

Synthesis and cytotoxic evaluation of ester analogues of (-)-curine

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Abstract

This study presents the synthesis and cytotoxic evaluation of ester analogues derived from (-)-curine, a natural compound isolated from the roots of *Cissampelos pareira* (Menispermaceae). The synthesis involved the preparation of mono- and di-acetylcurine, along with four other ester derivatives, through diverse chemical transformations. Structural characterization of the synthesized compounds was performed using spectroscopic techniques and high-resolution mass spectrometry. Cytotoxicity assessments were conducted across various cancer cell lines, including MCF-7, MDA-MB-231, and Huh-7, as well as non-cancerous HEK293T cells, utilizing the MTT assay. Notably, compounds **4** and **5** demonstrated significant cytotoxic activities superior to the parent compound **1** and even matched or exceeded the cytotoxic effects induced by cisplatin, a conventional chemotherapeutic agent, across all tested cancer cell lines. These findings highlight the potential of compounds **4** and **5** as promising candidates for further development as potent cytotoxic agents in cancer therapy.

Introduction

(-)-Curine, a bisbenzylisoquinoline (BBIQ) alkaloid, is the primary compound derived from the methanol extract of *Cissampelos pareira* roots. This plant, known for its diverse medicinal properties, has been historically used in traditional medicine to address a spectrum of ailments, including coughs, delirium, fever, madness, epilepsy, and convulsions [1]. Its global application extends to serving as a stimulant, sedative, analgesic, febrifuge, antioxidant, tonic, and narcotic agent [1]. The pharmacological potential of curine has attracted scientific interest, with earlier investigations uncovering its vasodilator effects attributed to the inhibition of calcium influx, possibly mediated by the direct blockade of L-type Ca^{2+} channels [2]. Additionally, curine has exhibited anti-inflammatory effects [3], anti-allergic properties [4–6] and cytotoxicity [7–8], suggesting its broad spectrum of biological activities. Despite its promising pharmacological profile, the chemical modification of curine has emerged as a strategy to enhance its therapeutic efficacy and selectivity. Chemical modification of natural compounds is a prevailing approach in medicinal chemistry aimed at developing more potent pharmacological agents. By employing straightforward synthetic methodologies, the production of modified curine analogs with robust bioactivity becomes feasible, thus presenting opportunities for the pharmaceutical industry. The current study focuses on the chemical modification of curine (**1**) to synthesize analogs (Scheme 1–2), with some exhibiting notable cytotoxic activities. This approach underscores the importance of structural manipulation in elucidating structure-activity relationships and enhancing the pharmacological properties of natural compounds like curine.

Results and discussion

Chemistry

The chemical modifications described in this study focus on the ester analogues at the C-7 and C-12 hydroxyl groups of (-)-curine (**1**). The first series of ester-(-)-curine (**1**) analogues were prepared as shown

in Scheme 1. Acetylation of the resulting (-)-curine (**1**) at C-7 and C-12 in the presence of acetic anhydride at room temperature for 3 h afforded the mono- and di-acetylcurine products 7,12-Diacetoxylcurine (**2**) and 7-Acetoxylcurine (**3**).

Compound **4**, 7-(1,6-dihydrocyclopenta[c]pyrazole-3-carboxyl)-curine, was synthesized by reacting compound **1** with 1,6-dihydrocyclopenta[c]pyrazole-3-carboxylic acid, N,N'-diisopropylcarbodiimide (DIC), in dichloromethane, employing 4-*N,N*-dimethylaminopyridine (DMAP) as a catalyst, resulting in a yield of 35.3% (Scheme 2). 7,12-Dithiophene-2-carbonylcurine (**5**) was obtained in excellent yield (94.1%) through the reaction of compound **1** with thiophene-2-carbonyl chloride, DIC, in dichloromethane, with DMAP serving as the catalyst (Scheme 2). The synthesis of 7-(4-Chlorocinamoyl)-curine (**6**) involved the reaction of compound **1** with 4-chlorocinamic acid, DIC, in dichloromethane, facilitated by DMAP, resulting in a yield of 35.2% (Scheme 2). 7,12-di-(3-nitrobenzoyl)-curine (**7**) was obtained by reacting compound **1** with 3-nitrobenzoic acid, DIC, in dichloromethane in the presence of DMAP, yielding 20.2% (Scheme 2). The preparation of 7,12-di-(trans-3-nitrocinaoyl)-curine (**8**) involved the reaction of compound **1** with trans-3-nitrocinaoyl acid, DIC, in dichloromethane, using DMAP as the catalyst, resulting in a yield of 47.0% (Scheme 2). All synthetic compounds were purified by column chromatography and characterized by spectroscopic techniques and high-resolution mass spectrometry (see Experimental).

Cytotoxic evaluation of (-)-curine and its analogs against cancer cell lines

The cytotoxic activities of (-)-curine (compound **1**) and its analogs (**2–8**), alongside the positive control cisplatin, were evaluated against triple-negative breast cancer (MDA-MB231), hepatocellular carcinoma (Huh-7), human breast cancer (MCF-7), and non-cancerous human embryonic kidney 293T cells (HEK293T), utilizing the MTT assay, as depicted in Table 1. Compounds **1**, **2**, **3**, **6**, **7**, and **8** demonstrated IC₅₀ values greater than 100 µM across all cell lines, indicating minimal cytotoxic effects within the tested concentration range. Conversely, compounds **4** and **5** exhibited varying degrees of cytotoxicity against the cell lines. Compound **4** displayed IC₅₀ values of 57.52 ± 1.20 µM, 37.44 ± 1.87 µM, and 45.33 ± 1.96 µM against MDA-MB-231, Huh-7, and MCF-7 cells, respectively, while showing no significant effect on HEK-293T cells (> 100 µM). Compound **5** demonstrated similar potency, with IC₅₀ values of 58.97 ± 3.07 µM, 33.00 ± 1.82 µM, and 23.33 ± 1.67 µM against MDA-MB-231, Huh-7, and MCF-7 cells, respectively, and 58.84 ± 3.06 µM against HEK-293T cells. Notably, compounds **4** and **5** exhibited comparable or superior cytotoxic effects against the tested cancer cell lines compared to the positive control cisplatin, indicating their potential as promising candidates for further investigation as cytotoxic agents. These findings suggest the importance of exploring structural modifications to enhance the pharmacological properties of (-)-curine and its analogs, potentially leading to the development of novel anticancer therapeutics.

Table 1
Cytotoxic activities of compounds 1–8

Compound	Cytotoxicity (IC ₅₀ , μ M)			
	MDA-MB-231	Huh-7	MCF-7	HEK-293T
1	> 100	> 100	> 100	> 100
2	> 100	> 100	> 100	> 100
3	> 100	> 100	> 100	> 100
4	57.52 $\pm$ 1.20	37.44 $\pm$ 1.87	45.33 $\pm$ 1.96	> 100
5	58.97 $\pm$ 3.07	33.00 $\pm$ 1.82	23.33 $\pm$ 1.67	58.84 $\pm$ 3.06
6	> 100	> 100	> 100	> 100
7	> 100	> 100	> 100	> 100
8	> 100	> 100	> 100	> 100
Cisplatin ^a	28.42 $\pm$ 1.73	46.54 $\pm$ 2.89	37.55 $\pm$ 1.81	60.22 $\pm$ 3.08
^a Positive control				

Conclusion

In conclusion, the study focused on the chemical modification of (-)-curine, a bisbenzylisoquinoline alkaloid derived from the roots of *Cissampelos pareira*, aiming to synthesize analogs with enhanced pharmacological properties, particularly cytotoxicity against various cancer cell lines. The synthesis of seven ester analogs of (-)-curine was successfully achieved through straightforward synthetic methodologies involving esterification reactions with different carboxylic acids. The compounds were purified and characterized using spectroscopic techniques and high-resolution mass spectrometry. Evaluation of cytotoxic potential across multiple cancer cell lines revealed compounds **4** and **5** to exhibit significant activity, surpassing (-)-curine and matching or exceeding cisplatin's effects. These results highlight the potential of chemical modification strategies to enhance natural compounds' pharmacological properties. Compounds **4** and **5**, particularly promising, warrant further investigation into their mechanisms of action and in vivo efficacy, suggesting potential clinical applications in cancer therapy. Future studies should delve into structure-activity relationships to optimize (-)-curine analogs' cytotoxicity and selectivity.

Experimental section

General experimental procedures

Optical rotations were measured on a JASCO-1020 polarimeter. IR spectra were obtained using a Frontier FT-IR Perkin-Elmer spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 FT-NMR spectrometer, operating at 400 MHz (^1H) and 100 MHz (^{13}C). ESTOFMS spectra were measured with a Bruker micrOTOF-II mass spectrometer. Unless otherwise indicated, column chromatography was carried out using Merck silica gel 60 (< 0.063 mm). For TLC, Merck precoated silica gel 60 F₂₅₄ plates were used. Spots on TLC were detected under UV light and by spraying with anisaldehyde-H₂SO₄ reagent followed by heating.

Extraction and isolation of (–)-curine

(–)-Curine was isolated from the roots of *Cissampelos pareira* (Menispermaceae). Its structure was confirmed by spectroscopic methods and comparison with the reported literature [1].

(–)-Curine (1) Pale yellow power; $[\alpha]_{\text{D}}^{26} -167.9^\circ$ (c 0.97, CHCl₃); IR ν_{max} : 3370, 2932, 2839, 2796, 1611, 1503, 1444, 1273, 1213, 1166, 1109, 1055, 907, 831, 801, 725 cm⁻¹; ^1H NMR (CDCl₃, 400 MHz): δ 7.08 (1H, d, J = 8.0 Hz, H-14'), 6.92 (1H, d, J = 8.0 Hz, H-14), 6.79 (1H, d, J = 8.0 Hz, H-13), 6.68 (1H, partially overlapping signal, H-13'), 6.66 (1H, s, H-5'), 6.63 (1H, d, J = 8.0 Hz, H-11'), 6.60 (1H, br s, H-10), 6.53 (1H, s, H-5), 6.44 (1H, d, J = 8.0 Hz, H-10'), 5.94 (1H, br s, H-8'), 3.88 (3H, s, 6'-OCH₃), 3.55 (1H, d, J = 7.6 Hz, H-1), 3.44 (1H, d, J = 9.2 Hz, H-1'), 3.31 (1H, m, H-3), 3.22 (1H, m, H-3'), 3.13, 2.53 (2H, br d, J = 11.8 Hz, H- α'), 2.97 (1H, overlapping signal, H-4), 2.92, 2.71 (2H, overlapping signal, H-4'), 2.87 (3H, s, 6-OCH₃), 2.85 (1H, overlapping signal, H-3), 2.81, 2.74 (2H, overlapping signal, H- α), 2.79 (1H, overlapping signal, H-3'), 2.49 (3H, s, N'-CH₃), 2.42 (1H, dd, J = 16.8, 3.8 Hz, H-4), 2.27 (3H, s, N-CH₃); ^{13}C NMR (CDCl₃, 100 MHz): δ 155.4 (C-12'), 148.6 (C-6'), 146.5 (C-6), 146.3 (C-12), 144.2 (C-11), 143.2 (C-7'), 138.5 (C-8), 137.3 (C-7), 133.7 (C-9), 132.4 (C-9'), 132.2 (C-10'), 129.6 (C-4a'), 129.5 (C-14'), 126.6 (C-14), 125.1 (C-4a), 124.5 (C-8a), 124.5 (C-8a'), 120.7 (C-10), 120.2 (C-8'), 115.5 (C-11'), 115.2 (C-13), 113.3 (C-13'), 112.3 (C-5'), 108.1 (C-5), 65.4 (C-1'), 60.4 (C-1), 56.1 (6-OCH₃), 56.0 (6'-OCH₃), 45.7 (C-3'), 43.9 (C-3), 42.2 (N'-CH₃), 41.7 (N-CH₃), 39.9 (C- α), 39.7 (C- α'), 25.3 (C-4'), 21.9 (C-4); ESI MS m/z 595.9 [M + H]⁺.

Synthesis of (–)-curine ester analogues

Preparation of Compounds 2 and 3

A mixture of compound **1** (50 mg, 0.08 mmol) was dissolved in CH₂Cl₂ (5 ml) and acetic anhydride (0.1 ml) was added. The reaction mixture was stirred at room temperature for 3 h. The mixture was poured into water (30 ml) and the solution was extracted with CH₂Cl₂ (100 ml). The organic phase was washed with water (3×20 ml) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the product was purified by column chromatography using 4% MeOH in CH₂Cl₂ as eluent to yield compounds **2** (22.0 mg, 37.5%) and **3** (15.1 mg, 25%).

7,12-Diacetoxylcurine (2) Pale-yellow powder; $[\alpha]_D^{27}$ -114.34 ($c = 0.32$, CH_2Cl_2); IR ν_{max} : 2931, 2845, 1764, 1609, 1503, 1418, 1367, 1271, 1214, 1186, 1112, 1055, 1011, 905, 834, 726 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.10 (1H, d, $J = 8.0$ Hz, H-14), 7.09 (1H, d, $J = 8.0$ Hz, H-14'), 6.91 (1H, d, $J = 8.0$ Hz, H-13), 6.69 (1H, d, $J = 8.0$ Hz, H-13'), 6.63 (1H, br s, H-5'), 6.62 (1H, d, $J = 8.0$ Hz, H-11'), 6.58 (1H, s, H-5), 6.58 (1H, br s, H-10), 6.40 (1H, d, $J = 8.0$ Hz, H-10'), 5.48 (1H, s, H-8'), 3.79 (3H, s, 6-OCH₃), 3.78 (3H, s, 6'-OCH₃), 3.55 (1H, d, $J = 10.8$ Hz, H-1'), 3.48 (1H, d, $J = 8.4$ Hz, H-1), 3.30 (2H, m, H-3,3'), 3.26, (2H, br d, $J = 11.6$ Hz, H- α , α'), 2.98 (2H, overlapping signal, H-4, 4'), 2.87 (1H, overlapping signal, H-3), 2.82 (1H, overlapping signal, H-3'), 2.66 (2H, d, $J = 14.4$ Hz, H- α), 2.53 (3H, s, N'-CH₃), 2.45 (1H, dd, $J = 18.0, 4.0$ Hz, H-4), 2.24 (3H, s, N-CH₃), 2.06 (3H, s, H-2''), 2.01 (3H, s, H-2'''); ^{13}C NMR (CDCl_3 , 100 MHz): δ 168.8 (C-1'''), 168.4 (C-1''), 155.2 (C-12'), 150.8 (C-6), 148.8 (C-6'), 146.7 (C-11), 144.5 (C-8), 143.8 (C-7'), 140.6 (C-12), 140.3 (C-9), 132.9 (C-10'), 132.2 (C-7), 131.3 (C-9'), 129.8 (C-14'), 126.9 (C-4a'), 126.5 (C-8a'), 125.8 (C-14), 124.1 (C-4a, C-8a), 122.7 (C-13), 122.2 (C-10), 117.7 (C-8'), 115.2 (C-11'), 114.1 (C-13'), 112.3 (C-5'), 108.9 (C-5), 64.9 (C-1'), 60.6 (C-1), 56.0 (6-OCH₃), 55.9 (6'-OCH₃), 44.8 (C-3'), 43.3 (C-3), 41.5 (N-CH₃), 41.1 (N'-CH₃), 40.2 (C- α), 39.5 (C- α'), 24.0 (C-4'), 21.9 (C-4), 20.4 (C-2'''), 20.1 (C-2''); HR-TOF-MS (ESI⁺): m/z 679.3029 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{40}\text{H}_{43}\text{N}_2\text{O}_8$, 679.3014). The physical and spectral data were in agreement with those reported in the literature [9].

7-Acetoxylcurine (3) Pale-yellow powder; $[\alpha]_D^{27}$ -132.57 ($c = 0.35$, CH_2Cl_2); IR ν_{max} : 3380, 2928, 2845, 1765, 1609, 1579, 1504, 1438, 1368, 1271, 1188, 1112, 1056, 1013, 902, 833, 726 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.12 (1H, d, $J = 7.6$ Hz, H-14'), 6.91 (1H, d, $J = 7.6$ Hz, H-14), 6.79 (1H, d, $J = 7.6$ Hz, H-13), 6.68 (1H, overlapping signal, H-5'), 6.68 (1H, overlapping signal, H-13'), 6.61 (1H, s, H-5), 6.65 (1H, br s, H-10), 6.64 (1H, overlapping signal, H-11'), 6.54 (1H, d, $J = 7.6$ Hz, H-10'), 5.92 (1H, s, H-8'), 3.89 (3H, s, 6'-OCH₃), 3.83 (3H, s, 6-OCH₃), 3.52 (1H, d, $J = 8.0$ Hz, H-1), 3.48 (1H, d, $J = 15.2$ Hz, H-1'), 3.36 (1H, m, H-3), 3.29 (1H, m, H-3'), 3.24 (1H, overlapping signal, H- α'), 2.98 (1H, m, H-4), 2.94 (1H, m, H-4'), 2.90 (1H, overlapping signal, H-3), 2.81 (1H, overlapping signal, H-4'), 2.77 (2H, overlapping signal, H- α , α'), 2.55 (1H, overlapping signal, H- α), 2.53 (3H, s, N'-CH₃), 2.48 (1H, dd, $J = 17.4, 4.2$ Hz, H-4), 2.25 (3H, s, N-CH₃), 2.11 (3H, s, H-2''); ^{13}C NMR (CDCl_3 , 100 MHz): δ 168.6 (C-1''), 155.3 (C-12'), 150.7 (C-6), 148.7 (C-6'), 146.4 (C-12), 144.6 (C-8), 144.0 (C-11), 143.4 (C-7'), 133.6 (C-9), 132.9 (C-7), 132.2 (C-10'), 131.7 (4a', C-9'), 129.6 (C-14'), 127.1 (C-8a'), 126.8 (C-14), 124.3 (C-4a), 123.6 (C-8a), 120.9 (C-10), 119.8 (C-8'), 115.7 (C-11'), 115.3 (C-13), 113.8 (C-13'), 112.3 (C-5'), 108.9 (C-5), 65.5 (C-1'), 60.4 (C-1), 56.1 (6-OCH₃, 6'-OCH₃), 45.7 (C-3'), 43.6 (C-3), 42.0 (N-CH₃), 41.7 (N'-CH₃), 39.8 (C- α , α'), 25.3 (C-4'), 22.2 (C-4), 20.2 (C-2''); HR-TOF-MS (ESI⁺): m/z 637.2918 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{38}\text{H}_{41}\text{N}_2\text{O}_7$, 637.2908).

Preparation of Compound 4

A mixture of compound 1 (100 mg, 0.17 mmol) was dissolved in CH_2Cl_2 (10 ml) and N,N'-diisopropylcarbodiimide (DIC) (0.1 ml), 4-N,N'-dimethylaminopyridine (DMAP) (1 mg, 0.01 mmol) and 1,6-dihydrocyclopenta[c]pyrazole-3-carboxylic acid (10 mg, 0.07 mmol) was added. The reaction mixture was stirred at room temperature for 72 h. The mixture was poured into water (30 ml) and the solution was

extracted with CH₂Cl₂ (100 ml). The organic phase was washed with water (3×20 ml) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the product was purified by column chromatography using 4% MeOH in CH₂Cl₂ to yield compound **4** (41.5 mg, 35.3%).

7-(1,6-dihydrocyclopenta[c]pyrazole-3-carboxyl)-curine (4) Pale-yellow powder; $[\alpha]_D^{27}$ -196.10 (c = 0.30, CH₂Cl₂); IR $\nu_{\max}$: 3312, 2932, 2849, 1740, 1609, 1504, 1441, 1272, 1209, 1108, 1023, 834, 765 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.07 (1H, d, *J* = 8.0 Hz, H-14'), 6.92 (1H, d, *J* = 8.0 Hz, H-14), 6.78 (1H, d, *J* = 8.0 Hz, H-13), 6.69 (1H, overlapping signal, H-13'), 6.65 (1H, br s, H-5'), 6.63 (1H, overlapping signal, H-5, 5''), 6.57 (1H, d, *J* = 8.0 Hz, H-11'), 6.55 (2H, overlapping signal, H-10,4''), 6.42 (1H, d, *J* = 7.6 Hz, H-10'), 5.91 (1H, s, H-8'), 3.86 (3H, s, 6'-OCH₃), 3.80 (3H, s, 6-OCH₃), 3.56 (1H, d, *J* = 7.6 Hz, H-1), 3.47 (1H, d, *J* = 5.6 Hz, H-1'), 3.35 (1H, m, H-3), 3.25 (1H, m, H-3'), 3.17, 2.55 (2H, br d, *J* = 11.6 Hz, H- α '), 3.01 (1H, m, H-4), 2.97 (1H, overlapping signal, H-4'), 2.90 (1H, overlapping signal, H-3), 2.81 (1H, overlapping signal, H-3'), 2.78 (1H, overlapping signal, H- α), 2.74 (1H, d, *J* = 12.4 Hz, H- α), 2.67 (1H, m, H-4'), 2.49 (1H, overlapping signal, H-4), 2.48 (3H, s, N'-CH₃), 2.34 (2H, m, H-6''), 2.27 (3H, s, N-CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 157.5 (C-7''), 155.4 (C-12'), 150.8 (C-6), 148.7 (C-6'), 146.3 (C-12), 144.7 (C-8), 144.0 (C-11), 143.2 (C-7'), 133.5 (C-7), 133.3 (C-9), 132.4 (C-9'), 132.0 (C-10'), 130.9 (C-3a''), 130.6 (C-4a'), 129.6 (C-14'), 129.3 (C-8a'), 129.1 (C-6a''), 126.5 (C-14), 124.6 (C-4a, 8a), 120.7 (C-10), 120.0 (C-8'), 115.4 (C-11'), 115.3 (C-13, 5''), 113.9 (C-13'), 112.3 (C-5'), 109.0 (C-5), 108.1 (C-4''), 65.3 (C-1'), 60.3 (C-1), 56.1 (6-OCH₃), 55.9 (6'-OCH₃), 45.6 (C-3'), 43.6 (C-3), 42.0 (N-CH₃), 41.8 (N'-CH₃), 39.7 (C- α , α '), 29.9 (C-6''), 25.1 (C-4'), 24.4 (C-4); HR-TOF-MS (ESI⁺): *m/z* 727.3105 [M + H]⁺ (calcd. for C₄₅H₄₃N₄O₇, 727.3126).

Preparation of Compound 5

A mixture of compound **1** (100 mg, 0.17 mmol) was dissolved in CH₂Cl₂ (10 ml). To this solution, N,N'-diisopropylcarbodiimide (DIC) (0.1 ml), 4-N,N'-dimethylaminopyridine (DMAP) (1 mg, 0.01 mmol), and thiophene-2-carbonyl chloride (10 mg, 0.08 mmol) were added and stirred at room temperature for 24 h. The mixture was poured into water (30 ml) and the solution was extracted with CH₂Cl₂ (100 ml). The organic phase was washed with water (3×20 ml) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the product was purified by column chromatography using 6% MeOH in CH₂Cl₂ to yield compound **5** (108.9 mg, 94.1%).

7,12-Dithiophene-2-carbonylcurine (5) Pale yellow powder; $[\alpha]_D^{27}$ -211.59 (c = 0.32, CH₂Cl₂); IR $\nu_{\max}$: 3341, 2929, 2841, 1729, 1609, 1502, 1414, 1359, 1250, 1201, 1112, 1050, 1014, 834, 734 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.74 (1H, br s, H-3'''), 7.72 (1H, br s, H-3''), 7.52 (2H, br s, H-5'', H-5'''), 7.19 (1H, d, *J* = 7.6 Hz, H-14), 7.06 (1H, d, *J* = 7.6 Hz, H-13), 7.05 (1H, overlapping signal, H-4'''), 7.01 (2H, overlapping signal, H-14',4''), 6.74 (1H, br s, H-11'), 6.65 (2H, overlapping signal, H-13', H-5), 6.70 (1H, br s, H-10), 6.44 (1H, br s, H-5'), 6.44 (1H, overlapping signal, H-10'), 5.49 (1H, s, H-8'), 3.82 (3H, s, 6-OCH₃), 3.53 (1H, d, *J* = 8.0 Hz, H-1), 3.42, 2.94 (2H, overlapping signal, H-3), 3.42 (3H, s, 6'-OCH₃), 3.36 (1H, d, *J* = 8.0 Hz, H-1'), 3.11 (1H, br d, *J* = 12.0 Hz, H- α '), 3.19 (1H, m, H-3'), 3.05, 2.49 (2H, overlapping signal, H-4), 2.91 (1H, overlapping

signal, H-4'), 2.86, 2.76 (2H, overlapping signal, H- α), 2.76 (1H, overlapping signal, H-3'), 2.63 (1H, m, H-4'), 2.49 (1H, overlapping signal, H- α'), 2.49 (3H, s, N'-CH₃), 2.29 (3H, s, N-CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 159.8 (C-6"), 159.5 (C-6"), 155.0 (C-12'), 151.1 (C-6), 148.2 (C-6'), 147.0 (C-12), 144.9 (C-8), 143.4 (C-11), 140.9 (C-7'), 134.7 (C-3"), 134.1 (C-3"), 133.2 (C-2", 5", 2"), 133.2 (C-9'), 132.8 (C-5"), 132.4 (C-9), 132.3 (C-10'), 131.2 (C-7), 129.5 (C-14'), 127.8 (C-4a', 8a'), 127.6 (C-4"), 127.5 (C-4"), 125.6 (C-14), 124.4 (C-4a, 8a), 122.7 (C-10, 13), 117.8 (C-8'), 115.2 (C-11'), 113.4 (C-13'), 111.8 (C-5'), 109.0 (C-5), 65.3 (C-1'), 60.8 (C-1), 56.1 (6-OCH₃), 55.1 (6'-OCH₃), 45.9 (C-3'), 43.4 (C-3), 42.4 (N'-CH₃), 41.6 (N-CH₃), 40.3 (C- α'), 39.3 (C- α), 25.5 (C-4'), 22.0 (C-4); HR-TOF-MS (ESI⁺): m/z 815.2452 [M + H]⁺ (calcd. for C₄₆H₄₃N₂O₈S₂, 815.2452).

Preparation of Compound 6

A mixture of compound **1** (100 mg, 0.17 mmol) was dissolved in CH₂Cl₂ (10 ml) and N,N-diisopropylcarbodiimide (DIC) (0.1 ml), 4-N,N-dimethylaminopyridine (DMAP) (1 mg, 0.01 mmol) and 4-Chlorocinamic acid (10 mg, 0.05 mmol) was stirred at room temperature for 96 h. The mixture was poured into water (30 ml) and the solution was extracted with CH₂Cl₂ (100 ml). The organic phase was washed with water (3×20 ml) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure, and the product was purified by column chromatography using 4% MeOH in CH₂Cl₂, yielding compound **6** (47.1 mg, 35.2%).

7-(4-Chlorocinamoyl)-curine (6) Pale yellow powder; $[\alpha]_D^{27}$ -208.18 (c = 0.32, CH₂Cl₂); IR $\nu_{\max}$: 2930, 2840, 1734, 1635, 1609, 1504, 1444, 1272, 1207, 1111, 1089, 820, 727 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.57 (1H, br d, J = 15.8 Hz, H-7"), 7.37 (1H, d, J = 7.6 Hz, H-2", 6"), 7.31 (1H, d, J = 7.6 Hz, H-3", 5"), 7.06 (1H, d, J = 7.6 Hz, H-14'), 6.92 (1H, d, J = 7.6 Hz, H-14), 6.79 (1H, d, J = 7.6 Hz, H-13), 6.72 (1H, d, J = 7.6 Hz, H-13'), 6.67 (1H, br s, H-5'), 6.64 (2H, overlapping signal, H-5, H-10), 6.61 (1H, overlapping signal, H-11'), 6.46 (1H, br d, J = 15.8 Hz, H-8"), 6.45 (1H, overlapping signal, H-10'), 5.94 (1H, s, H-8'), 3.88 (3H, s, 6'-OCH₃), 3.84 (3H, s, 6-OCH₃), 3.54 (1H, d, J = 7.6 Hz, H-1), 3.43 (1H, d, J = 10.4 Hz, H-1'), 3.35 (1H, m, H-3), 3.21 (1H, m, H-3'), 3.16 (1H, br d, J = 13.2 Hz, H- α'), 3.02 (1H, m, H-4), 2.90, 2.75 (2H, overlapping signal, H-4'), 2.88 (1H, overlapping signal, H-3), 2.79 (3H, overlapping signal, H- α , 3'), 2.57 (1H, overlapping signal, H- α'), 2.51 (1H, overlapping signal, H-4), 2.51 (3H, s, N'-CH₃), 2.27 (3H, s, N-CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 164.2 (C-9"), 155.4 (C-12'), 150.8 (C-6), 148.6 (C-6'), 146.4 (C-12), 145.1 (C-7"), 144.7 (C-8), 144.1 (C-11), 143.3 (C-7'), 136.4 (C-4"), 133.6 (C-9), 133.1 (C-1"), 132.6 (C-9'), 132.5 (C-10'), 131.6 (C-7), 129.6 (C-14'), 129.4 (C-2", C-6"), 129.2 (C-3", C-5"), 129.1 (C-4a', C-8a'), 126.7 (C-14), 124.4 (C-4a, C-8a), 120.9 (C-10), 119.9 (C-8'), 117.0 (C-8"), 115.7 (C-11'), 115.3 (C-13), 113.9 (C-13'), 112.3 (C-5'), 108.9 (C-5), 65.5 (C-1'), 60.3 (C-1), 56.1 (6-OCH₃), 56.0 (6'-OCH₃), 45.9 (C-3'), 43.6 (C-3), 42.3 (N'-CH₃), 41.7 (N-CH₃), 39.7 (C- α , α'), 25.5 (C-4'), 22.2 (C-4); HR-TOF-MS (ESI⁺): m/z 759.2820 [M + H]⁺ (calcd. for C₄₅H₄₄ClN₂O₇, 759.2932).

Preparation of Compound 7

A mixture of compound **1** (100 mg, 0.17 mmol) was dissolved in CH₂Cl₂ (10 ml) and N,N-diisopropylcarbodiimide (DIC) (0.1 ml), 4-N,N-dimethylaminopyridine (DMAP) (1 mg, 0.01 mmol) and 3-

Nitrobenzoic acid (10 mg, 0.06 mmol) was stirred at room temperature for 6 h. Subsequently, the mixture was poured into water (30 ml) and extracted with CH₂Cl₂ (100 ml). The organic phase was washed with water (3×20 ml) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the product was purified by column chromatography using 4% MeOH in CH₂Cl₂ to yield compound **7** (31.3 mg, 20.2%).

7,12-di-(3-nitrobenzoyl)-curine (7) Pale yellow powder; $[\alpha]_D^{27}$ -219.13 (*c* = 0.30, CH₂Cl₂); IR $\nu_{\max}$: 2933, 1747, 1612, 1530, 1503, 1435, 1348, 1252, 1205, 1110, 1050, 908, 816, 714 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 8.71 (1H, s, H-2"), 8.65 (1H, s, H-2"), 8.38 (1H, d, *J* = 8.0 Hz, H-4"), 8.36 (1H, d, *J* = 7.2 Hz, H-4"), 8.32 (1H, d, *J* = 8.0 Hz, H-6"), 8.22 (1H, d, *J* = 8.0 Hz, H-6"), 7.60 (1H, t, *J* = 8.0 Hz, H-5"), 7.55 (1H, t, *J* = 8.0 Hz, H-5"), 7.27 (1H, overlapping signal, H-14), 7.09 (1H, d, *J* = 8.0 Hz, H-13), 7.01 (1H, d, *J* = 8.0 Hz, H-14'), 6.78 (1H, overlapping signal, H-11'), 6.67 (1H, s, H-5), 6.67 (1H, overlapping signal, H-13'), 6.62 (1H, br s, H-10), 6.47 (1H, d, *J* = 8.0 Hz, H-10'), 6.53 (1H, br s, H-5'), 5.52 (1H, s, H-8'), 3.82 (3H, s, 6-OCH₃), 3.62 (1H, d, *J* = 8.0 Hz, H-1), 3.52 (1H, br s, H-1'), 3.41, 2.94 (2H, overlapping signal, H-3), 3.37 (3H, s, 6'-OCH₃), 3.22, 2.85 (2H, m, H-3'), 3.22, 2.54 (2H, overlapping signal, H- α), 3.10 (1H, m, H-4), 2.94, 2.78 (2H, overlapping signal, H- α'), 2.94, 2.73 (2H, overlapping signal, H-4'), 2.87, 2.72 (2H, d, *J* = 15.2 Hz, H-4'), 2.50 (1H, overlapping signal, H-4), 2.50 (3H, s, N'-CH₃), 2.35 (3H, s, N-CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 162.4 (C-7"), 162.1 (C-7"), 155.1 (C-12'), 150.8 (C-6), 148.3 (C-6'), 148.1 (C-3", C-3"), 146.8 (C-12), 144.6 (C-8), 143.2 (C-7'), 141.3 (C-11), 139.9 (C-9), 135.7 (C-6", C-6"), 133.6 (C-9'), 132.3 (C-10'), 131.4 (C-1"), 130.9 (C-1"), 130.7 (C-7), 129.7 (C-14'), 129.5 (C-5", C-5"), 128.7 (C-4a'), 127.7 (C-4"), 127.5 (C-4", C-8a'), 125.4 (C-14), 125.1 (C-2"), 124.9 (C-2"), 124.6 (C-4a, C-8a), 122.5 (C-13), 122.2 (C-10), 118.3 (C-8'), 115.0 (C-11'), 114.3 (C-13'), 112.0 (C-5'), 109.0 (C-5), 64.9 (C-1'), 60.6 (C-1), 56.1 (6-OCH₃), 55.2 (6'-OCH₃), 45.5 (C-3'), 43.4 (C-3), 41.9 (N'-CH₃), 41.7 (N-CH₃), 40.6 (C- α), 39.2 (C- α'), 25.0 (C-4'), 22.0 (C-4); HR-TOF-MS (ESI⁺): *m/z* 893.3039 [M + H]⁺ (calcd. for C₅₀H₄₅N₄O₁₂, 893.3028).

Preparation of Compound 8

A mixture of compound **1** (100 mg, 0.17 mmol) was dissolved in CH₂Cl₂ (10 ml) and N,N-diisopropylcarbodiimide (DIC) (0.1 ml), 4-N,N'-dimethylaminopyridine (DMAP) (1 mg, 0.01 mmol) and *trans*-3-Nitrocinnamic acid (10 mg, 0.05 mmol) was stirred at room temperature for 6 h. The mixture was poured into water (30 ml) and the solution was extracted with CH₂Cl₂ (100 ml). The organic phase was washed with water (3×20 ml) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the product was purified by column chromatography using 4% MeOH in CH₂Cl₂ to yield compound **8** (72.4 mg, 47%).

7,12-di-(trans-3-nitrocinnamoyl)-curine (8) Pale yellow powder; $[\alpha]_D^{27}$ -141.40 (*c* = 0.30, CH₂Cl₂); IR $\nu_{\max}$: 2931, 2845, 1735, 1640, 1610, 1503, 1350, 1268, 1196, 1112, 974, 805, 740 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 8.28 (1H, s, H-2"), 8.21 (1H, s, H-2"), 8.19 (1H, d, *J* = 8.0 Hz, H-4"), 8.18 (1H, d, *J* = 8.0 Hz, H-4"), 7.78 (1H, d, *J* = 7.9 Hz, H-6"), 7.70 (1H, d, *J* = 7.9 Hz, H-6"), 7.61 (1H, br d, *J* = 16.4 Hz, H-7"), 7.55 (1H, t, *J*

= 7.9 Hz, H-5"), 7.54 (1H, d, J = 7.9 Hz, H-5"), 7.45 (1H, overlapping signal, H-7"), 7.20 (1H, d, J = 8.0 Hz, H-14), 7.04 (1H, d, J = 8.0 Hz, H-13), 7.01 (1H, d, J = 8.0 Hz, H-14'), 6.69 (1H, d, J = 9.2 Hz, H-11'), 6.66 (1H, s, H-5), 6.64 (3H, overlapping signal, H-10, H-8", H-8"), 6.58 (2H, overlapping signal, H-5', H-13'), 6.48 (1H, d, J = 7.2 Hz, H-10'), 5.54 (1H, s, H-8'), 3.82 (3H, s, 6-OCH₃), 3.63 (1H, overlapping signal, H-1), 3.63 (3H, s, 6'-OCH₃), 3.46 (1H, d, J = 10.0 Hz, H-1'), 3.36 (1H, m, H-3), 3.23 (1H, m, H-3'), 3.13 (1H, br d, J = 11.2 Hz, H- α'), 3.04 (1H, m, H-4), 2.93 (1H, overlapping signal, H-3), 2.91, 2.72 (2H, overlapping signal, H-4'), 2.90 (1H, overlapping signal, H- α), 2.82 (1H, d, J = 10.0 Hz, H-3'), 2.72, 2.51 (2H, overlapping signal, H-4), 2.67 (1H, d, J = 13.6 Hz, H- α), 2.58 (1H, br d, J = 11.6 Hz, H- α'), 2.48 (3H, s, N'-CH₃), 2.32 (3H, s, N-CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 2.58 (1H, br d, J = 11.6 Hz, H- α'), 2.48 (3H, s, N'-CH₃), 2.32 (3H, s, N-CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 163.8 (C-9"), 163.3 (C-9"), 155.2 (C-12'), 150.8 (C-6), 148.6 (C-6', C-3", C-3"), 146.7 (C-12), 144.6 (C-8), 143.4 (C-7"), 143.2 (C-7'), 142.7 (C-7"), 140.9 (C-9), 139.9 (C-11), 136.0 (C-1"), 135.9 (C-1"), 133.5 (C-6", C-6"), 133.1 (C-9'), 132.2 (C-10', C-7), 130.0 (C-5"), 129.9 (C-14'), 129.6 (C-5"), 127.9 (C-4a', C-8a'), 125.2 (C-14), 124.7 (C-4", C-4"), 124.5 (C-4a, C-8a), 122.7 (C-13), 122.5 (C-2", C-2"), 121.9 (C-8", C-8"), 120.4 (C-10), 119.6 (C-13'), 118.2 (C-8'), 115.1 (C-11'), 112.2 (C-5'), 108.9 (C-5), 64.9 (C-1'), 60.5 (C-1), 56.1 (6'-OCH₃), 55.8 (6-OCH₃), 45.6 (C-3'), 43.4 (C-3), 42.1 (N'-CH₃), 41.6 (N-CH₃), 40.4 (C- α), 38.6 (C- α'), 25.3 (C-4'), 22.0 (C-4); HR-TOF-MS (ESI⁺): m/z 945.3359 [M + H]⁺ (calcd. for C₅₄H₄₉N₄O₁₂, 945.3341).

Cell lines and culture

Cell lines were cultured as previously reported [10]. Briefly, MCF-7, MDA-MB-231, Huh-7, and human embryonic kidney 293T (HEK293T) cells were cultured in Dulbecco's Modified Eagle's medium and Ham's F-12 nutrient mixture (DMEM/F12; Gibco, Thermo Fisher Scientific) with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific), 100 units/ml penicillin and 100 μ g/mL streptomycin at 37 °C under a humidified 5% CO₂ atmosphere. The cells were sub-cultured twice per week by following the standard trypsinization protocol.

Cytotoxicity assay

Cell lines at a density 5×10^3 cells/well were seeded in a 96-well plate for 24 h. Various test compound concentrations were added to the 96-well plate and incubated for 24 hours. Ten microliters of MTT solution (5 mg/ml) were added and incubated for 2 h. Then, cell supernatants were removed, and DMSO was added to dissolve the formazan product. A microplate spectrophotometer measured light absorbent at 570 nm. The IC₅₀ was calculated by using GraphPad Prism version 8. Cisplatin (Sigma) and 0.75% DMSO (RCI Labscan) were used as a positive and negative control.

Declarations

Conflict of interest:

The authors declare no competing interests.

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References

1. Rukachaisirikul T, Kumjun S, Suebsakwong P, Apiratikul N, Suksamrarna A (2019) A new pyrrole alkaloid from the roots of *Cissampelos pareira*. *Nat Prod Res* 35:80–87. <https://doi.org/10.1080/14786419.2019.1614576>
2. Medeiros MAA, Pinho JF, De-Lira DP, Barbosa-Filho JM, Araújo DAM, Cortes SF, Lemos VS, Cruz JS (2011) Curine, a bisbenzylisoquinoline alkaloid, blocks L-type Ca^{2+} channels and decreases intracellular Ca^{2+} transients in A7r5 cells. *Eur J Pharmacol* 669:100–107. <https://doi.org/10.1016/j.ejphar.2011.07.044>
3. Leite FC, Ribeiro-Filho J, Costa HF, Salgado PRR, Calheiros AS, Carneiro AB, Almeida RN, Dias CS, Bozza PT, Piuvezam MR (2014) Curine, an alkaloid Isolated from *Chondrodendron platyphyllum* inhibits prostaglandin E2 in experimental models of inflammation and pain. *Planta Med* 80:1072–1078. <https://doi.org/10.1055/s-0034-1382997>
4. Ribeiro-Filho J, Piuvezam MR, Bozza PT (2015) Anti-allergic properties of curine, a bisbenzylisoquinoline alkaloid. *Molecules* 20:4695–4697. <https://doi.org/10.3390/molecules20034695>
5. Ribeiro-Filho J, Leite FC, Costa HF, Calheiros AS, Torres RC, Azevedo CT, Martins MA, Dias CS, Bozza PT, Piuvezam MR (2014) Curine inhibits mast cell-dependent responses in mice. *J Ethnopharmacol* 155:1118–1124. <https://doi.org/10.1016/j.jep.2014.06.041>
6. Ribeiro-Filho J, Calheiros AS, Vieira-de-Abreu A, Carvalho KIM, Mendes DS, Melo CB, Martins MA, Dias CS, Piuvezam MR, Bozza PT (2013) Curine inhibits eosinophil activation and airway hyper-responsiveness in a mouse model of allergic asthma. *Toxicol Appl Pharmacol* 273:19–26. <https://doi.org/10.1016/j.taap.2013.08.015>
7. Gong S, Xu D, Zou F, Peng R (2017) (–)-Curine induces cell cycle arrest and cell death in hepatocellular carcinoma cells in a p53-independent way. *Biomed Pharmacother* 89:894–901. <http://dx.doi.org/10.1016/j.biopha.2017.01.148>
8. Dantas BB, Faheina-Martins GV, Coulidiati TH, Bomfim CCB, Dias CS, Barbosa-Filho JMB, Araújo DAM (2015) *J Nat Med* 69:218–223. <https://doi.org/10.1007/s11418-014-0881-5>
9. Koike L, Marsaioli AJ, Reis FAM (1981) Proton and carbon-13 nuclear magnetic resonance spectroscopy and conformational aspects of the curine class of bis(benzylisoquinoline) alkaloids. *J Org Chem* 46:2385–2389. <https://doi.org/10.1021/jo00324a036>
10. Chokchaisiri R, Chaichompoo W, Bureekaew S, Thepmalee C, Ganranoo L, Cheenpracha S, Suksamrarn A (2024) A new oligostilbenoid isolated from the stems of *Ochna integerrima*.

Scheme

Schemes 1 and 2 are available in the Supplementary Files section

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