



Research article

Synthesis and cytotoxic evaluation of amide derivatives of gambogic acid

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ABSTRACT

Gambogic acid (GA), a prominent cage-xanthonoid isolated from the resin of *Garcinia hanburyi*, has demonstrated significant pharmacological potential as a potent anticancer agent. Despite its efficacy, the clinical application of GA is constrained by its inherent lipophilicity, poor aqueous solubility, and non-selective toxicity toward normal cells. To address these challenges, a series of eight amide derivatives (**3a-3h**) were designed and synthesized through structural modification at the C-30 carboxyl group. The synthesis was performed under mild PyBOP/DIPEA-mediated coupling conditions, which provided superior selectivity and higher yields (66-85%) compared to other common coupling reagents. The chemical structures of the resulting analogues were elucidated using ¹H and ¹³C NMR spectroscopy. Evaluation of cytotoxic activity against human gastric carcinoma (HGC-27) and hepatocellular carcinoma (Hep-G2) cell lines via the MTT assay revealed that several derivatives exhibited enhanced potency compared to the parent compound. Notably, compounds **3h** (morpholinyl), **3f** (cyclohexyl), and **3b** (furfuryl) emerged as the most effective candidates against HGC-27 cells with IC₅₀ values of 11.63, 12.84, and 13.82 μM, respectively. These results suggest that the combination of amide moieties at the C-30 position can effectively modulate the electronic, steric, and lipophilic properties of the GA scaffold, thereby optimizing its anticancer performance. These findings establish therapeutic strategy for the further development of GA-based lead structures in targeted cancer therapy.

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INTRODUCTION

Gambogic acid (GA, Figure 1), a naturally occurring cage-xanthonoid derived from the resin of *Garcinia hanburyi* (*Clusiaceae*), a native plant of Southeast Asia, has attracted significant attention due to its potent anticancer properties and broad spectrum of anticancer properties [1]. These include the induction of apoptosis and autophagy, cell cycle arrest, and the suppression of tumor invasion, metastasis, and angiogenesis [2]. GA has demonstrated inhibitory effects on the proliferation of various cancer cell types, such as breast, pancreatic, prostate, lung cancers, and osteosarcoma, among others [3-5]. The underlying mechanisms are believed to involve the modulation of multiple cellular signaling pathways, including c-Jun N-terminal kinase (JNK-1), protein kinase B (AKT)/mammalian target of rapamycin (mTOR), AKT/forkhead box protein O1 (FOXO1)/BIM, and nuclear factor kappa B (NF- κ B) pathways [6, 7]. Despite its promising pharmacological potential, the clinical application of GA is limited by poor solubility, low bioavailability, and inherent cytotoxicity toward normal cells. To address these challenges and optimize its biological performance, structural modification of GA has emerged as an effective strategy. In particular, amide derivatization at the C-30 carboxyl group allows modulation of chemical stability, hydrogen-bonding interactions, and steric and electronic properties, thereby facilitating structure-activity relationship exploration rather than solely aiming to enhance aqueous solubility. Among various approaches, the synthesis of amide derivatives represents a particularly valuable route, as amide bond formation can enhance molecular stability, modulate lipophilicity, and influence target selectivity [8, 9].

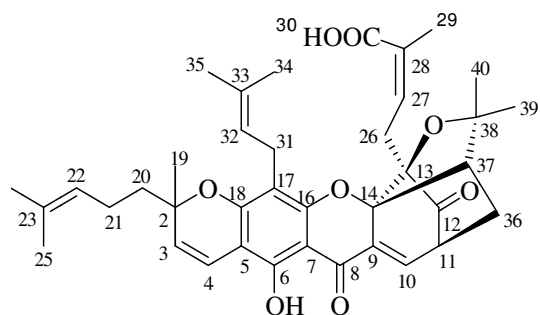


Figure 1. Chemical structure of gambogic acid

In this study, a series of novel amide derivatives of gambogic acid were designed and synthesized to investigate the structure-activity relationship and improve anticancer potency. The synthesized compounds were evaluated for their cytotoxic effects against a two human cancer cell lines (HGC-27 and Hep-G2), aiming to identify derivatives with enhanced selectivity and reduced toxicity.

MATERIALS AND METHODS

General experimental procedures

Provide NMR spectra were recorded on a Bruker Advance 600 ad 500 MHz spectrometer (Germany). Chemical shifts are reported in δ (ppm) with tetramethylsilane (TMS) as an internal reference and coupling constants (J) are given in Hertz (Hz). Column chromatography (CC) was carried out on silica gel 60 (Merck, 5-40 μ m), silica gel 100 (Merck, 63-200 μ m), Sephadex LH-20 (GE Healthcare), and C₁₈-reversed-phase silica gel (RP-18, Merck, 15-25 μ m). Thin-layer chromatography (TLC) was performed on silica gel 60 coated plates F254 (Merck). Visualization of TLC plates was performed under UV light (254 and 365 nm), staining with 10 % H₂SO₄ solution. Chemical reagents and solvents were purchased from Merck. Commercial solvents were distilled and dried, when necessary, by standard methods just prior to use.

Isolation of gambogic acid

The dried resin of *Garcinia hanburyi* (1 kg) collected from Phu Quoc island was cut into small pieces, ground in to a fine powder, and then dried in an oven at 50°C for 24 h. The resulting powder was extracted five times with ethyl acetate (EtOAc, 1L x5) at room temperature for 1h using ultrasound assistance. The filtrates were combined, filtered and evaporated under reduced pressure to obtain the crude extract as an orange powder (121 g).

A pyridine-water solution (85:15, v/v) was prepared by mixing 85 mL of pyridine with 15 mL of distilled water in an Erlenmeyer flask. Subsequently, 20 g of the *Garcinia hanburyi* resin extract was dissolved in 44.6 mL of the prepared pyridine-water solution. The mixture was stirred for 10 minutes at 60°C in a water bath to ensure complete dissolution. After stirring, the solution was allowed to cool to room temperature, then left to stand at 4°C for 24 hours to promote crystallization. The resulting crystals were collected by vacuum filtration using a Büchner funnel and washed with *n*-hexane to remove nonpolar impurities. The crude product was then recrystallized twice by dissolving it in 18 mL of the pyridine-water solution at 60°C, followed by standing at 4°C for 24 hours to allow crystallization. The crystals were collected, washed with cold acetone, and dried thoroughly under ambient conditions.

For further purification, the dried crystals were dissolved in 57 mL of diethyl ether and transferred to a separatory funnel. An equal volume (57 mL) of 15% aqueous hydrochloric acid was added, and the mixture was shaken to separate the organic and aqueous layers. This extraction step was repeated twice to ensure complete removal of

pyridine. The combined organic layers were then dried over anhydrous sodium sulfate (Na₂SO₄), filtered, and concentrated under reduced pressure. The final product, purified gambogic acid (1.45 g), was obtained from crystallization from acetone.

Gambogic acid (1): orange crystals. ¹H NMR (CDCl₃, 500 MHz) δ (ppm): 12.77 (s, 1H, OH-6), 7.55 (d, J = 7.0 Hz, 1H, H-10), 6.60 (d, J = 10.0 Hz, 1H, H-4), 6.09 (t, J = 7.5 Hz, 1H, H-27), 5.38 (d, J = 10.0 Hz, 1H, H-3), 5.04 (m, 2H, H-22, H-32), 3.47 (m, 1H, H-11), 3.29 (dd, J = 8.0, 15.0 Hz, 1H, H-31), 3.14 (dd, J = 5.0, 15.0 Hz, 1H, H-31), 2.95 (dd, J = 8.0, 15.5 Hz, 1H, H-26), 2.51 (d, J = 9.0 Hz, 1H, H-37), 2.31 (dd, J = 5.0, 13.5 Hz, 1H, H-36), 2.01 (m, 1H, H-21), 1.76 (m, 1H, H-20), 1.74 (s, 3H, H-30), 1.72 (s, 3H, H-34), 1.69 (s, 3H, H-39), 1.64 (s, 3H, H-24), 1.62 (s, 3H, H-35), 1.59 (m, 1H, H-20), 1.55 (s, 3H, H-25), 1.38 (s, 3H, H-19), 1.35 (m, 1H, H-36), 1.29 (s, 3H, H-40). ¹³C NMR (CDCl₃, 125 MHz) δ (ppm): 203.3 (C-12, C=O), 178.9 (C-8), 170.2 (C-30), 161.5 (C-6), 157.6 (C-16), 157.4 (C-18), 137.8 (C-27), 135.3 (C-10), 133.4 (C-9), 131.8 (C-23), 131.5 (C-33), 127.8 (C-28), 124.5 (C-3), 123.8 (C-22), 122.3 (C-32), 115.9 (C-4), 107.6 (C-17), 102.8 (C-5), 100.5 (C-7), 90.9 (C-14), 84.1 (C-38), 83.8 (C-13), 81.3 (C-2), 49.0 (C-37), 46.8 (C-11), 42.0 (C-20), 29.9 (C-39), 29.3 (C-26), 28.8 (C-40), 27.7 (C-19), 25.6 (C-24, C-35), 25.2 (C-36), 22.7 (C-21), 21.6 (C-31), 20.7 (C-29), 18.2 (C-34), 17.6 (C-25).

General protocol for the preparation of amide derivatives

To a solution of gambogic acid **1** (100 mg, 0.16 mmol, 1 eq.) in 7 mL of DCM were successively added PyBop (0.024 mmol, 1.5 eq.) and DIPEA (0.15 mmol, 0.6 eq.). After stirring for 30 minutes at room temperature, the corresponding amine **2a-2h** (0.19 mmol,

1.2 eq.), dissolved in 2 mL of DCM, was added. After 48 hours of reaction, the mixture was washed with 1 M HCl solution and then with a saturated NaCl solution. The organic phase was dried over MgSO_4 and evaporated under reduced pressure. The crude residue was then purified by silica gel chromatography (elution: *n*-hexane/acetone: 15/1) to afford the amide **3a-3h**.

***t*-Butyl gambogamide (3a):** Yield 72%. Orange oil. ^1H NMR (600 MHz, CDCl_3) δ_{H} (ppm): 12.85 (s, 1H, OH-6), 7.56 (d, $J = 6.6$ Hz, 1H, H-10), 6.69 (d, $J = 10.2$ Hz, 1H, H-4), 6.49 (brs, 1H, NH), 5.46 (d, $J = 10.2$ Hz, 1H, H-3), 5.18 (m, 1H, H-27), 5.05 (m, 2H, H-22, H-32), 3.46 (m, 1H, H-11), 3.31 (m, 1H, H-31), 3.20 (m, 1H, H-31), 2.74 (dd, $J = 9.6, 15.6$ Hz, 1H, H-26), 2.52 (d, $J = 9.6$ Hz, H-37), 2.30 (m, 1H, H-36), 2.19 (dd, $J = 7.2, 15.0$ Hz, 1H, H-26), 2.05 (m, 2H, H-21), 1.80 (m, 1H, H-20), 1.78 (s, 3H, H-29), 1.73 (s, 3H, H-34), 1.69 (s, 3H, H-39), 1.66 (s, 3H, H-24), 1.64 (s, 3H, H-35), 1.60 (m, 1H, H-20), 1.56 (s, 3H, H-25), 1.44 (s, 3H, H-19), 1.35 (s, 9H, 3Me-C), 1.30 (m, 1H, H-36), 1.28 (s, 3H, H-40). ^{13}C NMR (150 MHz, CDCl_3) δ_{C} (ppm): 203.8 (C-12), 175.5 (C-8), 168.7 (C-30), 161.9 (C-6), 157.6 (C-16), 157.2 (C-18), 137.3 (C-27), 135.5 (C-10), 133.3 (C-9), 131.9 (C-23), 131.7 (C-33), 124.8 (C-3), 123.8 (C-22), 122.5 (C-28), 122.2 (C-32), 115.9 (C-4), 107.8 (C-17), 102.9 (C-5), 100.0 (C-7), 91.3 (C-14), 84.1 (C-13), 83.6 (C-38), 81.5 (C-2), 51.4 (C-Me₃), 49.1 (C-37), 46.9 (C-11), 42.1 (C-20), 29.7 (C-39), 29.1 (C-26), 28.9 (C-40), 28.7 (Me₃), 27.9 (C-19), 25.6 (C-24, C-35), 25.2 (C-36), 22.7 (C-21), 21.6 (C-31), 21.1 (C-29), 18.1 (C-34), 17.6 (C-25).

Furfuryl gambogamide (3b): Yield 78%. Orange oil. ^1H NMR (600 MHz, CDCl_3) δ_{H} (ppm): 12.84 (s, 1H, OH-6), 7.53 (d, $J = 6.6$

Hz, 1H, H-10), 7.33 (dd, $J = 1.8, 1.2$ Hz, 1H, furfuryl), 6.90 (t, $J = 5.4$ Hz, 1H, NH), 6.68 (d, $J = 10.2$ Hz, 1H, H-4), 6.30 (dd, $J = 1.8, 3.0$ Hz, 1H, furfuryl), 6.20 (d, $J = 2.4$ Hz, 1H, furfuryl), 5.45 (d, $J = 10.2$ Hz, 1H, H-3), 5.38 (m, 1H, H-27), 5.05 (m, 2H, H-22, H-32), 4.46 (dd, $J = 5.4, 15.6$ Hz, 1H, furfuryl), 4.35 (dd, $J = 5.4, 15.6$ Hz, 1H, furfuryl), 3.3 (m, 1H, H-11), 3.32 (m, 1H, H-31), 3.19 (m, 1H, H-31), 2.60 (dd, $J = 8.4, 24.0$ Hz, 1H, H-26), 2.51 (d, $J = 9.6$ Hz, H-37), 2.43 (dd, $J = 8.4, 15.6$ Hz, 1H, H-26), 2.30 (m, 1H, H-36), 2.04 (m, 2H, H-21), 1.81 (s, 3H, H-29), 1.79 (m, 1H, H-20), 1.73 (s, 3H, H-34), 1.65 (s, 9H, H-24, 35, 39), 1.60 (m, 1H, H-20), 1.55 (s, 3H, H-25), 1.43 (s, 3H, H-19), 1.36 (m, 1H, H-36), 1.22 (s, 3H, H-40). ^{13}C NMR (150 MHz, CDCl_3) δ_{C} (ppm): 203.7 (C-12), 178.8 (C-8), 168.8 (C-30), 161.8, (C-6), 157.7 (C-16), 157.3 (C-18), 151.6 (C, furfuryl), 142.0 (CH, furfuryl), 135.7 (C-10), 135.1 (C-27), 133.2 (C-9), 131.9 (C-23), 131.8 (C-33), 124.9 (C-28), 124.8 (C-3), 123.8 (C-22), 122.1 (C-32), 115.8 (C-4), 110.4 (CH, furfuryl), 107.8 (C-17), 107.3 (CH, furfuryl), 102.8 (C-5), 100.4 (C-7), 91.1 (C-14), 84.0 (C-38), 83.6 (C-13), 81.5 (C-2), 49.0 (C-37), 47.0 (C-11), 42.1 (C-20), 36.1 (CH₂, furfuryl), 29.9 (C-39), 28.9 (C-40), 28.8 (C-26), 27.9 (C-19), 25.7 (C-24, C-35), 25.3 (C-36), 22.7 (C-21), 21.7 (C-31), 21.2 (C-29), 18.1 (C-34), 17.6 (C-25).

(S)-Methylbenzyl gambogamide (3c): Yield 77%. Orange oil. ^1H NMR (600 MHz, CDCl_3) δ_{H} (ppm): 12.85 (s, 1H, OH-6), 7.42 (d, $J = 6.6$ Hz, 1H, H-10), 7.31-7.37 (m, 4H, 4CH aromatic), 6.92 (d, $J = 7.8$ Hz, 1H, NH), 6.68 (d, $J = 10.2$ Hz, 1H, H-4), 5.45 (d, $J = 10.2$ Hz, 1H, H-3), 5.30 (m, 1H, H-27), 5.05 (m, 2H, H-22, H-32), 5.02 (m, 1H, CH-NH), 3.39 (m, 1H, H-11), 3.30 (dd, $J = 7.8, 14.4$ Hz, 1H, H-31), 3.19 (dd, $J = 6.0, 14.4$ Hz, 1H,

H-31), 2.61 (dd, $J = 8.4, 15.0$ Hz, 1H, H-26), 2.52 (d, $J = 9.0$ Hz, H-37), 2.35 (dd, $J = 7.8, 15.0$ Hz, 1H, H-36), 2.29 (dd, $J = 4.8, 13.8$ Hz, 1H, H-26), 2.05 (m, 2H, H-21), 1.79 (m, 1H, H-20), 1.78 (s, 3H, H-29), 1.73 (s, 3H, H-34), 1.69 (s, 3H, H-39), 1.66 (s, 3H, H-24), 1.64 (s, 3H, H-35), 1.60 (m, 1H, H-20), 1.56 (s, 3H, H-25), 1.48 (d, $J = 7.2$ Hz, 3H, Me-CH), 1.42 (s, 3H, H-19), 1.38 (m, 1H, H-36), 1.26 (s, 3H, H-40). ^{13}C NMR (150 MHz, CDCl_3) δ_{C} (ppm): 204.3 (C-12), 178.8 (C-8), 168.2 (C-30), 161.7 (C-6), 157.7 (C-16), 157.3 (C-18), 143.7 (C aromatic), 135.6 (C-27), 135.5 (C-10), 133.2 (C-9), 131.9 (C-23), 131.8 (C-33), 128.5 (2CH aromatic), 127.1 (1CH aromatic), 126.6 (2CH aromatic), 124.8 (C-3), 124.0 (C-22), 123.8 (C-28), 122.1 (C-32), 115.9 (C-4), 107.8 (C-17), 102.9 (C-5), 100.4 (C-7), 91.0 (C-14), 84.0 (C-13), 83.5 (C-38), 81.5 (C-2), 49.2 (CH-NH), 49.1 (C-37), 47.0 (C-11), 42.1 (C-20), 30.0 (C-39), 28.9 (C-40, C-26), 27.8 (C-19), 25.6 & 25.7 (C-24, C-35), 25.2 (C-36), 22.8 (C-21), 22.0 (Me), 21.6 (C-31), 21.3 (C-29), 18.1 (C-34), 17.6 (C-25).

4-Chlorobenzyl gambogamide (3d): Yield 68%. Orange oil. ^1H NMR (600 MHz, CDCl_3) δ_{H} (ppm): 12.84 (s, 1H, OH-6), 7.51 (d, $J = 6.6$ Hz, 1H, H-10), 7.29 (d, $J = 8.4$ Hz, 2H, H aromatic), 7.22 (d, $J = 8.4$ Hz, 2H, H aromatic), 6.95 (t, $J = 6.0$ Hz, 1H, NH), 6.67 (d, $J = 10.2$ Hz, 1H, H-4), 5.46 (d, $J = 10.2$ Hz, 1H, H-3), 5.33 (m, 1H, H-27), 5.05 (m, 2H, H-22, H-32), 4.43 (dd, $J = 6.0, 15.0$ Hz, 1H, $\text{CH}_2\text{-N}$), 4.32 (dd, $J = 5.4, 15.0$ Hz, 1H, $\text{CH}_2\text{-N}$), 3.43 (m, 1H, H-11), 3.32 (m, 1H, H-31), 3.17 (m, 1H, H-31), 2.63 (dd, $J = 9.0, 15.6$ Hz, 1H, H-26), 2.50 (d, $J = 9.6$ Hz, H-37), 2.39 (dd, $J = 8.4, 15.0$ Hz, 1H, H-26), 2.28 (dd, $J = 4.8, 13.2$ Hz, 1H, H-36), 2.04 (m, 2H, H-21), 1.81 (s, 3H, H-29), 1.79 (m, 1H, H-20), 1.73 (s, 3H, H-34), 1.65 (s, 3H, H-

24), 1.64 (s, 3H, H-35), 1.60 (m, 1H, H-20), 1.59 (s, 3H, H-39), 1.53 (s, 3H, H-25), 1.41 (s, 3H, H-19), 1.37 (m, 1H, H-36), 1.11 (s, 3H, H-40). ^{13}C NMR (150 MHz, CDCl_3) δ_{C} (ppm): 203.7 (C-12), 178.8 (C-8), 168.9 (C-30), 161.8 (C-6), 157.7 (C-16), 157.3 (C-18), 137.0 (C aromatic), 135.7 (C-27), 135.3 (C-10), 133.1 (C-9, C-aromatic), 131.9 (C-23), 131.8 (C-33), 129.5 & 128.7 (4CH aromatic), 124.9 (C-3), 124.7 (C-28), 123.7 (C-22), 122.0 (C-32), 115.8 (C-4), 107.8 (C-17), 102.9 (C-5), 100.4 (C-7), 91.1 (C-14), 84.0 (C-13), 83.6 (C-38), 81.6 (C-2), 49.0 (C-37), 46.9 (C-11), 42.7 (CH₂-N), 42.1 (C-20), 29.9 (C-39), 29.1 (C-26), 28.7 (C-40), 27.9 (C-19), 25.7 & 25.6 (C-24, C-35), 25.2 (C-36), 22.7 (C-21), 21.7 (C-31), 21.2 (C-29), 18.1 (C-34), 17.6 (C-25).

3,4-Dichloro benzyl gambogamide (3e): Yield 66%. Orange oil. ^1H NMR (600 MHz, CDCl_3) δ_{H} (ppm): 12.84 (s, 1H, OH-6), 7.53 (d, $J = 7.2$ Hz, 1H, H-10), 7.11 (m, 2H, 2H aromatic), 7.03 (m, 2H, 1H aromatic & NH), 6.66 (d, $J = 10.2$ Hz, 1H, H-4), 5.45 (d, $J = 10.2$ Hz, 1H, H-3), 5.33 (m, 1H, H-27), 5.05 (m, 1H, H-22), 5.03 (m, 1H, H-32), 4.42 (dd, $J = 5.4, 15.0$ Hz, 1H, CH_2 benzylic), 4.31 (dd, $J = 5.4, 15.0$ Hz, 1H, CH_2 benzylic), 3.45 (m, 1H, H-11), 3.31 (dd, $J = 7.8, 13.2$ Hz, 1H, H-31), 3.18 (dd, $J = 5.4, 15.0$ Hz, 1H, H-31), 2.64 (dd, $J = 8.4, 15.6$ Hz, 1H, H-26), 2.51 (d, $J = 9.0$ Hz, H-37), 2.38 (dd, $J = 9.6, 15.0$ Hz, 1H, H-26), 2.32 (m, 1H, H-36), 2.28 (dd, $J = 4.8, 13.8$ Hz, 1H, H-26), 2.03 (m, 2H, H-21), 1.82 (s, 3H, H-29), 1.78 (m, 1H, H-20), 1.73 (s, 3H, H-34), 1.65 (s, 3H, H-35), 1.64 (s, 3H, H-24), 1.60 (s, 3H, H-39), 1.60 (m, 1H, H-20), 1.55 (s, 3H, H-25), 1.42 (s, 3H, H-19), 1.37 (m, 1H, H-36), 1.14 (s, 3H, H-40). ^{13}C NMR (150 MHz, CDCl_3) δ_{C} (ppm): 203.7 (C-12), 178.8 (C-8), 169.0 (C-30), 161.8 (C-6), 157.7 (C-16), 157.2 (C-18),

149.5 (C aromatic), 135.7 (C-27), 135.4 (C-10), 133.2 (C-9), 131.9 (C-23), 131.88 (C-33), 124.9 (C-3), 124.8 (C-28), 124.0 (2C aromatic), 123.7 (C-22), 122.0 (C-32), 117.3, 117.2 & 117.1 (3C aromatic), 115.7 (C-4), 107.8 (C-17), 102.9 (C-5), 100.4 (C-7), 91.1 (C-14), 84.1 (C-13), 83.6 (C-38), 81.6 (C-2), 53.4, 49.0 (C-37), 46.9 (C-11), 42.5 (C-20), 42.1 (CH₂ benzylic), 29.9 (C-39), 29.1 (C-26), 28.7 (C-40), 27.9 (C-19), 25.7 (C-24), 25.6 (C-35), 25.2 (C-36), 22.7 (C-21), 21.7 (C-31), 21.2 (C-29), 18.1 (C-34), 17.6 (C-25).

Cyclohexyl gambogamide (3f): Yield 85%. Orange oil. ¹H NMR (600 MHz, CDCl₃) δ_H (ppm): 12.84 (s, 1H, OH-6), 7.55 (d, *J* = 6.6 Hz, 1H, H-10), 6.68 (d, *J* = 10.2 Hz, 1H, H-4), 6.44 (d, *J* = 7.8 Hz, 1H, NH), 5.46 (d, *J* = 10.2 Hz, 1H, H-3), 5.24 (m, 1H, H-27), 5.04 (m, 2H, H-22, H-32), 3.71 (m, 1H, CH, cyclohexyl), 3.47 (m, 1H, H-11), 3.32 (dd, *J* = 7.8, 15.0 Hz 1H, H-31), 3.20 (m, 1H, H-31), 2.69 (dd, *J* = 8.4, 15.6 Hz, 1H, H-26), 2.53 (d, *J* = 9.6 Hz, H-37), 2.32 (m, 1H, H-36), 2.29 (dd, *J* = 7.2, 15.6 Hz, 1H, H-26), 2.05 (m, 2H, H-21), 1.90 (m, 2H, CH₂, cyclohexyl), 1.80 (m, 1H, H-20), 1.78 (s, 3H, H-29), 1.73 (s, 3H, H-34), 1.69 (s, 3H, H-39), 1.67 (s, 3H, H-24), 1.65 (s, 3H, H-35), 1.61 (m, 1H, H-20), 1.56 (s, 3H, H-25), 1.44 (s, 3H, H-19), 1.41-1.30 (m, 6H, 3CH₂, cyclohexyl), 1.30 (s, 3H, H-40), 1.16 (m, 2H, CH₂, cyclohexyl), 1.15 (m, 1H, H-36). ¹³C NMR (150 MHz, CDCl₃) δ_C (ppm): 203.9 (C-12), 178.8 (C-8), 168.1 (C-30), 161.8 (C-6), 157.8 (C-16), 157.3 (C-18), 136.2 (C-27), 135.5 (C-10), 133.3 (C-9), 131.9 (C-23), 131.8 (C-33), 124.9 (C-3), 123.8 (C-22), 123.3 (C-28), 122.1 (C-32), 115.8 (C-4), 107.9 (C-17), 102.9 (C-5), 100.4 (C-7), 91.2 (C-14), 84.0 (C-13), 83.5 (C-38), 81.5 (C-2), 49.1 (C-37), 48.2 (CH, cyclohexyl), 47.0 (C-11), 42.1 (C-20), 33.1 & 32.9 (2CH₂,

cyclohexyl), 29.7 (C-39), 29.0 (C-26), 28.9 (C-40), 27.8 (C-19), 25.7 & 25.6 (C-24, C-35), 25.2 (C-36), 25.0 (2CH₂, cyclohexyl), 23.0 (CH₂, cyclohexyl), 22.7 (C-21), 21.6 (C-31), 21.2 (C-29), 18.1 (C-34), 17.6 (C-25).

Cyclohexanemethyl gambogamide (3g): Yield 72%. Orange oil. ¹H NMR (600 MHz, CDCl₃) δ_H (ppm): 12.83 (s, 1H, OH-6), 7.53 (d, *J* = 7.2 Hz, 1H, H-10), 6.67 (d, *J* = 10.2 Hz, 1H, H-4), 5.99 (m, 1H, H-27), 5.43 (d, *J* = 10.2 Hz, 1H, H-3), 5.05 (m, 2H, H-22, H-32), 3.68 (m, 1H, CH₂-N), 3.61 m (1H, CH₂-N), 3.46 (m, 1H, H-11), 3.29 (dd, *J* = 7.8, 15.0 Hz 1H, H-31), 3.18 (m, 1H, H-31), 2.97-3.02 (m, 2H, H-26), 2.51 (d, *J* = 9.6 Hz, H-37), 2.30 (m, 1H, H-36), 2.05 (m, 2H, H-21), 1.90 (m, 2H, CH₂, cyclohexyl), 1.78 (m, 1H, H-20), 1.75 (overlapped, 2H, cyclohexyl), 1.75 (s, 3H, H-34), 1.69 (s, 6H, H-29, H-39), 1.69-1.55 (overlapped, 4H, CH₂, cyclohexyl), 1.65 (s, 3H, H-24), 1.64 (s, 3H, H-35), 1.60 (overlapped, 1H, H-20), 1.55 (s, 3H, H-25), 1.49 (m, 1H, CH, cyclohexyl), 1.44 (s, 3H, H-19), 1.35 (m, 1H, H-36), 1.25 (s, 3H, H-40), 1.15 (m, 2H, CH₂, cyclohexyl), 0.8-0.9 (m, 2H, CH₂, cyclohexyl). ¹³C NMR (150 MHz, CDCl₃) δ_C (ppm): 203.6 (C-12), 179.0 (C-8), 167.0 (C-30), 161.3 (C-6), 157.6 (C-16, C-18), 136.1 (C-27), 135.0 (C-10), 133.6 (C-9), 131.9 (C-23), 131.5 (C-33), 128.1 (C-28), 124.4 (C-3), 123.8 (C-22), 122.4 (C-32), 116.1 (C-4), 107.6 (C-17), 102.5 (C-5), 100.5 (C-7), 91.0 (C-14), 83.7 (C-13, C-38), 81.2 (C-2), 69.3 (CH₂-N), 49.1 (C-37), 46.9 (C-11), 42.1 (C-20), 30.0 (C-39), 29.7 (2CH₂, cyclohexyl), 29.6 (C-26), 29.2 (C-40), 27.9 (C-19), 26.4 & 25.9 (3CH₂, cyclohexyl), 25.7 (C-24, C-35), 25.2 (C-36), 22.7 (C-21), 21.7 (C-31), 20.8 (C-29), 18.1 (C-34), 17.6 (C-25).

Morpholinyl gambogamide (3h): Yield 81%. Orange oil. ¹H NMR (500 MHz; CDCl₃) δ (ppm): 12.85 (s, 1H, OH-6), 7.55

(d, $J = 7.0$ Hz, 1H, H-10), 6.68 (d, $J = 10.0$ Hz, 1H, H-4), 5.45 (d, $J = 10.0$ Hz, 1H, H-3), 5.43 (overlapped, 1H, H-27), 5.09 (m, 2H, H-22, H-32), 3.63-3.22 (m, 8H, 4CH₂ morpholine), 3.45 (overlapped, 1H, H-11), 3.29 (overlapped, 2H, C-31), 2.51 (d, $J = 9.5$ Hz, 1H, H-37), 2.39 (dd, $J = 15.0, 6.0$ Hz, 1H, H-26), 2.29 (dd, $J = 13.5, 4.5$ Hz, 1H, H-36), 2.25 (dd, $J = 15.0, 7.0$ Hz, 1H, H-26), 2.06 (m, 2H, H-21), 1.79 (m, 1H, H-20), 1.74 (s, 6H, H-34, H-39), 1.68 (s, 3H, H-29), 1.65 (s, 6H, H-24, H-35), 1.62 (m, 1H, H-20), 1.56 (s, 3H, H-25), 1.44 (s, 3H, H-19), 1.36 (dd, 1H, $J = 13.5, 9.5$ Hz, H-36), 1.25 (s, 3H, H-40). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 203.4 (C-8), 179.1 (C-12), 169.7 (C-30), 161.7 (C-6), 157.8 (C-16), 157.5 (C-18), 135.5 (C-10), 133.2 (C-27), 133.1 (C-9), 131.9 (C-23), 131.6 (C-33), 124.7 (C-3), 123.8 (C-22), 122.6 (C-28), 122.2 (C-32), 115.9 (C-4), 107.6 (C-17), 102.8 (C-5), 100.5 (C-7), 91.1 (C-14), 83.6 (C-13), 82.9 (C-38), 81.4 (C-2), 67.2, 66.8 (4C morpholine), 49.0 (C-37), 47.0 (C-11), 42.1 (C-20), 41.3, 30.1 (C-39), 29.4 (C-26), 28.7 (C-40), 27.9 (C-19), 25.7 & 25.6 (C-24, C-35), 25.4 (C-36), 22.8 (C-21), 21.7 (C-31), 20.8 (C-29), 18.1 (C-34), 17.6 (C-25).

Cytotoxic assay

The cytotoxic activity assay against cancer cell lines was performed following the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] modified method described by Mosman [10-12]. The cytotoxicity assays were conducted at the Experimental Biology Laboratory - Institute of Chemistry - Vietnam Academy of Science and Technology. Ellipticine was used as a positive control. The results were measured using an Tecan Spark reader (Switzerland) at a wavelength range of 540/720 nm. Cell lines [human stomach carcinoma cell (HGC-27).

hepatocellular carcinoma (Hep-G2) and Vero cell (kidney cell, african green monkey) were provided by ATCC (American Type Culture Collection, USA) and CLS (Cell Lines Service GmbH, Germany).

Cells were cultured at 37°C with 5% CO₂ in suitable media: DMEM (Dulbecco's Modified Eagle Medium), EMEM (Eagle's Minimum Essential Medium, Sigma-Aldrich, USA), or RPMI 1640 (ThermoFisher, Waltham, Germany), supplemented with 2 mM L-glutamine, antibiotics (Penicillin + Streptomycin sulfate), and 5–10% fetal bovine serum. The cell suspension was added to 96-well microplates (1.5 × 10⁵ cells/well) and incubated with test samples at concentrations ranging from 128 → 8 µg/mL (for 48 hours). Each concentration tested in triplicate. Ellipticine was used as a positive control, and dimethyl sulfoxide (DMSO ≤ 1%; v/v) served as a negative control. Samples showing activity (% inhibition ≥ 50%) were further analyzed to determine the IC₅₀ value (µM), the concentration of the test sample that inhibits 50% of cell viability using TableCurve AISN Software (Jandel Scientific, San Rafael, CA, USA).

Statistical analysis

All experiments were performed at least in triplicate. IC₅₀ values were calculated by regression analysis of dose-response data. Data are presented as mean ± standard deviation (SD) of three independent experiments. Correlations and comparisons between methods were evaluated using appropriate statistical tests with a significance level of $P < 0.05$.

RESULTS AND DISCUSSIONS

RESULTS

The starting material, gambogic acid (GA), was isolated from the *Garcinia hanburyi* resin

collected in Phu Quoc island using a pyridine-water recrystallization method [13], which afforded highly pure GA suitable for subsequent structural modification. The synthesis of amide derivatives was conducted under mild coupling conditions using PyBOP/DIPEA as the activating system. Other coupling reagents such as EDC/DMAP, DCC/DMAP, and oxalyl chloride were also tested, but these reactions resulted in lower yields and partial isomerization at the C-28 position, indicating that PyBOP provides a more efficient and selective activation of the carboxyl group in GA.

Eight amide derivatives (**3a–3h**) were obtained in moderate to good yields (66–85%) through the reaction of GA with various primary and secondary amines, including *t*-butylamine (**2a**), furfurylamine (**2b**), (*S*)- α -methylbenzylamine (**2c**), 4-chlorobenzylamine (**2d**), 3,4-dichlorobenzylamine (**2e**), cyclohexylamine (**2f**), cyclohexanemethylamine (**2g**), and morpholine (**2h**) (Figure 2). The structures of these derivatives were confirmed by NMR spectra, showing the

expected chemical shifts and coupling patterns characteristic of the amide bond formation at the C-30 position.

The cytotoxic activities of compounds **3a–3h** and gambogic acid (**1**) were evaluated against two human cancer cell lines. HGC-27 (gastric carcinoma) and Hep-G2 (hepatocellular carcinoma), as well as the normal Vero cell line. The obtained IC₅₀ values (Table 1) indicate that the synthesized compounds exhibited moderate to good anticancer activity, with IC₅₀ values ranging from 11.63 to 57.43 μ M across the tested cell lines.

Among the tested derivatives, **3h** and **3f** displayed the most potent cytotoxic effects against HGC-27 cells, while **3b** and **3a** showed comparable activity with IC₅₀ values in the low micromolar range. These compounds showed stronger activity toward HGC-27 cells than HepG2 cells, suggesting a degree of cell line selectivity possibly related to differences in cellular uptake or target expression. On the other hand, **3d** exhibited better inhibition of Hep-G2 cells (IC₅₀ = 24.92 μ M), highlighting that specific

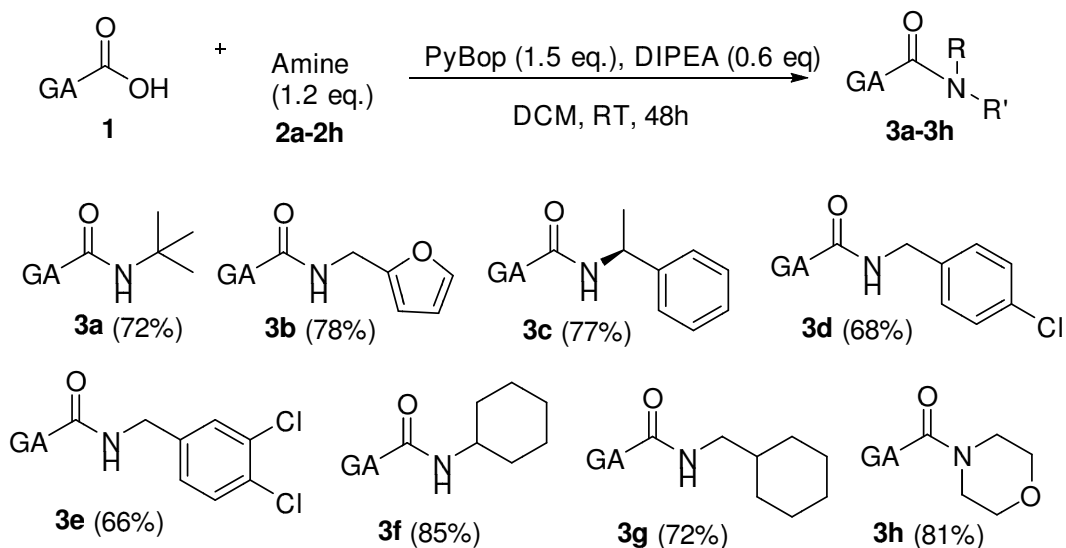


Figure 2. Synthesis of gambogamide **3a–3h**

structural modifications may favor activity against liver carcinoma.

DISCUSSIONS

These data revealed a consistent pattern: derivatives with balanced hydrophobic–hydrophilic properties and potential for hydrogen bonding (e.g., morpholine, cyclohexyl, *t*-butyl) exhibit higher activity and possibly better selectivity compared to GA itself. This suggests that both steric bulk and polarity at the amide nitrogen are crucial determinants of cytotoxic potency. Overall, modifying GA at the C-30 carboxyl position provides a viable route to optimize anticancer performance through modulation of electronic, steric, and lipophilic properties. Gambogic acid (GA) is limited by poor aqueous solubility and low bioavailability, primarily due to its rigid polycyclic xanthone core and high intrinsic lipophilicity. Amide formation at the C-30 carboxyl group provides a targeted structural modification to modulate these physicochemical constraints. Incorporation of an amide functionality

increases hydrogen-bond donor and acceptor capacity and alters molecular polarity, which can improve solvation and influence passive membrane transport. Furthermore, the electronic and steric characteristics of the amine substituent contribute to fine-tuning these effects. Polar or heterocyclic moieties, such as the morpholine group in compound **3h**, may enhance aqueous solubility through partial protonation under physiological conditions, whereas moderately lipophilic substituents (e.g., **3a** and **3f**) may maintain membrane permeability while reducing excessive hydrophobicity. Although solubility and pharmacokinetic parameters were not directly evaluated, the improved cytotoxic activity observed for several amide derivatives supports the hypothesis that C-30 amide modification favorably impacts physicochemical properties relevant to bioavailability.

CONCLUSION

This study successfully synthesized eight amide derivatives (**3a–3h**) of gambogic acid, among which compounds **3a–3g** are newly reported, using efficient PyBOP/DIPEA-mediated coupling reactions. Biological evaluation demonstrated that several derivatives exhibited enhanced cytotoxic activity against human gastric and liver carcinoma cell lines compared to the parent compound. The most promising candidates, **3h** (morpholinyl) and **3f** (cyclohexyl), showed the strongest potency and selective cytotoxicity toward cancer cells over normal Vero cells, while derivatives **3a** (*t*-butyl) and **3b** (furfuryl) displayed comparable activity in the low micromolar range. These findings establish a foundation for the further optimization of GA analogues as lead structures for anticancer drug development.

Table 1. Cytotoxicity (IC₅₀) of gambogic acid (1) and compounds **3a–3hb**

No	Compound	IC ₅₀ (μM)		
		HGC-27	Hep-G2	Vero
1	3a	14.33 ± 0.41	28.07 ± 1.52	20.80 ± 1.35
2	3b	13.82 ± 0.28	48.54 ± 2.67	25.53 ± 1.96
3	3c	40.26 ± 1.55	45.80 ± 2.84	26.98 ± 1.54
4	3d	45.41 ± 2.04	24.92 ± 1.81	26.17 ± 1.73
5	3e	15.80 ± 0.63	42.55 ± 2.49	16.99 ± 0.57
6	3f	12.84 ± 0.92	57.43 ± 2.76	17.19 ± 0.43
7	3g	40.74 ± 2.16	53.57 ± 2.65	49.08 ± 2.48
8	3h	11.63 ± 0.75	43.21 ± 2.90	17.39 ± 0.36
9	1	15.17 ± 0.47	40.79 ± 2.18	19.93 ± 0.47
10	Ellipticine	1.30 ± 0.21	1.38 ± 0.35	1.34 ± 0.24

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CONFLICTS OF INTEREST

None.

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