Pharmaceutical Sciences and Drug Design
ISSN: 3062-4428
2024, Volume 4, Page No: 262-277

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Galaxy Publication

Antiproliferative Activity of Novel Isosteviol-Derived 1,3-Aminoalcohols: Synthesis and Structure–Activity Relationships

Marco Rossi[1], Paolo Conti[1]*, Giulia Bianchi[1]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Milan, Milan, Italy.

***E-mail** ✉ paolo.conti@gmail.com

Received: 12 September 2024; **Revised:** 02 December 2024; **Accepted:** 06 December 2024

ABSTRACT

A series of diterpenoid 1,3-aminoalcohol derivatives was synthesised from isosteviol through stereoselective transformations. Isosteviol was obtained via acid-catalysed hydrolysis and rearrangement of natural stevioside and subsequently converted to the key methyl ester intermediate. A 1,3-aminoalcohol library was then constructed by reductive amination of the 3-hydroxyaldehyde intermediate derived from isosteviol in a two-step process. To evaluate the influence of the carboxylate ester at position 4, analogues bearing a free carboxylic acid, benzyl ester, or acryloyl ester were prepared as extended derivatives, building on our previous findings in this area. The antiproliferative activity of the compounds was assessed against human tumour cell lines (A2780, HeLa, MCF-7, and MDA-MB-231). In this preliminary investigation, the 1,3-aminoalcohol moiety featuring N-benzyl or (1H-imidazol-1-yl)-propyl substitution combined with a benzyl ester group appeared critical for consistent antiproliferative effects. These findings provide a promising foundation for further functionalisation aimed at developing more potent antiproliferative diterpenes.

Keywords: Isosteviol, Diterpene, Chiral, Aminoalcohol, Antiproliferative, SAR study

How to Cite This Article: Rossi M, Conti P, Bianchi G. Antiproliferative Activity of Novel Isosteviol-Derived 1,3-Aminoalcohols: Synthesis and Structure–Activity Relationships. Pharm Sci Drug Des. 2024;4:262-77. https://doi.org/10.51847/43aohcFff4

Introduction

Considerable interest is currently focused on the glycosides from the plant Stevia rebaudiana, owing not only to their intense sweetness compared to sucrose and their use as a zero-calorie artificial sweetener [1], but also to their diverse biological properties, including antibacterial, antiviral, and anticancer effects [2, 3]. Beyond the glycosides, isosteviol—a tetracyclic diterpenoid derived from the aglycone steviol of stevioside—has demonstrated various biological activities, such as hypoglycemic, anti-inflammatory, antihypertensive, and anticancer actions [4, 5]. Consequently, structural modifications of these compounds are frequently pursued to yield new derivatives with enhanced biological profiles, resulting in compounds exhibiting antiproliferative [6], antiviral [7], antimitotic [8], anticarcinogenic [9], cardioprotective [10], acetylcholinesterase inhibitory [11], antibacterial [12], and antituberculotic [13] activities.

The rising incidence of cancer represents a major global health challenge, with projections estimating 24 million cases by 2035 [14]. Despite advances in therapy, existing cancer treatments are limited by side effects and the high cost of anticancer drugs [15-17]. Thus, the development of novel, more affordable agents with improved efficacy and reduced toxicity remains essential. Natural products and their semi-synthetic analogues have played a crucial role as anticancer therapeutics, and diterpenoid metabolites have gained particular prominence in anticancer drug discovery over the last decade [18, 19]. Recent efforts employing microbial biotransformations and chemical modifications have produced cytotoxic isosteviol derivatives that have attracted substantial interest [20-22]. Notably, numerous isosteviol analogues have been generated through chemical alteration of reactive functional groups, with several displaying promising cytotoxic activity and potential as drug candidates [23]. Zhang *et al*. synthesised isosteviol derivatives incorporating a cyclopentanone ring with an exo-methylene bridge

and evaluated their antiproliferative effects against human gastric carcinoma MGC-803, HepG-2, and breast carcinoma MDA-MB-231 cell lines [24].

Among these, the 1,3-oxoallyl derivative emerged as one of the most potent and selective anticancer agents in the series, outperforming Adriamycin (IC50: 2.53, 2.08, and 2.26 μM) with an IC50 of 1.58 μM. At the C-19 position of the isosteviol skeleton, Khaybullin *et al*. performed structural modifications, synthesising 15 hybrid compounds combining a nitric oxide donor moiety with the isosteviol core, several of which exhibited antiproliferative activity [4]. The C-19 conjugated derivative proved most active against B16-F10 melanoma cells, with an IC50 of 0.02 μM.

Zhang *et al*. also prepared series of 1,2- and 1,3-aminoalcohol derivatives of isosteviol and examined their in vitro antitumour properties [25]. Their findings indicated that both hydroxyl and amino groups conferred advantages for anticancer activity in these isosteviol analogues. Comparison of diastereoisomeric and regioisomeric 1,3-aminoalcohols revealed no marked differences in cytotoxicity between epimers or regioisomers (IC50 values ranging from 2.47 μM to 12.25 μM against HCT-116, EC9706, and Eca109 cells), whereas alterations to the aminoalcohol unit led to reduced potency [23, 25]. Likewise, reaction of isosteviol-derived 1,3-aminoalcohols with isothiocyanates yielded thiourea derivatives with notable antiproliferative activity; the p-nitrophenyl-substituted compound displayed the highest cytotoxicity, with IC50 values of 1.45 μM, 2.35 μM, and 3.54 μM against HCT-116, HGC-27, and JEKO-1 cells, respectively [9].

In our prior research, we described the synthesis of isosteviol-based 1,3-aminoalcohols and highlighted the beneficial impact of N-benzyl substitution on the amino group for antiproliferative activity [26]. In the current work, we seek to broaden the structure–activity relationship study by exploring variations in N-substitution and modifications of the ester at position 4—replacing it with a free carboxylic acid or alternative esters, such as benzyl. Additionally, we aim to enhance antiproliferative potential by incorporating the aminoalcohol functionality alongside other bioactive groups, including acryloyl moieties at position 4 of the ent-kaurane framework.

Materials and Methods

Chemistry

General procedures: Reagents were purchased from commercial sources (Molar Chemicals Ltd., Halásztelek, Hungary; Merck Ltd., Budapest, Hungary; VWR International Ltd., Debrecen, Hungary) and used directly. Solvents underwent standard drying protocols. Specific optical rotations were determined in methanol at 20 °C using a Perkin-Elmer 341 polarimeter (PerkinElmer Inc., Shelton, CT, USA). TLC monitoring and column chromatography employed Merck Kieselgel 60 (Merck Ltd., Budapest, Hungary). Melting points were recorded on a Kofler hot-stage apparatus (Nagema, Dresden, Germany). NMR data (1H at 500 MHz, 13C at 125 MHz) were collected on a Bruker Avance DRX 500 instrument, with shifts referenced to TMS (δ = 0) and couplings reported in Hz. High-resolution mass analyses were obtained by flow injection on a Thermo Scientific Q Exactive Plus quadrupole-Orbitrap system (Thermo Fisher Scientific, Waltham, MA, USA) linked to a Waters Acquity I-Class UPLC™ (Waters, Manchester, UK).

Starting compounds: Stevioside originated from Molar Chemicals Ltd., Halásztelek, Hungary. Isosteviol 1 was derived in a single operation from commercial stevioside or related glycoside blends per reported methods, matching all prior spectroscopic descriptions [27].

Known intermediates 2 [28], 3 [29], 9 [30], 21 [31], and 22 [26] were accessed via documented routes, displaying physicochemical and spectral properties consistent with literature values.

(4R,4aS,6aS,7S,8R,9S,11bS)-Benzyl 7-formyl-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (4)

A solution of compound 3 (6.81 mmol, 3.00 g) in a 1:1 mixture of DCM/H_2O (300 mL) was treated with TEMPO (10 mol%, 106 mg), NBS (13.62 mmol, 2.42 g), and TBAB (6.81 mmol, 2.20 g). The mixture was refluxed for 12 h until completion (monitored by TLC), then extracted with DCM (3 × 100 mL). The combined organic layers were washed with water (1 × 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography using an appropriate eluent (n-hexane/EtOAc = 3:1). Yield: 2.48 g (83%); colourless oil; $[\alpha]D^{20} = -69$ (c 0.71, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.79 (s, 3H), 0.86–0.91 (m, 1H), 0.95 (s, 3H), 0.99–1.04 (m, 3H), 1.12–1.15 (m, 1H), 1.19 (s, 3H), 1.22–1.23 (m,

1H), 1.33–1.36 (m, 1H), 1.41–1.45 (m, 1H), 1.50–1.54 (m, 1H), 1.64–1.85 (m, 8H), 2.22 (d, 1H, J = 13.2 Hz), 2.85–2.86 (m, 1H), 4.27 (d, 1H, J = 4.9 Hz), 5.03 (d, 1H, J = 12.2 Hz), 5.14 (d, 1H, J = 12.2 Hz), 7.31–7.39 (m, 5H), 9.77 (d, 1H, J = 2.2 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.1 (CH_3), 18.8 (CH_2), 19.7 (CH_2), 21.8 (CH_2), 24.5 (CH_3), 28.8 (CH_3), 33.0 (CH_2), 36.0 (CH_2), 38.1 ($C_{(q)}$), 38.4 ($C_{(q)}$), 39.6 (CH_2), 41.1 ($C_{(q)}$), 43.9 ($C_{(q)}$), 46.5 ($C_{(q)}$), 53.9 (CH_2), 56.9 (CH), 57.3 (CH), 61.7 (CH), 66.3 (CH_2), 78.2 (CH), 128.3 (CH), 128.6 (2 × CH), 128.6 (2 × CH), 135.9 ($C_{(q)}$), 177.1 (C=O), 204.0 (CHO). $C_{28}H_{38}O_4$: 438.5989. HRMS (ESI+): m/z calcd for $C_{28}H_{39}O_4$ $[M + H]^+$ 439.2848; found 439.28230.

(4R,4aS,6aS,7R,8R,9S,11bS)-Benzyl 7-(((4-fluorobenzyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (5)

To a solution of compound 4 (1.14 mmol, 500 mg) in dry EtOH (20 mL) was added 4-fluorobenzylamine (1.14 mmol, 130 µL) in one portion. The resulting mixture was stirred at room temperature for 3 h and then concentrated to dryness. The residue was redissolved in dry EtOH (20 mL), stirred for an additional 1 h, and evaporated again. The material was then taken up in dry MeOH (20 mL), and $NaBH_4$ (2.28 mmol, 90 mg) was added portionwise under ice cooling. After stirring for 4 h at room temperature, the solvent was removed, the residue was partitioned between H_2O (50 mL) and DCM (3 × 50 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated. Purification of the crude product was performed by silica gel column chromatography ($CHCl_3$/MeOH = 19:1). Yield: 440 mg (70%); colourless oil; $[\alpha]D^{20} = -38$ (c 1.6, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.65 (s, 3H), 0.83–0.88 (m, 2H), 0.90 (s, 3H), 0.93–1.07 (m, 5H), 1.13–1.15 (m, 1H), 1.19 (s, 3H), 1.31 (d, 1H, J = 11.5 Hz), 1.40 (d, 1H, J = 14.2 Hz), 1.53–1.84 (m, 9H), 2.20 (d, 1H, J = 13.4 Hz), 2.33 (t, 1H, J = 11.9 Hz), 2.89 (dd, 1H, J = 3.7, 11.2 Hz), 3.44 (d, 1H, J = 4.8 Hz), 3.65 (d, 1H, J = 13.1 Hz), 3.85 (d, 1H, J = 13.1 Hz), 5.07 (dd, 2H, J = 12.4, 12.4 Hz), 7.01 (t, 2H, J = 8.5 Hz), 7.30–7.36 (m, 7H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.3 (CH_3), 18.9 (CH_2), 19.5 (CH_2), 22.2 (CH_2), 25.1 (CH_3), 29.0 (CH_3), 33.0 (CH_2), 35.0 (CH_2), 38.0 (CH_2), 38.2 ($C_{(q)}$), 39.6 (CH_2), 40.7 ($C_{(q)}$), 42.3 ($C_{(q)}$), 43.9 ($C_{(q)}$), 48.0 (CH), 51.6 (CH_2), 53.2 (CH_2), 54.2 (CH_2), 57.2 (CH), 57.7 (CH), 66.0 (CH_2), 88.3 (CH), 115.2 (CH), 115.3 (CH), 128.0 (CH), 128.3 (CH), 128.4 (2 × CH), 129.8 (2 × CH), 129.9 (CH), 135.4 ($C_{(q)}$-F), 136.1 (2 × $C_{(q)}$), 136.2 ($C_{(q)}$-F), 161.1 ($C_{(q)}$-F), 163.0 ($C_{(q)}$-F), 177.1 (C=O); ^{19}F-NMR (470 MHz, $CDCl_3$) δ (ppm): −116.2 ($C_{(q)}$-F). $C_{35}H_{46}FNO_3$: 547.7430. HRMS (ESI+): m/z calcd for $C_{35}H_{47}FNO_3$ $[M + H]^+$ 548.3540; found 548.3533.

(4R,4aS,6aS,7R,8R,9S,11bS)-Benzyl 7-(((tert-butoxycarbonyl)(4-fluorobenzyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (6)

A solution of compound 5 (0.91 mmol, 500 mg) in dry DCM (10 mL) was treated with di-tert-butyl dicarbonate (0.91 mmol, 200 mg) in one portion. The mixture was stirred at room temperature for 2 h and then concentrated to dryness. The residue was dissolved in DCM (10 mL) and washed with H_2O (3 × 10 mL). The organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude material was purified by silica gel column chromatography (n-hexane/EtOAc = 4:1). Yield: 470 mg (79%); colourless oil; $[\alpha]D^{20} = -1$ (c 2.80, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.70 (s, 3H), 0.83–0.87 (m, 1H), 0.90 (s, 3H), 0.92–1.06 (m, 5H), 1.12–1.18 (m, 4H), 1.37–1.42 (m, 2H), 1.47 (s, 9H), 1.56–1.84 (m, 8H), 2.14–2.21 (m, 2H), 3.03 (dd, 1H, J = 4.5 Hz, 13.8 Hz), 3.55 (t, 1H, J = 13.1 Hz), 3.64 (d, 1H, J = 4.4 Hz), 4.18 (d, 1H, J = 15.5 Hz), 4.66 (d, 1H, J = 15.5 Hz), 4.87 (d, 1H, J = 12.5 Hz), 5.18 (d, 1H, J = 12.5 Hz), 6.99 (t, 2H, J = 8.6 Hz), 7.23–7.31 (m, 7H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.5 (CH_3), 19.0 (CH_2), 19.5 (CH_2), 22.0 (CH_2), 25.1 (CH_3), 28.5 (3 × CH_3), 29.0 (CH_3), 33.2 (CH_2), 34.9 (CH_2), 37.9 (CH_2), 38.2 ($C_{(q)}$), 39.5 (CH_2), 41.0 ($C_{(q)}$), 42.9 ($C_{(q)}$), 43.8 ($C_{(q)}$), 45.6 (CH), 47.5 (CH_2), 49.3 (CH_2), 53.9 (CH_2), 57.3 (CH), 57.7 (CH), 65.8 (CH_2), 80.0 ($C_{(q)}$), 85.6 (CH), 115.3 (CH), 115.5 (CH), 127.9 (CH), 127.9 (2 × CH), 128.4 (2 × CH), 129.4 (CH), 129.5 (CH), 134.3 (2 × $C_{(q)}$), 136.3 ($C_{(q)}$), 156.5 ($C_{(q)}$-F), 161.1 ($C_{(q)}$-F), 163.1 ($C_{(q)}$-F), 176.8 (C=O); ^{19}F-NMR (470 MHz, $CDCl_3$) δ (ppm): −115.0 ($C_{(q)}$-F). $C_{40}H_{54}FNO_5$: 647.3986. HRMS (ESI+): m/z calcd for $C_{40}H_{55}FNO_5$ $[M + H]^+$ 648.4064; found 648.4074.

(4R,4aS,6aS,7R,8R,9S,11bS)-7-(((tert-butoxycarbonyl)(4-fluorobenzyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylic Acid (7)

To a suspension of 5% Pd/C (120 mg) in n-hexane/EtOAc (1:1, 20 mL) was added a solution of compound 6 (0.77 mmol, 500 mg) in n-hexane/EtOAc (1:1, 20 mL). The mixture was stirred under an atmosphere of H_2 (1 atm) at room temperature until completion (monitored by TLC, 24 h). The catalyst was removed by filtration through Celite, and the filtrate was concentrated to dryness. The crude product was purified by silica gel column

chromatography ($CHCl_3$/MeOH = 9:1). Yield: 350 mg (82%); colourless oil; $[\alpha]D^{20} = -9$ (c 0.67, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.85–0.89 (m, 1H), 0.90 (s, 3H), 0.92 (s, 3H), 0.94–1.05 (m, 4H), 1.09–1.11 (m, 1H), 1.13–1.18 (m, 1H), 1.20 (s, 3H), 1.37–1.42 (m, 1H), 1.49 (s, 9H), 1.58–1.81 (m, 9H), 2.16–2.19 (m, 2H), 2.99–3.02 (m, 1H), 3.56 (t, 1H, J = 12.5 Hz), 3.76 (d, 1H, J = 4.4 Hz), 3.95 (d, 1H, J = 15.3 Hz), 4.86–4.88 (m, 1H), 7.00 (t, 2H, J = 8.6 Hz), 7.24–7.26 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.6 (CH_3), 18.8 (CH_2), 19.6 (CH_2), 21.9 (CH_2), 25.2 (CH_3), 28.5 (3 × CH_3), 29.0 (CH_3), 33.2 (CH_2), 34.8 (CH_2), 37.8 (CH_2), 38.4 ($C_{(q)}$), 39.6 (CH_2), 41.0 ($C_{(q)}$), 42.9 ($C_{(q)}$), 43.7 ($C_{(q)}$), 45.9 (CH), 46.8 (CH_2), 48.6 (CH_2), 53.9 (CH_2), 57.2 (CH), 57.7 (CH), 80.1 ($C_{(q)}$), 115.4 (CH), 115.5 (CH), 129.8 (2 × CH), 156.4 ($C_{(q)}$-F), 161.2 ($C_{(q)}$-F), 163.2 ($C_{(q)}$-F), 171.3 ($C_{(q)}$), 183.4 (C=O); ^{19}F-NMR (470 MHz, $CDCl_3$) δ (ppm): −116.2 ($C_{(q)}$-F). $C_{33}H_{48}FNO_5$: 557.7363. HRMS (ESI+): m/z calcd for $C_{33}H_{49}FNO_5$ $[M + H]^+$ 558.3595; found 558.3607.

(4R,4aS,6aS,7R,8R,9S,11bS)-7-(((4-Fluorobenzyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylic Acid (8)

To a solution of compound 7 (0.54 mmol, 300 mg) in dry DCM (15 mL) cooled to 0 °C was added TFA (6.53 mmol, 0.50 mL). The cooling bath was removed, and the mixture was stirred at room temperature for 3 h. The solvent and excess TFA were evaporated in vacuo, and the residue was redissolved in dry DCM. The solution was cooled to 0 °C, triethylamine (1.23 mmol, 171.10 µL) was added, and the resulting mixture was warmed to room temperature and stirred for 1 h. The solvent was removed under reduced pressure, and the crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9:1). Yield: 190 mg (77%); white crystals; m.p. 149–150 °C; $[\alpha]D^{20} = -39$ (c 0.28, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.77 (s, 3H), 0.80–0.84 (m, 2H), 0.90 (s, 3H), 0.94–1.03 (m, 3H), 1.06–1.09 (m, 4H), 1.13–1.18 (m, 1H), 1.29 (d, 1H, J = 11.4 Hz), 1.35 (d, 1H, J = 14.1 Hz), 1.52–1.85 (m, 8H), 2.09 (d, 1H, J = 13.1 Hz), 2.21 (d, 1H, J = 12.0 Hz), 2.52 (d, 1H, J = 11.6 Hz), 3.10 (d, 1H, J = 8.4 Hz), 3.42 (s, 1H), 3.86 (d, 1H, J = 12.4 Hz), 3.97 (d, 1H, J = 12.4 Hz), 7.04 (t, 2H, J = 8.4 Hz), 7.39 (t, 2H, J = 6.9 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 14.6 (CH_3), 18.9 (CH_2), 19.4 (CH_2), 22.0 (CH_2), 25.1 (CH_3), 29.2 (CH_3), 33.0 (CH_2), 34.8 (CH_2), 37.8 (CH_2), 38.1 ($C_{(q)}$), 39.6 (CH_2), 41.5 ($C_{(q)}$), 43.3 ($C_{(q)}$), 43.9 ($C_{(q)}$), 46.0 (CH), 51.1 (CH_2), 52.3 (CH_2), 56.7 (CH), 56.9 (CH), 86.1 (CH), 115.6 (CH), 115.8 (CH), 130.1 ($C_{(q)}$), 131.4 (CH), 131.5 (CH), 161.8 ($C_{(q)}$-F), 163.8 ($C_{(q)}$-F), 182.5 (C=O). $C_{28}H_{40}FNO_3$: 457.6205. HRMS (ESI+): m/z calcd for $C_{28}H_{41}FNO_3$ $[M + H]^+$ 458.3070; found 458.3060.

4-Bromobutyl Acrylate (9) and 3-(4-Bromobutoxy)-3-oxopropyl Acrylate (10)

To a suspension of potassium carbonate (2.78 mmol, 380 mg) in dry acetone (50 mL) were added acrylic acid (containing approx. 20% dimer, 2.78 mmol, 200 mg) and 1,4-dibromobutane (2.78 mmol, 332 µL). The mixture was stirred at room temperature for 1 day. Upon completion (monitored by TLC, 24 h), the solids were removed by filtration, and the filtrate was concentrated to dryness. The crude material was purified by silica gel column chromatography (n-hexane/EtOAc = 3:1). Yield: 351 mg (product 9, 61%), 202 mg (product 10, 26%); colourless oil; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.18–1.84 (m, 2H), 1.91–1.96 (m, 2H), 2.69 (t, 2H, J = 6.3 Hz), 3.42 (t, 2H, J = 6.6 Hz), 4.16 (t, 2H, J = 6.2 Hz), 4.44 (t, 2H, J = 6.4 Hz), 5.83 (d, 1H, J = 10.5 Hz), 6.11 (dd, 1H, J = 10.5 Hz, 17.0 Hz), 6.40 (d, 1H, J = 17.1 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 27.3 (CH_2), 29.2 (CH_2), 32.7 (CH_2), 34.0 (CH_2), 59.9 (CH_2), 63.8 (CH_2), 128.1 (CH), 131.0 (CH_2), 165.8 ($C_{(q)}$), 170.5 ($C_{(q)}$); $C_{10}H_{15}BrO_4$: 279.1277. HRMS (ESI+): m/z calcd for $C_{10}H_{16}BrO_4$ $[M + H]^+$ 280.1356; found 280.1368.

(4R,4aS,6aS,7R,8R,9S,11bS)-4-(Acryloyloxy)butyl 7-(((tert-butoxycarbonyl)(4-fluorobenzyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (11)

To a suspension of potassium carbonate (0.44 mmol, 30 mg) in dry acetone (15 mL) were added compound 7 (0.22 mmol, 100 mg) and 4-bromobutyl acrylate (0.22 mmol, 35 µL). The mixture was stirred at room temperature for one day. Upon completion (monitored by TLC, 24 h), the solids were removed by filtration through filter paper, and the filtrate was concentrated to dryness. The crude material was purified by silica gel column chromatography (n-hexane/EtOAc = 3:1). Yield: 120 mg (83%); colourless oil; $[\alpha]D^{20} = -17$ (c 0.05, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.72 (s, 3H), 0.84–0.87 (m, 1H), 0.90 (s, 3H), 0.95–1.06 (m, 5H), 1.13 (s, 3H), 1.15–1.20 (m, 1H), 1.39–1.43 (m, 3H), 1.47 (s, 9H), 1.56–1.83 (m, 11H), 2.13–2.20 (m, 2H), 3.02 (dd, 1H, J = 4.6 Hz, 13.8 Hz), 3.58–3.65 (m, 2H), 3.81–3.86 (m, 1H), 4.08–4.17 (m, 4H), 4.78 (d, 1H, J = 15.6 Hz), 5.81 (d, 1H, J = 10.5 Hz), 6.11 (dd, 1H, J = 10.5 Hz, 17.6 Hz), 6.39 (d, 1H, J = 17.6 Hz), 7.01 (t, 2H, J = 8.7 Hz), 7.22–7.25 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.4 (CH_3), 19.0 (CH_2), 19.5 (CH_2), 22.1 (CH_2), 25.1 (CH_3),

25.3 (CH_2), 25.6 (CH_2), 28.5 (3 × CH_3), 29.0 (CH_3), 33.3 (CH_2), 34.9 (CH_2), 38.0 (CH_2), 38.2 ($C_{(q)}$), 39.5 (CH_2), 41.0 ($C_{(q)}$), 42.8 ($C_{(q)}$), 43.8 ($C_{(q)}$), 45.5 (CH), 47.1 (CH_2), 48.8 (CH_2), 54.0 (CH_2), 57.2 (CH), 57.8 (CH), 63.4 (CH_2), 64.0 (CH_2), 80.1 ($C_{(q)}$), 85.7 (CH), 115.4 (CH), 115.6 (CH), 128.5 (CH), 129.3 (2 × CH), 130.6 (CH_2), 134.2 ($C_{(q)}$), 134.3 ($C_{(q)}$), 156.5 ($C_{(q)}$-F), 161.2 ($C_{(q)}$-F), 163.1 ($C_{(q)}$-F), 166.2 (C=O), 177.1 (C=O); ^{19}F-NMR (470 MHz, $CDCl_3$) δ (ppm): −115.0 ($C_{(q)}$-F). $C_{40}H_{58}FNO_7$: 683.8894. HRMS (ESI+): m/z calcd for $C_{40}H_{59}FNO_7$ $[M + H]^+$ 684.4064; found 684.4283.

(4R,4aS,6aS,7R,8R,9S,11bS)-4-((3-(Acryloyloxy)propanoyl)oxy)butyl 7-(((tert-butoxycarbonyl)(4-fluorobenzyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (12)

To a suspension of potassium carbonate (0.44 mmol, 30 mg) in dry acetone (15 mL) were added compound 7 (0.22 mmol, 100 mg) and 3-(4-bromobutoxy)-3-oxopropyl acrylate (product 10, 0.22 mmol, 60 mg). The mixture was stirred at room temperature for one day. Upon completion (monitored by TLC, 24 h), the solids were removed by filtration through filter paper, and the filtrate was concentrated to dryness. The crude material was purified by silica gel column chromatography (n-hexane/EtOAc = 3:1). Yield: 130 mg (77%); colourless oil; $[\alpha]D^{20}$ = +8.7 (c 0.11, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.72 (s, 3H), 0.84–0.88 (m, 1H), 0.90 (s, 3H), 0.95–1.06 (m, 5H), 1.12 (s, 3H), 1.15–1.20 (m, 1H), 1.39–1.43 (m, 3H), 1.47 (s, 9H), 1.57–1.82 (m, 11H), 2.12–2.20 (m, 2H), 2.68 (t, 2H, J = 6.3 Hz), 3.02 (dd, 1H, J = 4.6 Hz, 13.8 Hz), 3.57–3.65 (m, 2H), 3.80–3.84 (m, 1H), 4.07–4.16 (m, 4H), 4.43 (t, 2H, J = 6.5 Hz), 4.79 (d, 1H, J = 15.6 Hz), 5.81 (d, 1H, J = 10.5 Hz), 6.10 (dd, 1H, J = 10.5 Hz, 17.6 Hz), 6.39 (d, 1H, J = 17.6 Hz), 7.01 (t, 2H, J = 8.9 Hz), 7.22–7.25 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.4 (CH_3), 19.0 (CH_2), 19.5 (CH_2), 22.1 (CH_2), 25.1 (CH_3), 25.2 (CH_2), 25.5 (CH_2), 28.5 (3 × CH_3), 29.1 (CH_3), 33.3 (CH_2), 34.0 (CH_2), 34.9 (CH_2), 38.0 (CH_2), 38.2 ($C_{(q)}$), 39.5 (CH_2), 41.0 ($C_{(q)}$), 42.8 ($C_{(q)}$), 43.8 ($C_{(q)}$), 45.5 (CH), 47.1 (CH_2), 48.8 (CH_2), 54.0 (CH_2), 57.2 (CH), 57.8 (CH), 59.9 (CH_2), 63.3 (CH_2), 64.3 (CH_2), 80.1 ($C_{(q)}$), 85.6 (CH), 115.4 (CH), 115.5 (CH), 128.2 (CH), 129.3 (2 × CH), 131.0 (CH_2), 134.2 ($C_{(q)}$), 134.3 ($C_{(q)}$), 156.5 ($C_{(q)}$-F), 161.2 ($C_{(q)}$-F), 163.1 ($C_{(q)}$-F), 165.8 (C=O), 170.5 (C=O), 177.1 (C=O); ^{19}F-NMR (470 MHz, $CDCl_3$) δ (ppm): −115.0 ($C_{(q)}$-F), −115.3 ($C_{(q)}$-F). $C_{43}H_{62}FNO_9$: 755.9521. HRMS (ESI+): m/z calcd for $C_{43}H_{63}FNO_9$ $[M + H]^+$ 756.9600; found 756.4495.

(4R,4aS,6aS,7R,8R,9S,11bS)-Prop-2-yn-1-yl 7-(((tert-butoxycarbonyl)(4-fluorobenzyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (13)

To a suspension of potassium carbonate (0.72 mmol, 100 mg) in dry acetone (15 mL) were added compound 7 (0.36 mmol, 200 mg) and propargyl bromide (0.36 mmol, 28 µL). The mixture was stirred at room temperature for one day. Upon completion (monitored by TLC, 24 h), the solids were removed by filtration through filter paper, and the filtrate was concentrated to dryness. The crude material was purified by silica gel column chromatography (n-hexane/EtOAc = 4:1). Yield: 190 mg (88%); colourless oil; $[\alpha]D^{20}$ = −17 (c 0.23, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.75 (s, 3H), 0.84–0.88 (m, 1H), 0.93 (s, 3H), 0.95–1.08 (m, 5H), 1.13–1.16 (m, 4H), 1.57–1.80 (m, 7H), 2.15–2.19 (m, 2H), 2.26 (s, 1H), 2.26–2.30 (m, 2H), 3.06 (dd, 1H, J = 4.2 Hz, 13.5 Hz), 3.60 (t, 1H, J = 12.9 Hz), 3.67 (m, 1H), 4.16 (d, 1H, J = 15.5 Hz), 4.51 (d, 1H, J = 15.6 Hz), 4.66 (d, 1H, J = 15.5 Hz), 4.74 (d, 1H, J = 15.7 Hz), 7.03 (t, 2H, J = 9.1 Hz), 7.25–7.28 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.6 (CH_3), 18.9 (CH_2), 19.5 (CH_2), 21.9 (CH_2), 25.1 (CH_3), 28.4 (3 × CH_3), 28.8 (CH_3), 33.2 (CH_2), 34.9 (CH_2), 37.9 (CH_2), 38.2 ($C_{(q)}$), 39.5 (CH_2), 41.0 ($C_{(q)}$), 42.9 ($C_{(q)}$), 43.8 ($C_{(q)}$), 45.7 (CH), 47.5 (CH_2), 49.1 (CH_2), 51.3 (CH_2), 53.9 (CH_2), 57.2 (CH), 57.7 (CH), 74.4 ($C_{(q)}$), 80.0 ($C_{(q)}$), 85.6 (CH), 115.4 (CH), 115.5 (CH), 129.3 (CH), 129.4 (CH), 134.3 (2 × $C_{(q)}$), 156.5 ($C_{(q)}$), 161.1 ($C_{(q)}$-F), 163.1 ($C_{(q)}$-F), 176.2 (C=O). $C_{36}H_{50}FNO_5$: 595.3673. HRMS (ESI+): m/z calcd for $C_{36}H_{51}FNO_3$ $[M + H]^+$ 596.3751; found 596.3759.

(4R,4aS,6aS,7R,8R,9S,11bS)-Prop-2-yn-1-yl 7-(((4-fluorobenzyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (14)

To a solution of compound 13 (0.34 mmol, 200 mg) in dry DCM (15 mL) cooled to 0 °C was added TFA (6.53 mmol, 0.50 mL). The cooling bath was removed, and the mixture was stirred at room temperature for 3 h. The solvent and excess TFA were evaporated in vacuo, and the residue was redissolved in dry DCM. The solution was cooled to 0 °C, triethylamine (0.68 mmol, 95 µL) was added, and the resulting mixture was warmed to room temperature and stirred for 1 h. The solvent was removed under reduced pressure, and the crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 19:1). Yield: 130 mg (79%); white crystals; m.p.

104–105 °C; [α]D²⁰ = −52 (c 1.06, MeOH); ¹H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.73 (s, 3H), 0.83–0.87 (m, 1H), 0.90 (s, 3H), 0.95–1.08 (m, 5H), 1.14–1.20 (m, 4H), 1.31 (d, 1H, J = 10.7 Hz), 1.42 (m, 1H, J = 14.2 Hz), 1.51–1.63 (m, 4H), 1.69–1.84 (m, 4H), 2.06 (d, 1H, J = 12.0 Hz), 2.17 (d, 1H, J = 13.3 Hz), 2.52–2.56 (m, 2H), 3.03 (dd, 1H, J = 3.4 Hz, 11.4 Hz), 3.53 (d, 1H, J = 4.7 Hz), 3.84 (d, 1H, J = 13.1 Hz), 4.01 (d, 1H, J = 13.1 Hz), 4.61–4.69 (m, 2H), 7.05 (t, 2H, J = 8.6 Hz), 7.42–7.45 (m, 2H); ¹³C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.5 (CH_3), 18.8 (CH_2), 19.5 (CH_2), 21.9 (CH_2), 24.9 (CH_3), 28.8 (CH_3), 33.0 (CH_2), 34.9 (CH_2), 37.8 (CH_2), 38.1 ($C_{(q)}$), 39.4 (CH_2), 41.0 ($C_{(q)}$), 42.5 ($C_{(q)}$), 43.8 ($C_{(q)}$), 46.2 (CH), 50.7 (CH_2), 51.4 (CH_2), 51.9 (CH_2), 53.8 (CH_2), 57.0 (CH), 57.6 (CH), 74.8 ($C_{(q)}$), 77.9 ($C_{(q)}$), 87.1 (CH), 115.5 (CH), 115.7 (CH), 130.9 (2 × CH), 131.8 ($C_{(q)}$), 161.5 ($C_{(q)}$-F), 163.5 ($C_{(q)}$-F), 176.3 (C=O); ¹⁹F-NMR (470 MHz, $CDCl_3$) δ (ppm): −113.9 ($C_{(q)}$-F). $C_{31}H_{42}FNO_3$: 495.6685. HRMS (ESI+): m/z calcd for $C_{31}H_{43}FNO_3$ $[M + H]^+$ 496.3227; found 496.3233.

General procedure for the preparation of aminoalcohols from primary amines and benzyl ester aldehyde

A solution of aldehyde 4 (0.23 mmol, 100 mg) in anhydrous EtOH (10 mL) was treated with the selected primary amine (0.23 mmol) in a single portion. The mixture was stirred at ambient temperature for 3 h before being concentrated to dryness under reduced pressure. The resulting residue was redissolved in dry EtOH (10 mL), stirred for an additional 1 h, and evaporated once more. The crude imine was then taken up in dry MeOH (10 mL), cooled in an ice bath, and NaBH4 (0.46 mmol, 0.02 g) was introduced portionwise. Stirring was continued for 4 h at room temperature, after which the solvent was removed in vacuo. The residue was partitioned between water (20 mL) and DCM (3 × 20 mL). The combined organic extracts were dried over Na2SO4, filtered, and concentrated. Purification of the crude material was performed by silica gel column chromatography (CHCl3/MeOH = 19:1).

(4R,4aS,6aS,7R,8R,9S,11bS)-Benzyl 7-((((R)-1-(4-fluorophenyl)ethyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (15)

The reaction was carried out starting from compound 4 with (R)-4-fluoro-α-methylbenzylamine (0.23 mmol, 38 µL) following the general procedure. Yield: 30 mg (21%); white crystals; m.p. 135–136 °C; [α]D²⁰ = −33 (c 0.97, MeOH); ¹H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.58 (s, 3H), 0.81–0.83 (m, 1H), 0.87 (s, 3H), 0.93–1.06 (m, 5H), 1.15 (s, 3H), 1.30–1.44 (m, 6H), 1.55–1.57 (m, 2H), 1.66 (d, 3H, J = 6.7 Hz), 1.72–1.80 (m, 3H), 2.13–2.19 (m, 2H), 2.82 (t, 1H, J = 12.5 Hz), 3.00 (d, 1H, J = 9.4 Hz), 3.70 (d, 1H, J = 4.5 Hz), 4.32–4.33 (m, 1H), 5.02 (d, 1H, J = 12.7 Hz), 5.20 (d, 1H, J = 12.7 Hz), 7.07 (t, 2H, J = 8.4 Hz), 7.31–7.39 (m, 5H), 7.56–7.58 (m, 2H); ¹³C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.2 (CH_3), 18.9 (CH_2), 19.2 (CH_3), 19.4 (CH_2), 21.7 (CH_2), 24.7 (CH_3), 29.0 (CH_3), 33.0 (CH_2), 34.8 (CH_2), 37.8 (CH_2), 38.0 ($C_{(q)}$), 39.5 (CH_2), 41.4 ($C_{(q)}$), 42.9 ($C_{(q)}$), 43.7 ($C_{(q)}$), 44.4 (CH), 48.4 (CH_2), 53.4 (CH_2), 57.0 (CH), 57.5 (CH), 57.7 ($C_{(q)}$), 65.8 (CH_2), 85.3 (CH), 116.2 (CH), 116.4 (CH), 127.8 (2 × CH), 128.0 (CH), 128.6 (2 × CH), 129.9 (CH), 129.9 (CH), 132.9 ($C_{(q)}$), 136.4 ($C_{(q)}$), 162.1 ($C_{(q)}$-F), 164.0 ($C_{(q)}$-F), 176.6 (C=O). $C_{36}H_{48}FNO_3$: 561.7696. HRMS (ESI+): m/z calcd for $C_{36}H_{49}FNO_3$ $[M + H]^+$ 562.3696; found 562.3702.

(4R,4aS,6aS,7R,8R,9S,11bS)-Benzyl 8-hydroxy-4,9,11b-trimethyl-7-((((R)-1-phenylpropyl)amino)methyl)tetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (16)

The reaction was carried out starting from compound 4 with (R)-(+)-α-ethylbenzylamine (0.23 mmol, 37 µL) following the general procedure. Yield: 60 mg (52%); white crystals; m.p. 154–155 °C; [α]D²⁰ = −24 (c 0.26, MeOH); ¹H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.56 (s, 3H), 0.79 (t, 3H, J = 7.4 Hz), 0.82–0.90 (m, 3H), 0.92 (s, 3H), 0.95–1.03 (m, 3H), 1.13 (s, 3H), 1.15–1.17 (m, 1H), 1.33–1.39 (m, 2H), 1.52–1.81 (m, 11H), 2.16 (d, 1H, J = 13.2 Hz), 2.32 (t, 1H, J = 11.6 Hz), 2.82 (dd, 1H, J = 4.1 Hz, 11.0 Hz), 3.42–3.48 (m, 2H), 4.94 (d, 1H, J = 12.7 Hz), 5.12 (d, 1H, J = 12.7 Hz), 7.18–7.22 (m, 1H), 7.25–7.33 (m, 9H); ¹³C-NMR (125 MHz, $CDCl_3$) δ (ppm): 10.5 (CH_3), 13.0 (CH_3), 18.9 (CH_2), 19.5 (CH_2), 22.2 (CH_2), 25.1 (CH_3), 28.9 (CH_3), 30.2 (CH_2), 33.0 (CH_2), 35.0 (CH_2), 38.1 (CH_2), 38.1 ($C_{(q)}$), 39.6 (CH_2), 40.6 ($C_{(q)}$), 42.3 ($C_{(q)}$), 43.8 ($C_{(q)}$), 49.0 (CH), 50.2 (CH_2), 54.4 (CH_2), 57.2 (CH), 57.9 (CH), 65.7 (CH), 65.7 (CH_2), 88.6 (CH), 127.0 (CH), 127.1 (2 × CH), 127.9 (CH), 128.1 (2 × CH), 128.4 (2 × CH), 128.4 (2 × CH), 136.3 ($C_{(q)}$), 144.4 ($C_{(q)}$), 177.0 (C=O). $C_{37}H_{51}NO_3$: 557.8057. HRMS (ESI+): m/z calcd for $C_{37}H_{52}NO_3$ $[M + H]^+$ 558.8137; found 558.3951.

(4R,4aS,6aS,7R,8R,9S,11bS)-Benzyl 8-hydroxy-4,9,11b-trimethyl-7-((((S)-1-phenylpropyl)amino)methyl)tetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (17)

The reaction was carried out starting from compound 4 with (S)-(+)-α-ethylbenzylamine (0.23 mmol, 37 µL) following the general procedure. Yield: 80 mg (68%); white crystals; m.p. 62–63 °C; $[\alpha]D^{20} = -32$ (c 0.19, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.67 (s, 3H), 0.78–0.82 (m, 4H), 0.84 (s, 3H), 0.84 (m, 1H), 0.90–1.04 (m, 4H), 1.15 (m, 1H), 1.16 (s, 3H), 1.39–1.43 (m, 2H), 1.56–1.79 (m, 11H), 2.02 (t, 1H, J = 11.9 Hz), 2.19 (d, 1H, J = 13.6 Hz), 2.75 (dd, 1H, J = 3.7 Hz, 10.8 Hz), 3.30 (d, 1H, J = 4.9 Hz), 3.48 (t, 1H, J = 6.8 Hz), 5.06 (d, 1H, J = 12.5 Hz), 5.10 (d, 1H, J = 12.5 Hz), 7.23–7.38 (m, 10H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 10.7 (CH_3), 13.3 (CH_3), 19.0 (CH_2), 19.6 (CH_2), 22.2 (CH_2), 25.1 (CH_3), 29.0 (CH_3), 31.7 (CH_2), 33.0 (CH_2), 35.0 (CH_2), 38.0 (CH_2), 38.2 ($C_{(q)}$), 39.6 (CH_2), 40.4 ($C_{(q)}$), 42.1 ($C_{(q)}$), 43.9 ($C_{(q)}$), 48.7 (CH), 49.8 (CH_2), 54.2 (CH_2), 57.0 (CH), 57.8 (CH), 65.3 (CH), 66.0 (CH_2), 88.5 (CH), 127.0 (CH), 127.6 (2 × CH), 128.0 (CH), 128.2 (2 × CH), 128.3 (2 × CH), 128.4 (2 × CH), 136.2 ($C_{(q)}$), 143.8 ($C_{(q)}$), 177.2 (C=O). $C_{37}H_{51}NO_3$: 557.8057. HRMS (ESI+): m/z calcd for $C_{37}H_{52}NO_3$ $[M + H]^+$ 558.8137; found 558.3947.

(4R,4aS,6aS,7R,8R,9S,11bS)-Benzyl 8-hydroxy-4,9,11b-trimethyl-7-((((R)-1-(naphthalen-1-yl)ethyl)amino)methyl)tetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (18)

The reaction was carried out starting from compound 4 with (R)-(+)-1-(1-naphthyl)ethylamine (0.23 mmol, 37 µL) following the general procedure. Yield: 60 mg (40%); white crystals; m.p. 118–119 °C; $[\alpha]D^{20} = -36$ (c 0.43, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.64 (s, 3H), 0.82–0.90 (m, 2H), 0.93 (s, 3H), 0.95–1.04 (m, 4H), 1.13 (s, 3H), 1.16–1.20 (m, 1H), 1.37 (t, 3H, J = 15.7 Hz), 1.49 (d, 3H, J = 6.5 Hz), 1.56–1.79 (m, 9H), 2.16 (d, 1H, J = 13.1 Hz), 2.41 (t, 1H, J = 11.5 Hz), 2.94 (dd, 1H, J = 3.1 Hz, 10.6 Hz), 3.52 (d, 1H, J = 4.4 Hz), 3.58–3.60 (m, 1H), 4.89 (d, 1H, J = 12.2 Hz), 5.09 (d, 1H, J = 12.2 Hz), 7.13–7.15 (m, 1H), 7.21–7.26 (m, 4H), 7.43–7.53 (m, 3H), 7.62 (d, 1H, J = 6.8 Hz), 7.72 (d, 1H, J = 7.9 Hz), 7.85 (d, 1H, J = 7.9 Hz), 8.18 (d, 1H, J = 8.4 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.1 (CH_3), 18.9 (CH_2), 19.5 (CH_2), 22.3 (CH_2), 23.1 (CH_3), 25.2 (CH_3), 28.9 (CH_3), 33.0 (CH_2), 35.1 (CH_2), 38.1 (CH), 38.2 ($C_{(q)}$), 39.6 (CH_2), 40.6 ($C_{(q)}$), 42.3 ($C_{(q)}$), 43.8 ($C_{(q)}$), 49.2 (CH), 50.3 (CH_2), 54.3 (CH), 54.4 (CH_2), 57.1 (CH), 57.8 (CH), 65.8 (CH_2), 88.8 (CH), 122.5 (CH), 123.1 (CH), 125.4 (CH), 125.7 (CH), 125.8 (CH), 127.3 (CH), 127.9 (CH), 128.2 (2 × CH), 128.4 (2 × CH), 129.0 (CH), 131.0 ($C_{(q)}$), 134.0 ($C_{(q)}$), 136.2 ($C_{(q)}$), 141.7 ($C_{(q)}$), 177.1 (C=O); $C_{40}H_{51}NO_3$: 593.8378. HRMS (ESI+): m/z calcd for $C_{40}H_{52}NO_3$ $[M + H]^+$ 594.3947; found 594.3953.

(4R,4aS,6aS,7R,8R,9S,11bS)-Benzyl 8-hydroxy-4,9,11b-trimethyl-7-((((S)-1-(naphthalen-1-yl)ethyl)amino)methyl)tetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (19)

The reaction was carried out starting from compound 4 with (S)-(+)-1-(1-naphthyl)ethylamine (0.23 mmol, 37 µL) following the general procedure. Yield: 60 mg (46%); white crystals; m.p. 126–127 °C; $[\alpha]D^{20} = -31$ (c 0.12, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.04 (s, 3H), 0.69–0.73 (m, 1H), 0.85–0.90 (m, 5H), 0.91 (s, 3H), 1.05 (s, 3H), 1.09–1.17 (m, 2H), 1.24–1.30 (m, 3H), 1.53–1.64 (m, 5H), 1.82 (d, 1H, J = 12.3 Hz), 2.05–2.07 (m, 4H), 2.26 (d, 1H, J = 12.8 Hz), 2.89 (t, 1H, J = 12.5 Hz), 3.15–3.17 (m, 1H), 3.87–3.88 (m, 1H), 4.86 (d, 1H, J = 12.5 Hz), 5.05 (m, 1H, J = 12.5 Hz), 5.60 (s, 1H), 7.36–7.63 (m, 8H), 7.84 (d, 2H, J = 8.2 Hz), 8.00 (d, 1H, J = 8.5 Hz), 8.24 (d, 1H, J = 6.9 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 12.3 (CH_3), 18.8 (CH_2), 19.3 (CH_2), 21.7 (CH_2), 22.1 (CH_3), 24.6 (CH_3), 28.9 (CH_3), 33.1 (CH_2), 34.6 (CH_2), 37.7 (CH), 37.8 ($C_{(q)}$), 39.4 (CH_2), 41.4 ($C_{(q)}$), 42.8 ($C_{(q)}$), 43.0 (CH), 43.5 ($C_{(q)}$), 48.7 (CH_2), 53.0 (CH), 53.4 (CH_2), 57.1 (CH), 58.0 (CH), 65.8 (CH_2), 84.6 (CH), 121.9 (CH), 124.5 (2 × CH), 126.1 (2 × CH), 127.1 (CH), 128.2 (2 × CH), 128.5 (2 × CH), 129.1 (CH), 129.3 (CH), 130.7 ($C_{(q)}$), 133.0 ($C_{(q)}$), 134.0 ($C_{(q)}$), 136.6 ($C_{(q)}$), 178.0 (C=O); $C_{40}H_{51}NO_3$: 593.8378. HRMS (ESI+): m/z calcd for $C_{40}H_{52}NO_3$ $[M + H]^+$ 594.3947; found 594.3945.

(4R,4aS,6aS,7R,8R,9S,11bS)-Benzyl 7-(((3-(1H-imidazol-1-yl)propyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (20)

The reaction was carried out starting from compound 4 with 1-(3-aminopropyl)imidazole (0.23 mmol, 27 µL) following the general procedure. Yield: 30 mg (33%); colourless oil; $[\alpha]D^{20} = -11$ (c 0.25, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.67 (s, 3H), 0.84–0.89 (m, 1H), 0.91 (s, 3H), 0.93–1.08 (m, 5H), 1.16–1.20 (m, 4H), 1.32–1.35 (m, 1H), 1.39–1.43 (m, 1H), 1.56–1.85 (m, 9H), 1.91–1.95 (m, 2H), 2.19–2.25 (m, 2H), 2.47–2.52 (m, 1H), 2.66–2.71 (m, 1H), 2.83 (dd, 1H, J = 4.0 Hz, 10.7 Hz), 3.40 (d, 1H, J = 5.0 Hz), 3.97–4.07 (m, 2H), 5.03 (d, 1H, J = 12.5 Hz), 5.10 (d, 1H, J = 12.5 Hz), 6.91 (s, 1H), 7.05 (s, 1H), 7.27–7.35 (m, 5H), 7.48 (s, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.3 (CH_3), 19.0 (CH_2), 19.5 (CH_2), 22.2 (CH_2), 25.1 (CH_3), 29.0 (CH_3), 31.5 (CH_2), 33.0 (CH_2), 35.0 (CH_2), 38.0 (CH_2), 38.2 ($C_{(q)}$), 39.6 (CH_2), 40.7 ($C_{(q)}$), 42.4 ($C_{(q)}$), 43.9 ($C_{(q)}$), 44.9 (CH_2), 46.9

(CH_2), 48.4 (CH), 52.4 (CH_2), 54.3 (CH_2), 57.2 (CH), 57.8 (CH), 66.0 (CH_2), 88.6 (CH), 118.8 (CH), 127.9 (CH), 128.2 (2 × CH), 128.4 (2 × CH), 129.5 ($C_{(q)}$), 136.2 ($C_{(q)}$), 137.2 (CH), 177.1 (C=O). $C_{34}H_{49}N_3O_3$: 547.7712. HRMS (ESI+): m/z calcd for $C_{34}H_{50}N_3O_3$ $[M + H]^+$ 548.7791; found 548.3862.

General Procedure for the Preparation of Aminoalcohols by the Reaction of Aldehyde 22 with Primary Amines
To a solution of 22 (100 mg, 0.28 mmol) in dry EtOH (10 mL), the appropriate primary amine (0.28 mmol) was added in one portion, and the solution was stirred at room temperature for 3 h and then evaporated to dryness. The residue was dissolved in dry EtOH (10 mL), stirred for a further 1 h, and evaporated to dryness. The product was dissolved in dry MeOH (10 mL), and $NaBH_4$ (0.56 mmol, 20 mg) was added in small portions to the mixture under ice cooling. After stirring for 4 h at room temperature, the mixture was evaporated to dryness, and the residue was dissolved in H_2O (20 mL) and extracted with DCM (3 × 20 mL). The combined organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The crude product obtained was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 19:1).

(4R,4aS,6aS,7R,8R,9S,11bS)-Methyl 7-((((R)-1-(4-fluorophenyl)ethyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (23)
The reaction was carried out starting from compound 22 with (R)-4-fluoro-α-methylbenzylamine (0.28 mmol, 38 µL) following the general procedure. Yield: 60 mg (50%); white crystals; m.p. 129–130 °C; $[\alpha]D^{20} = -56$ (c 0.17, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.58 (s, 3H), 0.81–0.91 (m, 3H), 0.93 (s, 3H), 0.95–1.01 (m, 3H), 1.11 (s, 3H), 1.14–1.19 (m, 1H), 1.33 (d, 3H, J = 6.5 Hz), 1.35 (m, 1H), 1.38–1.47 (m, 2H), 1.51–1.59 (m, 3H), 1.64–1.69 (m, 2H), 1.75–1.79 (m, 3H), 2.13 (d, 1H, J = 13.5 Hz), 2.34 (t, 1H, J = 11.6 Hz), 2.78 (dd, 1H, J = 4.3 Hz, 11.0 Hz), 3.47 (d, 1H, J = 5.0 Hz), 3.56 (s, 3H), 3.77 (m, 1H), 7.00 (t, 2H, J = 8.8 Hz), 7.28–7.31 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 12.8 (CH_3), 18.9 (CH_2), 19.5 (CH_2), 22.1 (CH_2), 24.3 (CH_3), 25.1 (CH_3), 28.8 (CH_3), 33.0 (CH_2), 34.9 (CH_2), 38.0 (CH_2), 38.0 ($C_{(q)}$), 39.6 (CH_2), 40.7 ($C_{(q)}$), 42.2 ($C_{(q)}$), 43.7 ($C_{(q)}$), 48.8 (CH), 50.0 (CH_2), 51.0 (CH_3), 54.3 (CH_2), 56.7 (CH), 57.7 (CH), 58.1 (CH), 88.5 (CH), 115.1 (CH), 115.3 (CH), 127.9 (CH), 128.0 (CH), 141.5 ($C_{(q)}$), 160.8 ($C_{(q)}$-F), 162.8 ($C_{(q)}$-F), 177.9 (C=O); ^{19}F-NMR (470 MHz, $CDCl_3$) δ (ppm): −116.3 ($C_{(q)}$-F). $C_{30}H_{44}FNO_3$: 485.6737. HRMS (ESI+): m/z calcd for $C_{30}H_{45}FNO_3$ $[M + H]^+$ 485.3305; found 485.3321.

(4R,4aS,6aS,7R,8R,9S,11bS)-Methyl 8-hydroxy-4,9,11b-trimethyl-7-((((R)-1-phenylpropyl)amino)methyl)tetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (24)
The reaction was carried out starting from compound 22 with (R)-(+)-α-ethylbenzylamine (0.28 mmol, 36 µL) following the general procedure. Yield: 120 mg (88%); white crystals; m.p. 144–145 °C; $[\alpha]D^{20} = -47$ (c 0.26, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.58 (s, 3H), 0.81 (t, 3H, J = 7.4 Hz), 0.81–0.91 (m, 3H), 0.93 (s, 3H), 0.95–1.02 (m, 3H), 1.10 (s, 3H), 1.14–1.20 (m, 1H), 1.32–1.79 (m, 14H), 2.12 (d, 1H, J = 13.3 Hz), 2.30 (t, 1H, J = 11.7 Hz), 2.82 (dd, 1H, J = 4.2 Hz, 11.0 Hz), 3.47–3.50 (m, 2H), 3.54 (s, 3H), 7.21–7.25 (m, 1H), 7.26–7.33 (m, 4H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 10.7 (CH_3), 12.8 (CH_3), 18.8 (CH_2), 19.5 (CH_2), 22.1 (CH_2), 25.1 (CH_3), 28.8 (CH_3), 30.7 (CH_2), 33.0 (CH_2), 34.9 (CH_2), 38.0 ($C_{(q)}$), 38.1 (CH_2), 39.6 (CH_2), 40.6 ($C_{(q)}$), 42.2 ($C_{(q)}$), 43.7 ($C_{(q)}$), 48.9 (CH), 50.2 (CH_2), 51.0 (CH_3), 54.4 (CH_2), 57.0 (CH), 57.8 (CH), 65.7 (CH), 88.8 (CH), 126.9 (2 × CH), 127.1 (2 × CH), 128.3 (CH), 144.6 ($C_{(q)}$), 178.0 (C=O); $C_{31}H_{47}NO_3$: 481.7098. HRMS (ESI+): m/z calcd for $C_{31}H_{48}NO_3$ $[M + H]^+$ 482.3634; found 482.3644.

(4R,4aS,6aS,7R,8R,9S,11bS)-Methyl 8-hydroxy-4,9,11b-trimethyl-7-((((S)-1-phenylpropyl)amino)methyl)tetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (25)
The reaction was carried out starting from compound 22 with (S)-(+)-α-ethylbenzylamine (0.28 mmol, 36 µL) following the general procedure. Yield: 60 mg (48%); white crystals; m.p. 115–116 °C; $[\alpha]D^{20} = -23$ (c 0.27, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.71 (s, 3H), 0.78–0.83 (m, 4H), 0.85–0.88 (m, 4H), 0.91–1.02 (m, 4H), 1.13 (s, 3H), 1.15–1.21 (m, 2H), 1.39–1.83 (m, 14H), 2.08–2.17 (m, 2H), 2.85 (dd, 1H, J = 3.9 Hz, 11.1 Hz), 3.34 (d, 1H, J = 4.9 Hz), 3.53 (t, 1H, J = 7.1 Hz), 3.62 (s, 3H), 7.23–7.24 (m, 1H), 7.28–7.34 (m, 4H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 10.7 (CH_3), 13.1 (CH_3), 19.0 (CH_2), 19.6 (CH_2), 22.1 (CH_2), 25.1 (CH_3), 29.0 (CH_3), 31.6 (CH_2), 33.0 (CH_2), 34.9 (CH_2), 37.9 (CH), 38.0 ($C_{(q)}$), 39.6 (CH_2), 40.4 ($C_{(q)}$), 42.1 ($C_{(q)}$), 43.7 ($C_{(q)}$), 48.5 (CH), 49.6 (CH_2), 51.3 (CH_3), 54.3 (CH_2), 57.1 (CH), 57.9 (CH), 64.9 (CH), 88.5 (CH), 126.9 (CH), 127.5

(2 × CH), 128.3 (2 × CH), 144.0 ($C_{(q)}$), 177.9 (C=O); $C_{31}H_{47}NO_3$: 481.7098. HRMS (ESI+): m/z calcd for $C_{31}H_{48}NO_3$ $[M + H]^+$ 482.3634; found 482.3631.

(4R,4aS,6aS,7R,8R,9S,11bS)-Methyl 8-hydroxy-4,9,11b-trimethyl-7-((((S)-1-(naphthalen-2-yl)ethyl)amino)methyl)tetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (26)

The reaction was carried out starting from compound 22 with (S)-(+)-1-(1-naphthyl)ethylamine (0.28 mmol, 45 µL) following the general procedure. Yield: 60 mg (42%); white crystals; m.p. 194–195 °C; $[\alpha]D^{20} = -65$ (c 0.47, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.66 (s, 3H), 0.81–0.87 (m, 2H), 0.89 (s, 3H), 0.91–1.01 (m, 4H), 1.10 (s, 3H), 1.14–1.23 (m, 2H), 1.37–1.40 (m, 1H), 1.43–1.46 (m, 1H), 1.48 (d, 3H, J = 6.6 Hz), 1.59–1.82 (m, 6H), 1.87–1.89 (m, 1H), 2.12 (d, 1H, J = 13.4 Hz), 2.23 (t, 1H, J = 12.0 Hz), 2.31 (s, 1H), 2.95 (dd, 1H, J = 3.9 Hz, 11.0 Hz), 3.43 (s, 3H), 3.50 (d, 1H, J = 4.8 Hz), 4.67–4.71 (m, 1H), 7.45–7.52 (m, 3H), 7.69 (d, 1H, J = 7.0 Hz), 7.74 (d, 1H, J = 8.2 Hz), 7.86 (d, 1H, J = 7.8 Hz), 8.20 (d, 1H, J = 8.4 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.1 (CH_3), 18.9 (CH_2), 19.6 (CH_2), 22.1 (CH_2), 24.5 (CH_3), 25.1 (CH_3), 28.9 (CH_3), 33.0 (CH_2), 34.9 (CH_2), 37.9 (CH), 38.1 ($C_{(q)}$), 39.6 (CH_2), 40.5 ($C_{(q)}$), 42.2 ($C_{(q)}$), 43.7 ($C_{(q)}$), 48.6 (CH_2), 50.2 (CH_2), 51.0 (CH_3), 53.7 (CH), 54.3 (CH_2), 57.1 (CH), 57.8 (CH), 88.6 (CH), 122.6 (CH), 122.9 (CH), 125.3 (CH), 125.7 (2 × CH), 127.1 (CH), 128.9 (CH), 131.5 ($C_{(q)}$), 134.0 ($C_{(q)}$), 141.2 ($C_{(q)}$), 177.9 (C=O); $C_{34}H_{47}NO_3$: 517.7419. HRMS (ESI+): m/z calcd for $C_{34}H_{48}NO_3$ $[M + H]^+$ 518.3634; found 518.3629.

(4R,4aS,6aS,7R,8R,9S,11bS)-Methyl 8-hydroxy-4,9,11b-trimethyl-7-((((R)-1-(naphthalen-2-yl)ethyl)amino)methyl)tetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (27)

The reaction was carried out starting from compound 22 with (R)-(+)-1-(1-naphthyl)ethylamine (0.28 mmol, 45 µL) following the general procedure. Yield: 60 mg (38%); white crystals; m.p. 153–154 °C; $[\alpha]D^{20} = -40$ (c 0.75, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.66 (s, 3H), 0.84–0.92 (m, 2H), 0.93 (s, 3H), 0.95–1.02 (m, 3H), 1.09 (s, 3H), 1.15–1.22 (m, 1H), 1.37–1.47 (m, 3H), 1.51 (d, 3H, J = 6.7 Hz), 1.57–1.86 (m, 9H), 2.12 (d, 1H, J = 13.3 Hz), 2.42 (t, 1H, J = 11.3 Hz), 2.96 (dd, 1H, J = 3.9 Hz, 10.9 Hz), 3.50 (s, 3H), 3.52 (d, 1H, J = 5.0 Hz), 4.63 (m, 1H), 7.44–7.54 (m, 3H), 7.64 (d, 1H, J = 7.2 Hz), 7.74 (d, 1H, J = 8.3 Hz), 7.87 (d, 1H, J = 7.6 Hz), 8.19 (d, 1H, J = 8.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 12.8 (CH_3), 18.9 (CH_2), 19.5 (CH_2), 22.2 (CH_2), 23.5 (CH_3), 25.1 (CH_3), 28.8 (CH_3), 33.0 (CH_2), 34.9 (CH_2), 38.1 (CH), 38.1 ($C_{(q)}$), 39.6 (CH_2), 40.7 ($C_{(q)}$), 42.3 ($C_{(q)}$), 43.7 ($C_{(q)}$), 49.1 (CH_2), 50.4 (CH_2), 51.0 (CH_3), 54.3 (CH_2), 54.4 ($C_{(q)}$), 57.0 (CH), 57.8 (CH), 88.8 (CH), 122.4 (CH), 123.0 (CH), 125.4 (CH), 125.7 (CH), 125.8 (CH), 127.2 (CH), 129.0 (CH), 131.2 ($C_{(q)}$), 134.1 ($C_{(q)}$), 141.6 ($C_{(q)}$), 178.0 (C=O); $C_{34}H_{47}NO_3$: 517.7419. HRMS (ESI+): m/z calcd for $C_{34}H_{48}NO_3$ $[M + H]^+$ 518.3634; found 518.3632.

(4R,4aS,6aS,7R,8R,9S,11bS)-Methyl 7-(((3-(1H-imidazol-1-yl)propyl)amino)methyl)-8-hydroxy-4,9,11b-trimethyltetradecahydro-6a,9-methanocyclohepta[a]naphthalene-4-carboxylate (28)

The reaction was carried out starting from compound 22 with 1-(3-aminopropyl)imidazole (0.28 mmol, 33 µL) following the general procedure. Yield: 60 mg (42%); white crystals; m.p. 107–108 °C; $[\alpha]D^{20} = -39$ (c 0.20, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.71 (s, 3H), 0.84–0.90 (m, 1H), 0.92 (s, 3H), 0.95–1.08 (m, 5H), 1.16 (s, 3H), 1.18–1.22 (m, 1H), 1.34–1.42 (m, 2H), 1.64–1.81 (m, 9H), 1.94–1.99 (m, 2H), 2.16 (d, 1H, J = 13.3 Hz), 2.31 (t, 1H, J = 11.8 Hz), 2.55–2.60 (m, 1H), 2.71–2.76 (m, 1H), 2.95 (dd, 1H, J = 4.0 Hz, 11.0 Hz), 3.43 (d, 1H, J = 4.8 Hz), 3.62 (s, 3H), 4.00–4.09 (m, 2H), 6.92 (s, 1H), 7.05 (s, 1H), 7.48 (s, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.2 (CH_3), 19.0 (CH_2), 19.6 (CH_2), 22.2 (CH_2), 25.1 (CH_3), 29.0 (CH_3), 31.4 (CH_2), 33.1 (CH_2), 35.1 (CH_2), 38.0 (CH_2), 38.2 ($C_{(q)}$), 39.7 (CH_2), 40.8 ($C_{(q)}$), 42.4 ($C_{(q)}$), 43.8 ($C_{(q)}$), 44.9 (CH_2), 46.9 (CH_2), 48.3 (CH), 51.2 (CH_3), 52.5 (CH_2), 54.3 (CH_2), 57.3 (CH), 57.8 (CH), 88.7 (CH), 118.9 (CH), 129.6 (CH), 137.3 (CH), 177.9 (C=O); $C_{28}H_{45}N_3O_3$: 471.6752. HRMS (ESI+): m/z calcd for $C_{28}H_{46}N_3O_3$ $[M + H]^+$ 472.3539; found 472.3551.

Determination of the antiproliferative effect

The growth-suppressive properties of the isosteviol-derived 1,3-aminoalcohols were assessed using a conventional MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay across a panel of five cell lines, comprising HeLa (cervical carcinoma), MDA-MB-231 and MCF-7 (breast carcinomas), and A2780 (ovarian carcinoma) cells [32]. All cell lines were sourced from the European Collection of Authenticated Cell Cultures (Salisbury, UK). Cultures were maintained in minimal essential medium augmented with 10% foetal

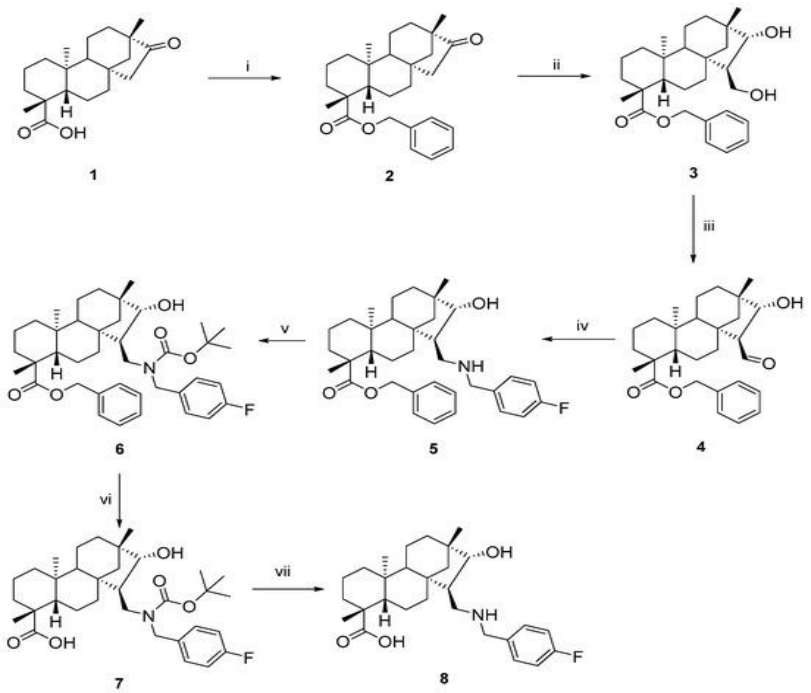
bovine serum, 1% non-essential amino acids, and 1% penicillin–streptomycin mixture, incubated at 37 °C in a humidified incubator with 5% CO2. Media components and supplements were supplied by Lonza Group Ltd. (Basel, Switzerland). Tumour cells were plated in 96-well microplates at a density of 5000 cells per well. Following overnight incubation, test compounds were introduced at two concentrations (10 μM and 30 μM) and cultured for an additional 72 h under standard conditions. Subsequently, 20 μL of MTT solution (5 mg/mL) was added to each well, followed by a 4 h incubation. Culture medium was aspirated, and the resulting formazan crystals were solubilised in DMSO with shaking for 60 min at 37 °C. Absorbance readings were taken at 545 nm on a microplate reader. Vehicle-treated cells served as controls. For compounds demonstrating strong inhibition (exceeding 50% at 10 μM or 85% at 30 μM), full dose-response experiments were conducted over a concentration range of 1.0–30 μM to calculate IC50 values. Each assay was replicated in two independent runs, with five replicate wells per condition. Data analysis and IC50 determination were carried out using GraphPad Prism 10.0 software (GraphPad Software Inc., San Diego, CA, USA).

Results and Discussion

Synthesis of isosteviol-based 1,3-Aminoalcohols

Our prior structure–activity investigations showed that the most potent antiproliferative effects against tumour cell lines were achieved with an isosteviol methyl ester incorporating a 1,3-aminoalcohol unit and an N-(4-fluorobenzyl) substituent. This lead structure was therefore selected for additional structural optimisation [26]. Two main strategies were evaluated: appending novel groups to the secondary amine or transforming the ester functionality at C-19. Further alkylation of the nitrogen was avoided, since earlier data established that a secondary NH was vital for maintaining cytotoxicity. Consequently, variations on the existing benzylamino substituent offered the most potential. Published studies indicated that the C-19 carboxyl moiety had little direct influence on biological activity, positioning it as an ideal handle for diversification.

Efforts to access a free carboxylic acid analogue via demethylation of the ester were unsuccessful, necessitating a full revision of the synthetic plan. Introducing an unprotected carboxylic acid too early caused interfering side reactions during amine incorporation, producing mixtures that resisted purification. A benzyl ester was adopted instead, enabling clean late-stage hydrogenolytic cleavage and improved intermediate handling during chromatography. The crucial intermediate 7 was secured through a six-step sequence in reasonable overall yield **(Scheme 1).**



Scheme 1. Synthesis of isosteviol-based N-(4-fluorobenzyl)-1,3-aminoalcohol 8. (i) BnBr, K2CO3, dry acetone, 4 h, 60 °C, 87%; (ii) HCHO, NaOEt, dry EtOH, 1 h, 60 °C, 78%; (iii) 10 mol% TEMPO, NBS, TBAB, DCM/H2O, 12 h, reflux, 83%; (iv) (1) 4-FBnNH2, dry EtOH, 3 h, 25 °C; (2) MeOH, NaBH4, 4 h, 25

°C, 70%; (v) Boc2O, dry DCM, 1 h, 25 °C, 79%; (vi) EtOAc/n-hexane, 5% Pd/C, H2 (1 atm), 24 h, 25 °C, 82%; (vii) (1) TFA, dry DCM, 3 h, 25 °C; (2) Et3N, dry DCM, 3 min, 25 °C, 77%.

Starting material isosteviol 1 was generated from a natural stevioside mixture by acid-promoted hydrolysis accompanied by Wagner–Meerwein rearrangement, as described previously [9]. Esterification with benzyl bromide delivered benzyl ester 2 efficiently.

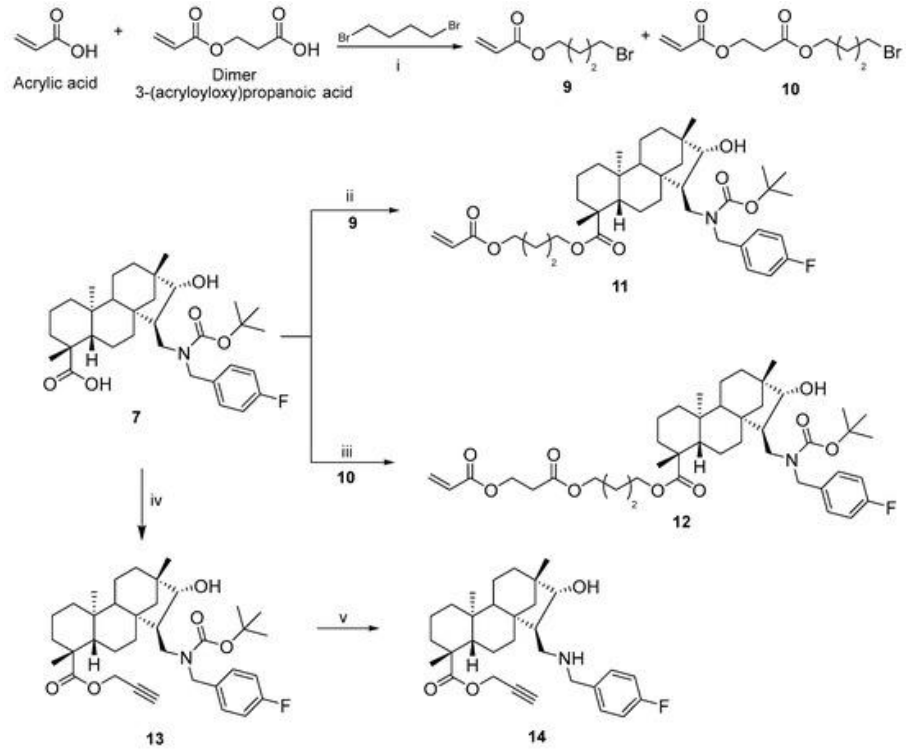
The diol benzyl ester 3 was accessed in high yield through a stereocontrolled one-pot aldol-Cannizzaro reaction, following established protocol [33]. Regioselective conversion to aldehyde 4 proceeded smoothly under TBAB-assisted TEMPO/NBS oxidation conditions. Construction of the N-(4-fluorobenzyl)-1,3-aminoalcohol benzyl ester 5 involved a sequential reductive amination: condensation of aldehyde 4 with 4-fluorobenzylamine to the imine, then in situ reduction using NaBH4 at ambient temperature.

Progress toward target 8 required selective removal of the C-19 benzyl ester without affecting the N-benzyl group. Temporary Boc protection of the amine circumvented this issue. Hydrogenolytic cleavage of protected intermediate 6 using Pd/C in an EtOAc/n-hexane mixture provided carboxylic acid 7 in high yield, leaving the fluorobenzyl intact.

Acid-mediated Boc cleavage with TFA generated the corresponding ammonium salt, which upon treatment with triethylamine in dichloromethane afforded the pure free amine 8 **(Scheme 1).**

(Acryloyloxy)butyl and propargyl ester derivatives of N-Substituted 1,3-Aminoalcohols

An acrylate-containing spacer was constructed via direct esterification of acrylic acid with 1,4-dibromobutane, employing excess dibromide to favour monoester formation. Extended reaction times and acrylic acid's tendency to oligomerise nevertheless produced both mono- and di-acrylate products, which were separated and carried forward **(Scheme 2).** This serendipitous outcome enabled examination of alkyl chain extension on biological performance.



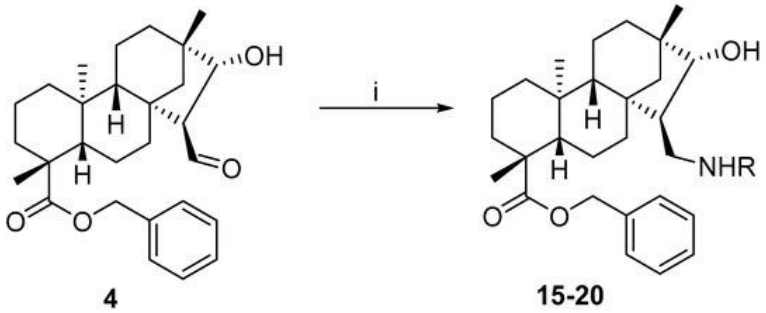
Scheme 2. Synthesis of allyl (11, 12) and acetylene derivatives (13, 14). (i) Acrylic acid, K2CO3, 1,4-dibromobutane, dry acetone, 24 h, 25 °C, 61% (9), 26% (10); (ii) 4-bromobutyl acrylate, K2CO3, dry acetone, 24 h, 25 °C, 83%; (iii) 3-(4-bromobutoxy)-3-oxopropyl acrylate, K2CO3, dry acetone, 24 h, 25 °C, 77%; (iv) propargyl bromide, K2CO3, dry acetone, 24 h, 25 °C, 88%; (v) (1) TFA, dry DCM, 3 h, 25 °C; (2) Et3N, dry DCM, 3 min, 25 °C, 79%.

Acrylate bromides bearing one or two vinyl units were appended to acid 7 by standard esterification, yielding protected intermediates 11 and 12. The Boc group was retained throughout to suppress competitive N-alkylation. Subsequent attempts at deprotection triggered complex mixtures, likely arising from aza-Michael additions involving the secondary amine and acrylate moieties **(Scheme 2).**

In contrast, installation of a terminal alkyne at C-19 succeeded: alkylation of 7 with propargyl bromide followed by clean Boc removal furnished compound 14 **(Scheme 2).**

Isosteviol-Based 1,3-Aminoalcohols with Diverse O and N Functions

Isosteviol-derived 1,3-aminoalcohols consistently outperformed other analogues in antiproliferative assays, prompting expansion of this scaffold. Hydroxyaldehyde benzyl ester 4 was treated with six varied primary amines under optimised reductive amination conditions to deliver N-diversified products 15–20. Each sequence comprised imine formation followed by mild NaBH4 reduction, providing the targets in acceptable yields. Crystallisation ensured high purity (98%) despite moderate recoveries. Structures and yields are summarised in **Scheme 3 and Table 1.**

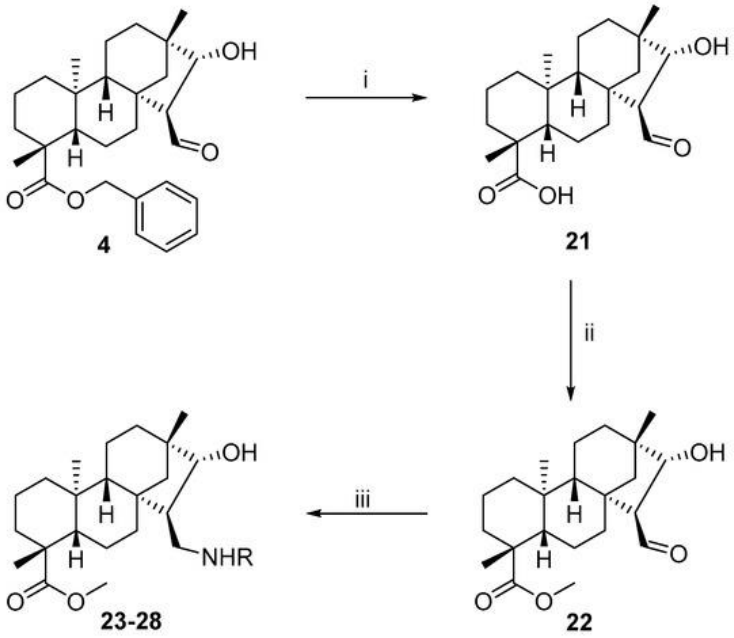


Scheme 3. Synthesis of isosteviol-based 1,3-aminoalcohols. (i) (1) RNH2 (1 equ.), dry EtOH, 3 h, 25 °C; (2) MeOH, NaBH4 (2 equ.), 4 h, 25 °C, 21–68%.

Table 1. Synthesis of 1,3-aminoalcohols 14–19 via Schiff intermediates.

Entry	RNH_2	Product	Yield (%)
1	(R)-4-fluoro-α-methylbenzylamine	**15**	21
2	(R)-(+)-α-ethylbenzylamine	**16**	52
3	(S)-(+)-α-ethylbenzylamine	**17**	68
4	(S)-(+)-1-(1-naphthyl)ethylamine	**18**	40
5	(R)-(+)-1-(1-naphthyl)ethylamine	**19**	46
6	1-(3-aminopropyl)imidazole	**20**	33

To probe how different nitrogen substituents modulate cytotoxicity, an additional set of six 1,3-aminoalcohols (23–28) was assembled. Benzyl ester 4 was first converted to carboxylic acid 21 by hydrogenolysis over Pd/C in n-hexane/EtOAc, then remethylated with diazomethane to give aldehyde 22 **(Scheme 4).**



Scheme 4. Synthesis of the library of 23–28. (i) EtOAc/n-hexane 1:1, 5% Pd/C, H2 (1 atm), 24 h, 25 °C, 63%; (ii) CH2N2, Et2O, 5 min, 25 °C, 81%; (iii) (1) RNH2 (1 equ.), dry EtOH, 3 h, 25 °C; (2) MeOH, NaBH4 (2 equ.), 4 h, 25 °C, 38–88%.

Reductive amination of methyl ester aldehyde 22 with the same panel of primary amines produced library members 23–28 **(Scheme 4 and Table 2).**

Table 2. Synthesis of 1,3-aminoalcohols 23–28 via Schiff products.

Entry	RNH_2	Product	Yield (%)
1	(R)-4-fluoro-α-methylbenzylamine	**23**	50
2	(R)-(+)-α-ethylbenzylamine	**24**	88
3	(S)-(+)-α-ethylbenzylamine	**25**	48
4	(S)-(+)-1-(1-naphthyl)ethylamine	**26**	42
5	(R)-(+)-1-(1-naphthyl)ethylamine	**27**	38
6	1-(3-aminopropyl)imidazole	**28**	58

In vitro antiproliferative studies of steviol-based aminoalcohols and structure–activity relationship

The growth-inhibitory potential of the new diterpene compounds was assessed via MTT assays across several human adherent tumour lines derived from cervical (HeLa), breast (MDA-MB-231, MCF-7), and ovarian (A2780) carcinomas, with detailed data shown in **Figure 1**. A healthy mouse fibroblast line (NIH/3T3) was included to probe initial selectivity toward malignant cells. The resulting potency profiles allowed derivation of key structure–activity correlations. Notably, neither the hydroxyaldehyde benzyl ester intermediate 4, nor the Boc-protected aminoalcohols (6, 7, 11, 12), nor the free-acid analogue 8 exhibited meaningful growth suppression (aside from moderate effects of 12 on A2780 cells). These observations underscored the necessity of combining an ester group with a protonatable secondary amine for robust antiproliferative action. Direct comparisons between benzyl ester amines (15–20) and corresponding methyl esters (23–28) showed no clear or significant potency variations, suggesting the ester's alcoholic component exerted negligible influence. Unfortunately, potent derivatives also markedly impaired non-cancerous cell growth, revealing limited tumour selectivity and tempering enthusiasm for most of these molecules as anticancer leads.

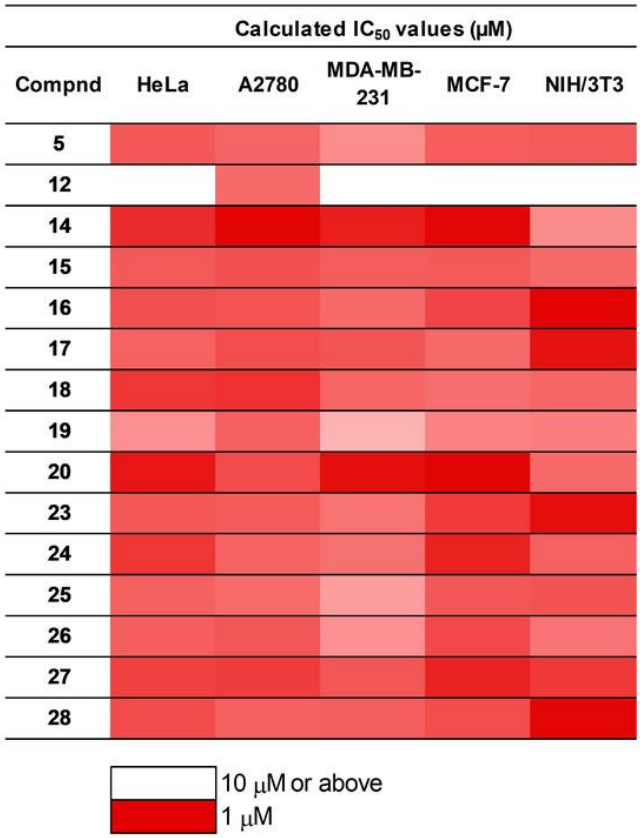


Figure 1. Antiproliferative properties showing IC50 values of the prepared isosteviol analogues against cancer cells and NIH/3T3 fibroblasts.

Modifying the ester in our prior top performer (methyl ester with N-4-fluorobenzyl aminoalcohol, top IC50: 2.14 μM on MCF-7 cells) [26] to propargyl (14, top IC50: 1.54 μM on MCF-7 cells) boosted activity, while benzyl substitution maintained similar levels—possibly from enhanced aromatic stacking interactions. Conversely, conversion to a free acid eliminated potency altogether.

Replacing the 4-fluorobenzyl unit with different aromatic motifs offered no gains. Remarkably, the analogue 20 carrying an N-(1H-imidazol-1-yl)propyl appendage proved the standout performer (top IC50: 1.37 μM on MCF-7 cells), even though past work indicated alkyl-type nitrogen substituents typically weakened effects [26, 34]. Outperforming the standard agent cisplatin in these assays, this derivative stands out as a valuable lead worthy of deeper exploration.

Conclusion

A range of diterpenoid 1,3-aminoalcohol derivatives was synthesised from isosteviol employing stereoselective reactions. To explore the influence of the ester moiety at position 4, analogues incorporating a free carboxylic acid, benzyl, propargyl, or acryloyl esters were developed, extending our prior work in this area. Antiproliferative effects of the compounds were evaluated against human cancer cell lines (A2780, HeLa, MCF-7, and MDA-MB-231). Substitution of the methyl ester with a propargyl group enhanced potency, attributable to potential π–π interactions and greater lipophilicity, whereas benzyl ester replacement offered no significant benefit. Notably, derivative 20 featuring an N-(1H-imidazol-1-yl)propyl group emerged as the most potent compound, contrary to earlier findings that N-alkyl substituents diminished activity [26, 34]. As this molecule outperformed the standard chemotherapeutic agent cisplatin, it represents a promising lead candidate. Future studies could profitably examine further aminoalcohol- or aminodiol-based diterpenes for antiproliferative potential. Furthermore, the Boc-protected aminoalcohol 12 displayed marked and selective inhibition against A2780 cells (IC50: 4.78 μM), suggesting this scaffold may serve as a basis for designing novel agents targeting ovarian cancer.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Carrera-Lanestosa, A.; Moguel-Ordóñez, Y.; Segura-Campos, M. Stevia Rebaudiana Bertoni: A Natural Alternative for Treating Diseases Associated with Metabolic Syndrome. J. Med. Food **2017**, 20, 933–943.
2. Singh, S.D.; Rao, G.P. Stevia: The Herbal Sugar of 21st Century. Sugar Tech **2005**, 7, 17–24.
3. Sanches Lopes, S.M.; Francisco, M.G.; Higashi, B.; De Almeida, R.T.R.; Krausová, G.; Pilau, E.J.; Gonçalves, J.E.; Gonçalves, R.A.C.; Oliveira, A.J.B.D. Chemical Characterization and Prebiotic Activity of Fructo-Oligosaccharides from Stevia Rebaudiana (Bertoni) Roots and in Vitro Adventitious Root Cultures. Carbohydr. Polym. **2016**, 152, 718–725.
4. Khaybullin, R.; Zhang, M.; Fu, J.; Liang, X.; Li, T.; Katritzky, A.; Okunieff, P.; Qi, X. Design and Synthesis of Isosteviol Triazole Conjugates for Cancer Therapy. Molecules **2014**, 19, 18676–18689.
5. , Y.; Badshah, S. Bioactivity Profile of the Diterpene Isosteviol and Its Derivatives. Molecules **2019**, 24, 678.
6. Luan, T.; Cao, L.-H.; Deng, H.; Shen, Q.-K.; Tian, Y.-S.; Quan, Z.-S. Design and Synthesis of C-19 Isosteviol Derivatives as Potent and Highly Selective Antiproliferative Agents. Molecules **2018**, 24, 121.
7. Huang, T.-J.; Chou, B.-H.; Lin, C.-W.; Weng, J.-H.; Chou, C.-H.; Yang, L.-M.; Lin, S.-J. Synthesis and Antiviral Effects of Isosteviol-Derived Analogues against the Hepatitis B Virus. Phytochemistry **2014**, 99, 107–114.
8. Strobykina, I.Y.; Belenok, M.G.; Semenova, M.N.; Semenov, V.V.; Babaev, V.M.; Rizvanov, I.K.; Mironov, V.F.; Kataev, V.E. Triphenylphosphonium Cations of the Diterpenoid Isosteviol: Synthesis and Antimitotic Activity in a Sea Urchin Embryo Model. J. Nat. Prod. **2015**, 78, 1300–1308.
9. Liu, C.-J.; Yu, S.-L.; Liu, Y.-P.; Dai, X.-J.; Wu, Y.; Li, R.-J.; Tao, J.-C. Synthesis, Cytotoxic Activity Evaluation and HQSAR Study of Novel Isosteviol Derivatives as Potential Anticancer Agents. Eur. J. Med. Chem. **2016**, 115, 26–40.
10. Testai, L.; Strobykina, I.; Semenov, V.V.; Semenova, M.; Pozzo, E.D.; Martelli, A.; Citi, V.; Martini, C.; Breschi, M.C.; Kataev, V.E.; et al. Mitochondriotropic and Cardioprotective Effects of Triphenylphosphonium-Conjugated Derivatives of the Diterpenoid Isosteviol. Int. J. Mol. Sci. **2017**, 18, 2060.
11. Korochkina, M.G.; Nikitashina, A.D.; Khaybullin, R.N.; Petrov, K.A.; Strobykina, I.Y.; Zobov, V.V.; Kataev, V.E. Unfolded and Macrocyclic Ammonium Derivatives of Diterpenoids Steviol and Isosteviol Having Choline Moieties. Synthesis and Inhibitory Activities toward Acetylcholine- and Butyrylcholinesterases. MedChemComm **2012**, 3, 1449–1454.
12. Wu, Y.; Liu, C.-J.; Liu, X.; Dai, G.-F.; Du, J.-Y.; Tao, J.-C. Stereoselective Synthesis, Characterization, and Antibacterial Activities of Novel Isosteviol Derivatives with D-Ring Modification. Helv. Chim. Acta **2010**, 93, 2052–2069.
13. Kataev, V.E.; Strobykina, I.Y.; Andreeva, O.V.; Garifullin, B.F.; Sharipova, R.R.; Mironov, V.F.; Chestnova, R.V. Synthesis and Antituberculosis Activity of Derivatives of Stevia Rebaudiana Glycoside Steviolbioside and Diterpenoid Isosteviol Containing Hydrazone, Hydrazide, and Pyridinoyl Moieties. Russ. J. Bioorganic Chem. **2011**, 37, 483–491.
14. McGuire, S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv. Nutr. **2016**, 7, 418–419.
15. Vera-Badillo, F.E.; Al-Mubarak, M.; Templeton, A.J.; Amir, E. Benefit and Harms of New Anti-Cancer Drugs. Curr. Oncol. Rep. **2013**, 15, 270–275.
16. Chen Eh, L.D. Anticancer Drug Development, a Matter of Money or a Matter of Idea? J. Postgenomics Drug Biomark. Dev. **2015**, 5, e134.
17. Wefel, J.S.; Schagen, S.B. Chemotherapy-Related Cognitive Dysfunction. Curr. Neurol. Neurosci. Rep. **2012**, 12, 267–275.

18. Newman, D.J.; Cragg, G.M. Natural Products as Sources of New Drugs over the Last 25 Years. J. Nat. Prod. **2007**, 70, 461–477.
19. Gordaliza, M. Natural Products as Leads to Anticancer Drugs. Clin. Transl. Oncol. **2007**, 9, 767–776.
20. Lohoelter, C.; Weckbecker, M.; Waldvogel, S.R. (–)-Isosteviol as a Versatile Ex-Chiral-Pool Building Block for Organic Chemistry. Eur. J. Org. Chem. **2013**, 2013, 5539–5554.
21. Huang, T.-J.; Yang, C.-L.; Kuo, Y.-C.; Chang, Y.-C.; Yang, L.-M.; Chou, B.-H.; Lin, S.-J. Synthesis and Anti-Hepatitis B Virus Activity of C4 Amide-Substituted Isosteviol Derivatives. Bioorg. Med. Chem. **2015**, 23, 720–728.
22. Heise, N.V.; Heisig, J.; Meier, K.; Csuk, R.; Mueller, T. F16 Hybrids Derived from Steviol or Isosteviol Are Accumulated in the Mitochondria of Tumor Cells and Overcome Drug Resistance. Molecules **2024**, 29, 381.
23. Yang, Y.; Zhao, L.; Wang, T.; Zheng, X.; Wu, Y. Biological Activity and Structural Modification of Isosteviol over the Past 15 Years. Bioorganic Chem. **2024**, 143, 107074.
24. Abdullah Al-Dhabi, N.; Valan Arasu, M.; Rejiniemon, T.S. In Vitro Antibacterial, Antifungal, Antibiofilm, Antioxidant, and Anticancer Properties of Isosteviol Isolated from Endangered Medicinal Plant Pittosporum tetraspermum. Evid. Based Complement. Alternat. Med. **2015**, 2015, 164261.
25. Zhang, T.; Lu, L.-H.; Liu, H.; Wang, J.-W.; Wang, R.-X.; Zhang, Y.-X.; Tao, J.-C. D-Ring Modified Novel Isosteviol Derivatives: Design, Synthesis and Cytotoxic Activity Evaluation. Bioorg. Med. Chem. Lett. **2012**, 22, 5827–5832.
26. Ozsvár, D.; Nagy, V.; Zupkó, I.; Szakonyi, Z. Synthesis and Biological Application of Isosteviol-Based 1,3-Aminoalcohols. Int. J. Mol. Sci. **2021**, 22, 11232.
27. Melis, M.S. Renal Excretion of Stevioside in Rats. J. Nat. Prod. **1992**, 55, 688–690.
28. Li, N.; Li, X.; Deng, M.; Zhu, F.; Wang, Z.; Sheng, R.; Wu, W.; Guo, R. Isosteviol Derivatives as Protein Tyrosine Phosphatase-1B Inhibitors: Synthesis, Biological Evaluation and Molecular Docking. Bioorg. Med. Chem. **2023**, 83, 117240.
29. Li, J.; Zhang, D.; Wu, X. Synthesis and Biological Evaluation of Novel Exo-Methylene Cyclopentanone Tetracyclic Diterpenoids as Antitumor Agents. Bioorg. Med. Chem. Lett. **2011**, 21, 130–132.
30. Malik, S.; Kawano, S.; Fujita, N.; Shinkai, S. Pyridine-Containing Versatile Gelators for Post-Modification of Gel Tissues toward Construction of Novel Porphyrin Nanotubes. Tetrahedron **2007**, 63, 7326–7333.
31. Jayachandra, R.; Zhao, H.; Cheng, Z.; Luo, L.; Sun, T.; Tan, W. Synthesis of Isosteviol Analogues as Potential Protective Agents against Doxorubicin-Induced Cardiomyopathy in Zebrafish Embryos. Bioorg. Med. Chem. Lett. **2019**, 29, 1705–1709.
32. Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. J. Immunol. Methods **1983**, 65, 55–63.
33. Wu, Y.; Yang, J.-H.; Dai, G.-F.; Liu, C.-J.; Tian, G.-Q.; Ma, W.-Y.; Tao, J.-C. Stereoselective Synthesis of Bioactive Isosteviol Derivatives as α-Glucosidase Inhibitors. Bioorg. Med. Chem. **2009**, 17, 1464–1473.
34. Ozsvár, D.; Nagy, V.; Zupkó, I.; Szakonyi, Z. Stereoselective Synthesis and Antiproliferative Activity of Steviol-Based Diterpen Aminodiols. Int. J. Mol. Sci. **2020**, 21, 184.