 Hz, 1H, ArH), 7.51 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.90, 22.02, 25.37, 27.21, 28.71, 31.20, 38.83, 42.35, 101.39, 101.77, 104.84, 105.56, 107.69, 108.51, 114.79, 120.01, 122.58, 129.86, 130.80, 134.40, 144.90, 145.84, 146.96, 148.19, 152.70, 177.51; HRESIMS m/z 447.1916 $[M + H]^+$ (calcd for $C_{26}H_{27}N_2O_5$, 447.1920).

*(E)-N-(6,7-Dihydro-4H-[1,3]dioxolo[4′,5′:7,8]isoquinolino-[3,2-a][1,3]dioxolo[4,5-g]isoquinolin-4-ylidene)octanamide (**6g**).* Yellow amorphous solid; yield: 60.9%; IR (neat) ν_{max} 2929, 1612, 1528, 1483, 1276, 1040, 880 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.88 (t, J = 6.4 Hz, 3H, $COCH_2CH_2CH_2CH_2CH_2CH_2\underline{CH_3}$), 1.31 (m, 8H, $COCH_2CH_2\underline{CH_2CH_2CH_2CH_2}CH_3$), 1.61 (m, 2H, $COCH_2\underline{CH_2}CH_2CH_2CH_2CH_2CH_3$), 2.35 (t, J = 7.6 Hz, 2H, $CO\underline{CH_2}CH_2CH_2CH_2CH_2CH_2CH_3$), 2.90 (t, J = 6.0 Hz, 2H, Ar$\underline{CH_2}CH_2N$), 4.19 (t, J = 6.0 Hz, 2H, Ar$CH_2\underline{CH_2}N$), 6.08 (s, 2H, OCH_2O), 6.12 (s, 2H, OCH_2O), 6.93 (s, 1H, ArH), 7.26 (d, J = 8.4 Hz, 1H, ArH), 7.40 (s, 1H, ArH), 7.42 (d, J = 8.4 Hz, 1H, ArH), 7.51 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.89, 22.02, 25.42, 27.21, 28.61, 29.00, 31.21, 38.84, 42.32, 101.38, 101.76, 104.83, 105.49, 107.70, 108.49, 114.76, 120.00, 122.59, 129.85, 130.79, 134.38, 144.91, 145.83, 146.96, 148.18, 152.72, 177.54; HRESIMS m/z 461.2078 $[M + H]^+$ (calcd for $C_{27}H_{29}N_2O_5$, 461.2076).

In Vitro Cytotoxicity and XBP1-Activation Activity Assay. An in vitro cytotoxicity assay with IEC-6 cells, a dual luciferase reporter assay, and an EC_{50} value assay were carried out as described previously.[9b]

Evaluation of Curative Effects in Vivo. The animal experiments were approved by the Institutional Ethical Committee for Animal Care and Use of the Chinese Academy of Medical Sciences (CAMS). The experimental procedure was modeled after previous work.[9b]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00807.

^{1}H and ^{13}C NMR spectra for all of the target compounds and 1D difference NOE spectra for compounds **4d** and **6c** (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

* (L.Q.W.) Tel: 0086-010-63031589. Fax: 0086-010-63017757. E-mail: wlq@imm.ac.cn.

* (H.L.Q.) Tel: 0086-010-83172503. Fax: 0086-010-63017757. E-mail: qinhailin@imm.ac.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by grants from NSFC (No. 81373269; 81202546) and from the Fundamental Research Funds for the Central Universities of China (No. 2015TD02).

■ REFERENCES

(1) Guazelli, C. F. S.; Fattori, V.; Colombo, B. B.; Georgetti, S. R.; Vicentini, F. T. M. C.; Casagrande, R.; Baracat, M. M.; Verri, W. A., Jr. *J. Nat. Prod.* **2013**, *76*, 200−208.

(2) Xavier, R. J.; Podolsky, D. K. *Nature* **2007**, *448*, 427−434.

(3) (a) Ekbom, A.; Helmick, C.; Zack, M.; Adami, H. O. *N. Engl. J. Med.* **1990**, *323*, 1228−1233. (b) Prior, P.; Gyde, S. N.; Macartney, J. C.; Thompson, H.; Waterhouse, J. A. H.; Allan, R. N. *Gut* **1982**, *23*, 490−497.

(4) (a) Jiang, L.; Xia, B.; Li, J.; Ye, M.; Yan, W.; Deng, C.; Ding, Y.; Luo, H.; Hou, W.; Zhao, Q.; Liu, N.; Ren, H.; Hou, X.; Xu, H. *Inflammatory Bowel Dis.* **2006**, *12*, 212−217. (b) Jiang, X. L.; Cui, H. F. *World J. Gastroenterol.* **2002**, *8*, 158−161. (c) Wang, Y.; Ouyang, Q. *J. Gastroenterol. Hepatol.* **2007**, *22*, 1450−1455.

(5) (a) Hotoleanu, C.; Popp, R.; Trifa, A. P.; Nedelcu, L.; Dumitrascu, D. L. *World J. Gastroenterol.* **2008**, *14*, 6636−6640. (b) Loftus, E. V., Jr.; Sandborn, W. J. *Clin. Gastroenterol.* **2002**, *31*, 1−20. (c) Andoh, A.; Yagi, Y.; Shioya, M.; Nishida, A.; Tsujikawa, T.; Fujiyama, Y. *World J. Gastroenterol.* **2008**, *14*, 5154−5161.

(6) Kaser, A.; Lee, A. H.; Franke, A.; Glickman, J. N.; Zeissig, S.; Tilg, H.; Nieuwenhuis, E. E. S.; Higgins, D. E.; Schreiber, S.; Glimcher, L. H.; Blumberg, R. S. *Cell* **2008**, *134*, 743−756.

(7) (a) Yoshida, H.; Matsui, T.; Yamamoto, A.; Okada, T.; Mori, K. *Cell* **2001**, *107*, 881−891. (b) Ron, D.; Walter, P. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 519−529.

(8) Niederreiter, L.; Fritz, T. M. J.; Adolph, T. E.; Krismer, A. M.; Offner, F. A.; Tschurtschenthaler, M.; Flak, M. B.; Hosomi, S.; Tomczak, M. F.; Kaneider, N. C.; Sarcevic, E.; Kempster, S. L.; Raine, T.; Esser, D.; Rosenstiel, P.; Kohno, K.; Iwawaki, T.; Tilg, H.; Blumberg, R. S.; Kaser, A. *J. Exp. Med.* **2013**, *210*, 2041−2056.

(9) (a) Zhang, Z. H.; Wu, L. Q.; Deng, A. J.; Yu, J. Q.; Li, Z. H.; Zhang, H. J.; Wang, W. J.; Qin, H. L. *J. Asian Nat. Prod. Res.* **2014**, *16*, 841−846. (b) Zhang, Z. H.; Zhang, H. J.; Deng, A. J.; Wang, B.; Li, Z. H.; Liu, Y.; Wu, L. Q.; Wang, W. J.; Qin, H. L. *J. Med. Chem.* **2015**, *58*, 7557−7571.

(10) (a) Jung, H. A.; Yoon, N. Y.; Bae, H. J.; Min, B. S.; Choi, J. S. *Arch. Pharmacal Res.* **2008**, *31*, 1405−1412. (b) Yan, D.; Jin, C.; Xiao, X. H.; Dong, X. P. *J. Biochem. Biophys. Methods* **2008**, *70*, 845−849. (c) Li, H. L.; Han, T.; Liu, R. H.; Zhang, C.; Chen, H. S.; Zhang, W. D. *Chem. Biodiversity* **2008**, *5*, 777−783. (d) Lin, C. C.; Ng, L. T.; Hsu, F. F.; Shieh, D. E.; Chiang, L. C. *Clin. Exp. Pharmacol. Physiol.* **2004**, *31*, 65−69. (e) Gong, L. L.; Fang, L. H.; Wang, S. B.; Sun, J. L.; Qin, H. L.; Li, X. X.; Wang, S. B.; Du, G. H. *Atherosclerosis* **2012**, *222*, 50−58. (f) Guo, J.; Wang, S. B.; Yuan, T. Y.; Wu, Y. J.; Yan, Y.; Li, L.; Xu, X. N.; Gong, L. L.; Qin, H. L.; Fang, L. H.; Du, G. H. *Atherosclerosis* **2013**, *231*, 384−391.

DOI: 10.1021/acs.jnatprod.5b00807
J. Nat. Prod. XXXX, XXX, XXX−XXX

(11) Zhang, Z. H.; Deng, A. J.; Wu, L. Q.; Fang, L. H.; Yu, J. Q.; Li, Z. H.; Yuan, T. Y.; Wang, W. J.; Du, G. H.; Qin, H. L. *Eur. J. Med. Chem.* **2014**, *86*, 542−549.
(12) Fischbeck, A.; Leucht, K.; Frey-Wagner, I.; Bentz, S.; Pesch, T.; Kellermeier, S.; Krebs, M.; Fried, M.; Rogler, G.; Hausmann, M.; Humpf, H. U. *Gut* **2011**, *60*, 55−65.
(13) Okayasu, I.; Hatakeyama, S.; Yamada, M.; Ohkusa, T.; Inagaki, Y.; Nakaya, R. *Gastroenterology* **1990**, *98*, 694−702.
(14) Luk, H. H.; Ko, J. K. S.; Fung, H. S.; Cho, C. H. *Eur. J. Pharmacol.* **2002**, *443*, 197−204.

DOI: 10.1021/acs.jnatprod.5b00807
J. Nat. Prod. XXXX, XXX, XXX−XXX