Semi-Synthesis and Cytotoxicity Evaluation of Pyrimidine, Thiazole, and Indole Analogues of Argentatins A−C from Guayule (Parthenium Argentatum) Resin

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Semi-synthesis and cytotoxicity evaluation of pyrimidine, thiazole, and indole analogues of argentatins A–C from guayule (*Parthenium argentatum*) resin

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Abstract

Argentatins A–C (**1–3**), the major cycloartane-type triterpenoids of guayule resin, a byproduct of commercial rubber production, were converted into their pyrimidine (**7–12**), thiazole (**13–15**), and indole (**16–18**) analogues by a molecular hybridization approach. The cytotoxic activities of these fused heterocyclic analogues **7–18** were compared with those of argentatins A–C (**1–3**) against a panel of three sentinel human cancer cell lines [NCI-H460 (non-small cell lung), MCF-7 (breast adenocarcinoma), and SF-268 (central nervous system glioma)], and normal human fibroblast (WI-38) cells. The cytotoxicity data suggest that the pyrimidine analogues **7** and **8** (derived from **1**), **9** and **10** (derived from **2**), and **12** (derived from **3**) had significantly enhanced activity compared to the parent compounds or their thiazole (**13–15**) and indole (**16–18**) analogues. These findings indicate that triterpenoid constituents of guayule resin may be exploited to obtain value-added products with potential applications in anticancer drug discovery.

Graphical abstract
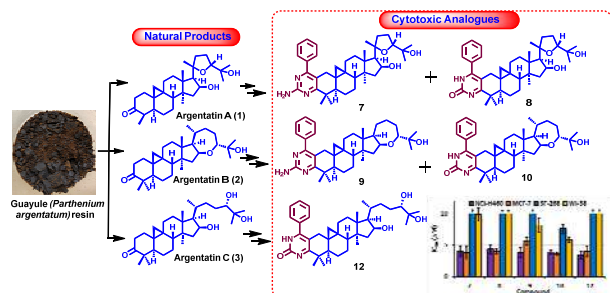
2

Introduction

Natural products (NPs) continue to be valuable sources of structurally diverse and biologically active compounds for drug discovery [1–3]. Many NPs have also served as promising lead compounds for drug development, especially as anticancer agents, after chemical modifications [4, 5]. Triterpenoids, one of the major classes of NPs, have been reported to exhibit a variety of biological activities [6, 7]. Among these, cycloartane-type triterpenoids are found widely in plants and algae, some of which are known to display cytotoxic activity [8, 9]. Cycloartane-type triterpenoids, such as argentatins A–C, have been found to be the major chemical components in *Parthenium argentatum* Gray (guayule) resin [10, 11], which is one of the byproducts in the manufacture of natural rubber from guayule, a widely-investigated arid land crop of potential industrial importance [12−14]. Among argentatins, argentatin A has been found to have antimicrobial activity [15], whereas argentatin B is known to inhibit proliferation of colon (HCT-15) and prostate (PC-3) cancer cells [16]. Detailed studies on argentatin B has revealed that it is a non-competitive inhibitor of 3H-estradiol-binding receptors of hormone-dependent breast cancer [17]. In addition, ring A modification of argentatins A and B have provided several analogues with enhanced cytotoxic activity against some selected cancer cell lines [18, 19]. Therefore, it was of interest to investigate the effect of structural modifications of argentatins A–C (**1–3**) on their potential anticancer activity for the purpose of obtaining value-added products from this byproduct of guayule rubber production.

Heteroaromatic moieties such as pyrimidines, thiazoles, and indoles are important fragments representing useful pharmacophores for the enhancement of therapeutic activity. Compounds containing these moieties have been found to exhibit a wide variety of biological activities, including anticancer, anti-inflammatory, antimicrobial, anti-HIV, anti-tubercular, antioxidant, and anti-hypertensive activities [20–25]. Molecular hybridization involving the linking of two structural moieties as a single hybrid is an important strategy in medicinal chemistry to enhance biological activity and pharmacokinetic properties

[26, 27]. We envisaged that argentatins A–C (**1–3**) with a carbonyl group at C-3 in ring A and some differences in ring D and the side chain could serve as suitable starting materials for conversion into the corresponding heteroaromatic hybrids which may then display enhanced biological activities. Herein we report the semi-synthesis of novel pyrimidine, thiazole, and indole analogues **7–18** of argentatins A–C (**1–3**) and the investigation of their cytotoxic activity against three sentinel human cancer cell lines and normal human fibroblast cells.

Results and discussion

Chemistry

Argentatins A–C (**1–3**) obtained from guayule resin [28], were used for the synthesis of their target pyrimidine, thiazole, and indole analogues as outlined in Schemes 1 and 2. Depicted in Scheme 1 is the synthesis of pyrimidine analogues (**7–12**). Claisen-Schmidt condensation [22] of **1–3** with benzaldehyde afforded their corresponding benzylidene intermediates **4–6** in 74–77% yield, which were the key precursors for the synthesis of their pyrimidine analogues. Presence of the benzylidene moiety in **4–6** were inferred from their characteristic absorption bands of the α,β-unsaturated carbonyl group at 1678 cm^{-1} in their IR spectra and the presence of a signal at δ_C ~208 in their ^{13}C NMR spectra. Intermediates **4–6** were separately treated with guanidine hydrochloride, thiourea, and urea in the presence of ethanolic KOH under reflux to give crude products which on treatment with DDQ (2,3-dichloro-5,6-dicyano-*p*-benzoquinone) in dry 1,4-dioxane at room temperature afforded 2'-amino-pyrimidine analogues (**7**, **9**, and **11**) in 49–79% yield and the 2'-oxo-3'H-pyrimidine analogues (**8**, **10**, and **12**) in 31–58% yield. The structures of pyrimidine analogues were confirmed by the characteristic peaks in their ^{13}C NMR spectra. Signals around δ_C 175, 166, 161, and 116 ppm in 2'-amino-pyrimidine analogues **7**, **9**, and **11** were assigned to C-3, C-4', C-2' and C-2, respectively by the HMBC correlations of H$_3$-29/C-3, H$_3$-30/C-3, H$_2$-1/C-3, H$_2$-1/C-4', and H$_2$-1/C-2. The presence of the 2'-amino-pyrimidine moiety in these was further

confirmed by the ^{1}H NMR signal (δ_H 4.80-4.90, brs, 2H) due to the NH_2 group. It is noteworthy that the pyrimidine analogues **8**, **10**, and **12** exhibited only two ^{13}C NMR signals for the pyrimidine moiety (at δ_C ~159 and 110–112 ppm), assigned for C-2' and C-2 respectively, and the chemical shift of C-2 indicated the presence of 2'-oxo-substituted pyrimidine moiety in these [29]. The actual tautomer of pyrimidine moiety was suggested to be 3'H-pyrimidine because C-3 of **10** was detected at δ_C 164.4 ppm by the HMBC experiment although the signals due to C-3 and C-4' of the pyrimidine ring were not detected or appeared as very weak peaks [30].

Synthesis of thiazole and indole analogues (**13–18**) of argentatins A–C (**1–3**) is outlined in Scheme 2. The target thiazole analogues were obtained by a two-step protocol. Compounds **1–3** were brominated at C-2 by the reaction with liquid bromine in acetic acid at 0 ºC–25 ºC [18]. The resulting mixture of bromoketones (α and β epimers) were used without further purification for the next step involving cyclization with thiourea [31] in ethanol at reflux temperature to afford fused amino-thiazoles (**13–15**) in 46–58% yield. The identities of these products were confirmed by their NMR data. The characteristic broad singlet proton signals at δ_H ~ 4.78 ppm of these in their ^{1}H NMR spectra were assigned to the NH_2 group, whereas the carbon signals in their ^{13}C NMR spectra at δ_C ~164–168 (C-2'), ~154 (C-3), and ~116 (C-2) corresponding to thiazole rings were assigned with the help of HMBC correlations of H_2-1/C-3, H_2-1/C-2 and H_3-29/C-3, H_3-30/C-3.

The desired fused indole analogues (**16–18**) were prepared by Fries indole synthesis [31] involving condensation of argentatins A–C (**1–3**) with phenylhydrazine hydrochloride in acetic acid at 50 ºC. The structures of the resulting indole analogues were confirmed by their NMR data. In their ^{1}H NMR spectra, indole aromatic protons appeared as two pairs of doublets (H-2' ~7.29 ppm; H-5' ~7.38 ppm) and two pairs of triplets (H-3' ~7.10 ppm; H-4' ~7.04 ppm), and the NH protons appeared as singlets at δ_H ~7.76 ppm. The ^{13}C NMR spectra exhibited eight characteristic signals in the range of δ_C

~108–143 ppm which were assigned by HMBC correlations of H$_2$-1/C-3, C-2 and C-7'; H-1'/C-2 and C-7'; H-2'/C-4' and C-7'; H-3'/C-5' and C-6'; H-4'/C-2' and C-7'; H-5'/C-3' and C-6'; H$_3$-29/C-3; and H$_3$-30/C-3. The presence of cyclopropane ring of the argentatin moieties of **16–18** was confirmed by the proton signals at δ_H ~0.5 and 0.7 in their ^{1}H NMR spectra and the carbon signals at δ_C ~30 in their ^{13}C NMR spectra. The structures of all synthesized compounds were further supported by their HRMS data.

Cytotoxicity evaluation

All twelve synthetic analogues (**7–18**) and their parent argentatins A–C (**1–3**) were evaluated for their cell proliferation inhibitory activity against a panel of three human sentinel cancer cell lines [NCI-H460 (non-small cell lung cancer), MCF-7 (breast adenocarcinoma), SF-268 (CNS cancer, glioma)] and normal human primary fibroblast (WI-38) cells by using the MTT assay [32]. Doxorubicin and DMSO were used as positive and negative controls, respectively. The results obtained are summarized in Table 1. These results revealed that compounds **7**, **8**, **9**, **10**, and **12** were active below a 10 μM concentration against most of the tested cell lines. Interestingly, amino-pyrimidine analogues **7** and **9** and oxo-pyrimidine analogues **8**, **10**, and **12** showed moderate cytotoxic effect against NCI-H460 and MCF-7 and were about 4–9-fold more active than the parent compounds **1–3**. Additionally, the analogue **10** derived from argentatin B (**2**) showed moderate cytotoxicity against SF-268 cells and was ca. 4-fold more active than the parent compound. Significantly, most of the active compounds exhibited less cytotoxic activity towards the normal human fibroblast (WI-38) cells.

Conclusion

In summary, we designed and synthesized twelve new fused heterocyclic analogues **7–18** of argentatins A–C (**1–3**) by utilizing the C-3 keto functionality of ring A of the parent compounds. All analogues were evaluated for their cell proliferation inhibitory activity against the three sentinel cancer cell lines NCI-H460, MCF-7, SF-268, and towards normal fibroblast cells, WI-38. The bioassay results demonstrated that

that the analogues **7**, **8**, **9**, **10**, and **12** exhibited enhanced cytotoxic activity against NCI-H460 and MCF-7 cells compared to the parent argentatins **1**–**3**. Among all compounds tested, the pyrimidine analogue **10** was unique in displaying single-digit micro-molar activity against SF-268 cells. The preliminary structure-activity relationship (SAR) study suggested that the 2'-amino-pyrimidine and 2'-oxo-3'H-pyrimidine derivatives were more active than the thiazole and the indole analogues against the cell lines tested. These findings support the possibility of development of potential anticancer agents from some triterpenoid constituents of the guayule resin.

Experimental

Chemistry

General

Reagents for chemical synthesis were purchased from Sigma-Aldrich and Fischer Scientific (USA). All solvents were distilled prior to use. The progress of all reactions was monitored by thin-layer chromatography (TLC) using silica gel 60 F_{254} plates (Merck). Visualization was accomplished under UV light (254 nm) and spraying with p-anisaldehyde in $H_2SO_4/H_2O/CH_3CO_2H$ followed by heating. Purification of compounds was carried out by column chromatography using silica gel 40 μm flash chromatography packing (J. T. Baker, Jackson, TN, USA). 1D and 2D NMR spectra were recorded in $CDCl_3$ with a Bruker Avance III 400 spectrometer at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR using residual $CHCl_3$ as the internal standard. The chemical shift (δ) values are expressed in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz). Optical rotations were measured with a JASCO Dip-370 polarimeter using MeOH as the solvent. Preparative HPLC purification was performed on a Waters Delta Prep 4000 equipped with a PDA 996 detector using a 10 × 250 mm Phenomenex Luna 5 μm C-18 column for reversed-phase (RP) chromatography. High Resolution Mass Spectra (HRMS) were recorded on Agilent G6224A TOF mass spectrometer. The following

abbreviations were used to indicate the NMR signals: s = singlet, brs = broad singlet, d = doublet, t = triplet, m = multiplet, J = coupling constant.

Isolation and identification of argentatins A–C (1–3)

Argentatins A–C (**1**–**3**) used for the semi-synthesis of their analogues **7**–**18** were obtained from guayule (*Parthenium argentatum* AZ-2) resin as described previously [26].

General procedure for synthesis of compounds 4–6

To a solution of appropriate compounds **1** (1.0 eq, 50.0 mg), **2** (1.0 eq, 50.0 mg), and **3** (1.0 eq, 60.0 mg) in absolute EtOH (5.0 mL) were added benzaldehyde (2.0 eq) and KOH (2.0 eq), and the resulting mixture was stirred at 45 °C for 12 h. After completion of the reaction (monitored by TLC), it was neutralized with 10% aq. HCl and extracted (3 x) with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4, evaporated under reduced pressure, and the residue was passed through a silica gel column with CH_2Cl_2-*i*-PrOH (97:3) and hexane-EtOAc (85:15) as eluents to afford **4** (44.0 mg, 74%)**, 5** (46.0 mg, 77%), and **6** (46.6 mg, 79%) respectively.

2-Benzylidene-(16β,20S,24R)-20,24-epoxy-16,25-dihydroxycycloartan-3-one (**4**) Light yellow solid; $[\alpha]_D^{25}$ +136.0° (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3394 (O-H), 2970 (C-H), 1678 (α,β-unsaturated ketone), 1593 (Ar C=C), 1461, 1380, 1164 (C–O), 1018, 952, 694 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.44 (d, J = 4.6 Hz, 1H, H-19a), 0.70 (d, J = 4.6 Hz, 1H, H-19b), 0.91 (s, 3H, H$_3$-28), 0.92 (m, 1H, H-6a), 1.07 (s, 3H, H$_3$-29), 1.21 (m, 1H, H-7a), 1.11 (s, 3H, H$_3$-26), 1.17 (s, 3H, H$_3$-30), 1.22 (s, 3H, H$_3$-27), 1.25 (s, 3H, H$_3$-18), 1.38 (m, 1H, H-7b), 1.40 (s, 3H, H$_3$-21), 1.51 (m, 1H, H-15a), 1.60 (m, 1H, H-8), 1.64 (m, 1H, H-6b), 1.70 (m, 1H, H-22a), 1.76 (m, 2H, H$_2$-12), 1.98 (m, 1H, H-5), 1.94 (m, 2H, H$_2$-23), 2.03 (m, 1H, H-15b), 2.13 (d, J = 7.8 Hz, 1H, H-17), 2.16 (m, 2H, H$_2$-11), 2.24 (m, 1H, H-22b), 2.51 (d, J = 16.2 Hz, 1H, H-1a), 2.85 (d, J = 16.2 Hz, 1H, H-1b), 3.82 (t, J = 15.0 Hz, 1H, H-24), 4.59 (m, 1H, H-16), 7.27-7.36 (m, 5H, Ar-H), 7.37 (s, 1H, H-2'); ^{13}C NMR (100 MHz, $CDCl_3$): δ 208.1 (C-3), 135.9 (C-2'),

135.8 (C-2), 130.1 (Ar-C), 128.3 (Ar-C), 128.2 (Ar-C), 87.1 (C-20), 84.5 (C-24), 73.3 (C-16), 70.8 (C-25), 55.6 (C-17), 48.7 (C-4), 48.6 (C-15), 48.4 (C-8), 46.5 (C-14), 46.3 (C-13), 45.4 (C-5), 37.3 (C-22), 36.6 (C-1), 33.1 (C-12), 30.3 (C-19), 27.3 (C-27), 26.2 (C-11), 26.1 (C-26), 25.5 (C-7), 25.3 (C- 18), 24.2 (C-30), 24.1 (C-10), 23.7 (C-23), 21.9 (C-6), 21.3 (C-29), 21.1 (C-21), 20.5 (C-28), 19.3 (C-9); HRESIMS m/z calcd for $C_{37}H_{53}O_4$ 561.3941, found 561.3938 $[M+H]^+$.

2-benzylidene-(16β,20R,24R)-16,24-epoxy-25-hydroxy-cycloartan-3-one (**5**) Light yellow solid; $[\alpha]_D^{25}$ +109.5° (c 0.1, CHCl$_3$); IR (KBr) ν_{max} 3409 (O-H), 2939 (C-H), 1678 (α,β unsaturated ketone), 1593 (Ar C=C), 1461, 1377, 1164 (C–O), 1033, 694 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.43 (d, J = 4.0 Hz, 1H, H-19a), 0.70 (d, J = 4.0 Hz, 1H, H-19b), 0.90 (s, 3H, H$_3$-28), 0.91 (d, J = 6.4 Hz, 3H, H$_3$-21), 1.03 (m, 1H, H-7a), 1.08 (s, 3H, H$_3$-29), 1.07 (s, 6H, H$_3$-26, H$_3$-27), 1.13 (s, 3H, H$_3$-18), 1.17 (s, 3H, H$_3$-30), 1.38 (m, 1H, H-7b), 1.39 (m, 1H, H-22a), 1.50 (m, 1H, H-15a), 1.52 (m, 1H, H-23a), 1.60 (m, 1H, H-8), 1.62 (m, 1H, H-17), 1.63 (m, 2H, H$_2$-6), 1.64 (m, 2H, H$_2$-12), 1.74 (m, 1H, H-22b), 1.87 (m, 1H, H-15b), 1.92 (m, 1H, H-23b), 1.98 (m, 1H, H-5), 2.05 (m, 1H, H-20), 2.14 (m, 2H, H$_2$-11), 2.52 (d, J = 16.6 Hz, 1H, H-1a), 2.85 (d, J = 16.6 Hz, 1H, H-1b), 3.57 (dd, 1H, H-24), 4.60 (m, 1H, H-16), 7.27-7.36 (m, 5H, Ar-H), 7.37 (s, 1H, H-2'); ^{13}C NMR (100 MHz, CDCl$_3$): δ 208.2 (C-3), 135.9 (Ar-C, C-2'), 135.8 (C-2), 130.1 (Ar-C), 128.3 (Ar-C), 128.2 (Ar-C), 82.5 (C-24), 74.8 (C-16), 73.2 (C-25), 57.4 (C-17), 48.6 (C-4), 48.1 (C-8), 45.8 (C-14), 45.6 (C-13), 45.3 (C-5), 45.0 (C-15), 36.7 (C-1), 35.4 (C-22), 32.7 (C-12), 30.0 (C-19), 29.0 (C-20), 25.6 (C-11), 25.9 (C-27), 25.6 (C-7), 24.2 (C-30), 24.3 (C-10), 23.8 (C-26), 23.4 (C-23), 21.9 (C-6), 21.3 (C-29), 20.9 (C-21), 19.7 (C-28), 19.4 (C-9), 19.0 (C- 18); HRESIMS m/z calcd for $C_{37}H_{53}O_3$ 545.3938, found 545.3989 $[M+H]^+$.

2-benzylidene-(16β,20R,24R)-16,24,25-trihydroxy-cycloartan-3-one (**6**) Light yellow solid; $[\alpha]_D^{25}$ +148.0° (c 0.1, MeOH); IR (KBr) ν_{max} 3421 (O-H), 2943 (C-H), 1678 (α,β-unsaturated ketone), 1596 (Ar C=C), 1458, 1377, 1157 (C–O), 1026, 694 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.44 (d, J = 4.2 Hz, 1H, H-19a), 0.70 (d, J = 4.2 Hz, 1H, H-19b), 0.90 (d, J = 6.4 Hz, 3H, H$_3$-21), 0.91 (s, 3H, H$_3$-28), 1.07

(s, 3H, H$_3$-29), 1.09 (m, 1H, H-22a), 1.13 (s, 6H, H$_3$-18, H$_3$-26,), 1.17 (s, 3H, H$_3$-30), 1.20 (s, 3H, H$_3$-27), 1.33 (m, 1H, H-15a), 1.37 (m, 2H, H$_2$-7), 1.40 (m, 1H, H$_2$-23), 1.57 (m, 1H, H-8), 1.62 (m, 2H, H$_2$-6), 1.64 (m, 1H, H-17), 1.66 (m, 2H, H$_2$-12), 1.80 (m, 1H, H-22b), 1.90 (m, 1H, H-20), 1.98 (m, 1H, H-5), 2.06 (m, 1H, H-15b), 2.13 (m, 2H, H$_2$-11), 2.52 (d, $J = 16.4$ Hz, 1H, H-1a), 2.85 (d, $J = 16.4$ Hz, 1H, H-1b), 3.55 (dd, 1H, H-24), 4.48 (m, 1H, H-16), 7.27-7.36 (m, 5H, Ar-H), 7.37 (s, 1H, H-2'); ^{13}C NMR (100 MHz, CDCl$_3$): δ 208.2 (C-3), 135.9 (C-2'), 135.8 (C-2), 130.1 (Ar-C), 128.3 (Ar-C), 128.2 (Ar-C), 75.2 (C-24), 73.0 (C-16), 72.8 (C-25), 56.8 (C-17), 48.6 (C-8), 48.5 (C-4), 47.9 (C-15), 46.6 (C-14), 45.3 (C-5), 45.2 (C-13), 36.6 (C-1), 32.6 (C-12), 31.1 (C-22), 30.1 (C-19), 26.8 (C-27), 26.7 (C-20), 26.5 (C-11), 26.0 (C-7), 25.6 (C-23), 25.3 (C-10), 24.3 (C-30), 22.9 (C-26), 22.0 (C-6), 21.9 (C-29), 20.1 (C-28), 19.5 (C-9), 19.2 (C-18), 17.6 (C-21). HRESIMS m/z calcd for C$_{37}$H$_{55}$O$_4$ 563.4078, found 563.4094 [M+H]$^+$.

Synthesis of argentatin analogues 7, 9 and 11

To stirred solutions of appropriate intermediates **4** (1.0 eq, 15.0 mg), **5** (1.0 eq, 10.0 mg) and **6** (1.0 eq, 20.0 mg) in absolute EtOH (1.5 mL) were added guanidine hydrochloride (2.0 eq) and KOH (2.0 eq), and the resulting reaction mixture was refluxed for 12 h (monitored by TLC). The reaction mixtures were neutralized with 10% aq. HCl and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure to give crude products. Crude products were then dissolved in dry 1,4-dioxane (2.0 mL), DDQ (1.5 eq) was added and the resulting reaction mixtures were stirred at 25 °C for 1 h (TLC control). The reaction mixtures were then quenched with 10% aq. NaHCO$_3$ solution, and extracted with EtOAc (3 x 5 mL) and evaporated under *vacuo*. The resulting residues were purified by column chromatography using silica gel with toluene-EtOAc (90:10) and CH$_2$Cl$_2$-i-PrOH (95:5) to afford the argentatin analogues **7** (12.8 mg, 79%), **9** (6.4 mg, 60%), and **11** (10.5 mg, 49%).

(16β,20S,24R)-20,24-epoxy-16,25-dihydroxy-2'-amino-4'-phenylpyrimidino[5',6':2,3]cycloartane (7) White

solid; $[\alpha]_D^{25}$ +162.4° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3495 (O-H), 3402 (N-H), 2939 (C-H), 1608, 1550 (Ar

C=C), 1450, 1369, 1180 (C–O), 1056 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.32 (d, *J* = 4.3 Hz, 1H, H-19a),

0.64 (d, *J* = 4.3 Hz, 1H, H-19b), 0.89 (s, 3H, H$_3$-28), 0.96 (m, 1H, H-6a), 1.13 (m, 1H, H-7a), 1.10 (s, 3H,

H$_3$-26), 1.19 (s, 3H, H$_3$-30), 1.21 (s, 3H, H$_3$-18), 1.22 (s, 3H, H$_3$-27), 1.38 (m, 1H, H-7b), 1.37 (s, 3H, H$_3$-29),

1.38 (s, 3H, H$_3$-21), 1.49 (m, 1H, H-15a), 1.67 (m, 2H, H$_2$-12), 1.66 (m, 1H, H-22a), 1.67 (m, 1H, H-8), 1.68

(m, 1H, H-5), 1.78 (m, 1H, H-6b), 1.92 (m, 2H, H$_2$-23), 1.99 (d, *J* = 16.0 Hz, 1H, H-1a), 2.05 (m, 1H, H-

15b), 2.02 (m, 2H, H$_2$-11), 2.12 (d, *J* = 7.6 Hz, 1H, H-17), 2.23 (m, 1H, H-22b), 2.85 (d, *J* = 16.0 Hz, 1H, H-

1b), 3.82 (t, *J* = 15.2 Hz, 1H, H-24), 4.57 (q, 1H, H-16), 4.87 (s, 2H, -NH$_2$), 7.35-7.43 (m, 5H, Ar-H); ^{13}C

NMR (100 MHz, CDCl$_3$): δ 175.3 (C-3), 166.5 (C-4'), 161.0 (C-2'), 139.0 (Ar-C), 128.5 (Ar-C), 128.3 (Ar-

C), 128.2 (Ar-C), 115.8 (C-2), 87.2 (C-20), 84.5 (C-24), 73.4 (C-16), 70.9 (C-25), 55.6 (C-17), 48.5 (C-5),

48.4 (C-15), 46.2 (C-8), 46.5 (C-14), 45.8 (C-13), 42.3 (C-4), 37.3 (C-22), 34.9 (C-1), 33.1 (C-12), 30.0 (C-

19), 27.4 (C-29), 27.3 (C-27), 26.1 (C-26), 25.8 (C-11), 25.6 (C-18), 25.3 (C-10), 25.2 (C-7), 24.4 (C-30),

23.7 (C-23), 22.3 (C-6), 21.2 (C-21), 20.6 (C-28),18.9 (C-9); HRESIMS *m/z* calcd for C$_{38}$H$_{54}$N$_3$O$_3$ 600.4120,

found 600.4156 [M+H]$^+$.

(16β,20R,24R)-16,24-epoxy-25-hydroxy-2'-amino-4'-phenyl-pyrimidino[5',6':2,3]cycloartane (9) White

solid; $[\alpha]_D^{25}$ +31.5° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3490 (O-H), 3375 (N-H), 2939 (C-H), 1612, 1550 (Ar

C=C), 1442, 1369, 1168 (C–O), 1060, 702 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.32 (d, *J* = 4.4 Hz, 1H,

H-19a), 0.63 (d, *J* = 4.4 Hz, 1H, H-19b), 0.87 (d, *J* = 6.4 Hz, 3H, H$_3$-21), 0.89 (s, 3H, H$_3$-28), 1.07 (s,

6H, H$_3$-26, H$_3$-27), 1.10 (s, 3H, H$_3$-18), 1.16 (m, 1H, H-7a), 1.19 (s, 3H, H$_3$-30), 1.38 (m, 1H, H-22a),

1.40 (m, 1H, H-7b), 1.48 (m, 1H, H-15a), 1.37 (s, 3H, H$_3$-29), 1.54 (m, 1H, H-23a), 1.76 (m, 2H, H$_2$-6),

1.57 (m, 1H, H-17), 1.60 (m, 2H, H$_2$-12), 1.55 (m, 1H, H-5), 1.85 (m, 1H, H-8), 1.74 (m, 1H, H-22b),

1.97 (d, *J* = 16.2 Hz, 1H, H-1a), 1.88 (m, 1H, H-15b), 1.90 (m, 1H, H-23b), 2.02 (m, 2H, H$_2$-11), 2.03

(m, 1H, H-20), 2.84 (d, *J* = 16.2 Hz, 1H, H-1b), 3.56 (dd, 1H, H-24), 4.57 (m, 1H, H-16), 4.85 (Brs, 2H,

-NH$_2$), 7.33-7.45 (m, 5H, Ar-H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 175.2 (C-3), 166.6 (C-4'), 160.9 (C-2'), 138.9 (Ar-C), 128.5 (Ar-C), 128.3 (Ar-C), 128.2 (Ar-C), 115.9 (C-2), 82.5 (C-24), 74.8 (C-16), 73.2 (C-25), 57.4 (C-17), 48.5 (C-5), 45.8 (C-14), 45.6 (C-8), 45.6 (C-13), 45.1 (C-15), 42.3 (C-4), 35.4 (C-22), 34.9 (C-1), 32.6 (C-12), 29.8 (C-19), 28.8 (C-20), 27.4 (C-29), 25.7 (C-7), 25.6 (C-10), 25.4 (C-27), 24.3 (C-11), 23.8 (C-26), 23.6 (C-23), 23.4 (C-30), 22.3 (C-6), 20.8 (C-21), 19.7 (C-9), 19.0 (C-28), 19.0 (C- 18); HRESIMS m/z calcd for C$_{38}$H$_{54}$N$_3$O$_2$ 584.4216, found 584.4214 [M+H]$^+$.

(16β,20R,24R)-16,24,25-trihydroxy-2'-amino-4'-phenyl-pyrimidino[5',6':2,3]cycloartane (**11**) White solid; $[\alpha]_D^{25}$ +127.0° (c 0.1, MeOH); IR (KBr) ν_{max} 3504 (O-H), 3406 (N-H), 2933 (C-H), 1622, 1556 (Ar C=C), 1456, 1380, 1022, 702 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.32 (d, J = 4.2 Hz, 1H, H-19a), 0.64 (d, J = 4.2 Hz, 1H, H-19b), 0.87 (d, J = 6.3 Hz, 3H, H$_3$-21), 0.90 (s, 3H, H$_3$-28), 1.12 (s, 3H, H$_3$-26), 1.14 (s, 3H, H$_3$-18), 1.18 (m, 1H, H-22a), 1.19 (s, 6H, H$_3$-30, H$_3$-27), 1.20 (m, 1H, H-23a), 1.38 (m, 1H, H-15a), 1.42 (m, 1H, H-23b), 1.37 (s, 3H, H$_3$-29), 1.59 (m, 2H, H$_2$-6), 1.60 (m, 1H, H-5), 1.62 (m, 2H, H$_2$-12), 1.64 (m, 1H, H-17), 1.77 (m, 1H, H-22b), 1.80 (m, 2H, H$_2$-7), 1.86 (m, 1H, H-20), 1.98 (d, J = 16.5 Hz, 1H, H-1a), 1.97 (m, 1H, H-8), 2.04 (m, 2H, H$_2$-11), 2.08 (m, 1H, H-15b), 2.85 (d, J = 16.2 Hz, 1H, H-1b), 3.55 (dd, 1H, H-24), 4.47 (m, 1H, H-16), 4.82 (brs, 2H, -NH$_2$), 7.33-7.43 (m, 5H, Ar-H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 175.3 (C-3), 166.5 (C-4'), 160.8 (C-2'), 138.6 (Ar-C), 128.6 (Ar-C), 128.1 (Ar-C), 115.8 (C-2), 75.2 (C-24), 72.8 (C-25), 72.6 (C-16), 56.7 (C-17), 48.8 (C-5), 47.5 (C-15), 46.4 (C-8), 45.6 (C-14), 45.1 (C-13), 42.3 (C-4), 34.8 (C-1), 32.5 (C-12), 31.1 (C-22), 29.6 (C-19), 27.2 (C-27), 26.5 (C-20), 26.3 (C-11), 26.1 (C-7), 25.6 (C-23), 25.5 (C-10), 24.2 (C-26), 23.5 (C-29), 22.7 (C-6), 22.2 (C-30), 20.0 (C-9), 19.0 (C- 18, C-28), 17.6 (C-21); HRESIMS m/z calcd for C$_{38}$H$_{56}$N$_3$O$_3$ 602.4322, found 602.4344 [M+H]$^+$.

Synthesis of argentatin analogues 8, 10 and 12

To stirred solutions of the appropriate intermediates **4** (1.0 eq, 15.0 mg), **5** (1.0 eq, 16.0 mg), and **6** (1.0 eq, 15.0 mg) in absolute EtOH (1.5 mL) were added urea (2.0 eq) and KOH (2.0 eq), and the resulting mixtures were refluxed for 12 h (TLC control). The reaction mixtures were then neutralized with 10% aq. HCl and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residues were dissolved in dry 1,4-dioxane (2.0 mL), DDQ (1.5 eq) was added and stirred at 25 °C for 1 h (TLC control). The reaction mixtures were quenched with 10% aq. $NaHCO_3$ solution, extracted with EtOAc (3 x 5 mL), dried (anhydrous Na_2SO_4), and evaporated under reduced pressure to provide the crude products which were then purified by column chromatography using silica gel eluting with toluene-EtOAc (15:10) and CH_2Cl_2-*i*-PrOH (95:10) to afford the argentatin analogues **8** (5.0 mg, 31%), **10** (10.0 mg, 58%), and **12** (6.5 mg, 40%).

(16β,20S,24R)-20,24-epoxy-16,25-dihydroxy-2'-oxo-4'-phenyl-3'H-pyrimidino[5',6':2,3] cycloartane (8)

White solid; $[\alpha]_D^{25}$ +20.7° (*c* 0.05, MeOH); IR (KBr) ν_{max} 3429 (OH), 2941 (C-H), 1645 (C=O), 1600 (Ar C=C), 1450, 1379, 1110 (C-O), 1056 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.34 (d, J = 4.3 Hz, 1H, H-19a), 0.65 (d, J = 4.3 Hz, 1H, H-19b), 0.88 (s, 3H, H$_3$-28), 0.96 (m, 1H, H-6a), 1.13 (m, 1H, H-7a), 1.10 (s, 3H, H$_3$-26), 1.21 (s, 6H, H$_3$-18, H$_3$-30), 1.39 (m, 1H, H-7b), 1.37 (s, 6H, H$_3$-27, H$_3$-29), 1.51 (s, 3H, H$_3$-21), 1.49 (m, 1H, H-15a), 1.67 (m, 2H, H$_2$-12), 1.66 (m, 1H, H-22a), 1.67 (m, 1H, H-8), 1.68 (m, 1H, H-5), 1.78 (m, 1H, H-6b), 1.92 (m, 2H, H$_2$-23), 1.87 (d, J = 16.0 Hz, 1H, H-1a), 2.05 (m, 1H, H-15b), 2.02 (m, 2H, H$_2$-11), 2.10 (d, J = 7.6 Hz, 1H, H-17), 2.22 (m, 1H, H-22b), 2.80 (d, J = 16.0 Hz, 1H, H-1b), 3.81 (t, J = 15.2 Hz, 1H, H-24), 4.57 (q, 1H, H-16), 7.38-7.41 (m, 5H, Ar-H); ^{13}C NMR (100 MHz, CDCl$_3$): δ 158.6 (C-2'), 138.9 (Ar-C), 129.6 (Ar-C), 128.3 (Ar-C), 128.2 (Ar-C), 110.3 (C-2), 87.2 (C-20), 84.5 (C-24), 73.3 (C-16), 70.9 (C-25), 55.6 (C-17), 48.7 (C-5), 48.6 (C-15), 46.5 (C-8), 46.2 (C-4, C-14), 45.6 (C-13), 37.3 (C-22), 34.9 (C-1), 33.1 (C-12), 29.8 (C-19), 27.2 (C-27, C-29), 26.5 (C-26),

26.1 (C-11, C-18), 25.8 (C-10), 25.4 (C-7), 25.2 (C-30), 23.7 (C-23), 23.6 (C-6), 21.8 (C-21), 20.5 (C-28), 19.6 (C-9); HRESIMS m/z calcd for $C_{38}H_{53}N_2O_4$ 601.4005, found 601.4015 $[M+H]^+$.

(16β,20R,24R)-16,24-epoxy-25-hydroxy-2'-oxo-4'-phenyl-3'H-pyrimidino[5',6':2,3]cycloartane (10)

White solid; $[\alpha]_D^{25}$ +34.2° (c 0.1, MeOH); IR (KBr) ν_{max} 3427 (O-H), 2939 (C-H), 1645 (C=O), 1600 (Ar C=C), 1452, 1377, 1110 (C-O), 1056 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.33 (d, J = 4.4 Hz, 1H, H-19a), 0.63 (d, J = 4.4 Hz, 1H, H-19b), 0.86 (d, J = 6.4 Hz, 3H, H$_3$-21), 0.87 (s, 3H, H$_3$-28), 1.09 (s, 3H, H$_3$-18), 1.06 (s, 6H, H$_3$-26, H$_3$-27), 1.16 (m, 1H, H-7a), 1.37 (s, 3H, H$_3$-30), 1.38 (m, 1H, H-22a), 1.40 (m, 1H, H-7b), 1.48 (m, 1H, H-15a), 1.52 (s, 3H, H$_3$-29), 1.54 (m, 1H, H-23a), 1.76 (m, 2H, H$_2$-6), 1.57 (m, 1H, H-17), 1.60 (m, 2H, H$_2$-12), 1.55 (m, 1H, H-5), 1.85 (m, 1H, H-8), 1.74 (m, 1H, H-22b), 1.88 (d, J = 16.2 Hz, 1H, H-1a), 1.88 (m, 1H, H-15b), 1.90 (m, 1H, H-23b), 2.02 (m, 2H, H$_2$-11), 2.03 (m, 1H, H-20), 2.80 (d, J = 16.2 Hz, 1H, H-1b), 3.56 (dd, 1H, H-24), 4.57 (m, 1H, H-16), 7.33-7.46 (m, 5H, Ar-H), 12.90 (brs, 1H, -NH); ^{13}C NMR (100 MHz, CDCl$_3$): δ 164.4 (C-3), 158.9 (C-2'), 129.5 (Ar-C), 128.3 (Ar-C), 128.1 (Ar-C), 110.4 (C-2), 82.5 (C-24), 74.8 (C-16), 73.2 (C-25), 57.4 (C-17), 48.5 (C-5), 45.7 (C-14), 45.6 (C-8), 45.5 (C-13), 45.5 (C-4), 45.1 (C-15), 35.4 (C-22), 34.9 (C-1), 32.6 (C-12), 29.8 (C-19), 28.8 (C-20), 26.5 (C-29), 25.6 (C-7, C-10), 25.5 (C-27), 25.3 (C-11), 23.8 (C-26), 23.4 (C-23), 23.4 (C-30), 21.8 (C-6), 20.8 (C-21), 19.7 (C-9), 19.3 (C-28), 19.0 (C-18); HRESIMS m/z calcd for $C_{38}H_{53}N_2O_3$ 585.4056, found 585.4067 $[M+H]^+$.

(16β,20R,24R)-16,24,25-trihydroxy-2'-oxo-4'-phenyl-3'H-pyrimidino[5',6':2,3]cycloartane (**12**) White solid; $[\alpha]_D^{25}$ +111.0° (c 0.1, MeOH); IR (KBr) ν_{max} 3406 (O-H), 2937 (C-H), 1645 (C=O), 1598 (Ar C=C), 1444, 1380 cm^{-1}; ^{1}H NMR (400 MHz, CD$_3$OD): δ 0.40 (d, J = 4.2 Hz, 1H, H-19a), 0.70 (d, J = 4.2 Hz, 1H, H-19b), 0.91 (d, J = 6.4 Hz, 3H, H$_3$-21), 0.95 (s, 3H, H$_3$-28), 1.14 (s, 3H, H$_3$-26), 1.16 (s, 3H, H$_3$-18), 1.16 (s, 3H, H$_3$-27), 1.18 (m, 1H, H-22a), 1.20 (m, 1H, H-23a), 1.29 (s, 3H, H$_3$-30), 1.38 (m, 1H, H-15a), 1.42 (m, 1H, H-23b), 1.44 (s, 3H, H$_3$-29), 1.59 (m, 2H, H$_2$-6), 1.60 (m, 1H, H-5), 1.62 (m,

2H, H_2-12), 1.67 (m, 1H, H-17), 1.77 (m, 1H, H-22b), 1.80 (m, 2H, H_2-7), 1.86 (m, 1H, H-20), 1.90 (d, J = 16.5 Hz, 1H, H-1a), 1.97 (m, 1H, H-8), 2.02 (m, 2H, H_2-11), 2.04 (m, 1H, H-15b), 2.85 (d, J = 16.5 Hz, 1H, H-1b), 3.36 (dd, 1H, H-24), 4.43 (m, 1H, H-16), 7.49 (m, 5H, Ar-H); ^{13}C NMR (100 MHz, CD$_3$OD): δ 164.0 (C-3), 159.8 (C-2'), 131.3 (Ar-C), 129.8 (Ar-C), 129.5 (Ar-C), 112.6 (C-2), 78.6 (C-24), 73.9 (C-25), 73.4 (C-16), 58.2 (C-17), 50.5 (C-5), 49.3 (C-15), 47.8 (C-14), 46.9 (C-8), 46.5 (C-4), 46.5 (C-13), 35.4 (C-1), 33.9 (C-12), 33.8 (C-22), 30.6 (C-19), 29.9 (C-20), 28.5 (C-6), 27.0 (C-29), 26.9 (C-11), 26.8 (C-23), 25.5 (C-27), 25.5 (C-10), 25.4 (C-26), 24.8 (C-30), 23.7 (C-7), 20.8 (C-28), 20.7 (C-9), 19.9 (C-18), 18.5 (C-21); HRESIMS m/z calcd for $C_{38}H_{55}N_2O_4$, 603.4162, found 603.4169 $[M+H]^+$.

Synthesis of argentatin analogues 13, 14, and 15

To each of the stirred solutions of **1** (10.0 mg, 1.0 eq), **2** (10.0 mg, 1.0 eq,), and **3** (1.0 eq, 15.0 mg) in CH_3CO_2H (1.0 mL) was added a bromine solution (1.5 eq, 2 mL) and the resulting mixtures were stirred at 0° C-25 °C for 1 h (TLC control). The reaction mixtures were quenched with (10%) aq. $NaHCO_3$ solution and extracted with EtOAc (3 x 5 mL). The combined EtOAc extracts were dried (anhydrous Na_2SO_4) and concentrated under reduced pressure. The resulting residues were dissolved in absolute EtOH (0.5 mL) to which thiourea (1.5 eq) was added and refluxed for 12 h (TLC control). The reaction mixtures were extracted with EtOAc (3 x 5 mL), dried (anhydrous Na_2SO_4), and evaporated under reduced pressure. The residues thus obtained were purified by column chromatography using silica gel. Elution with hexane-EtOAc (60-40) followed by CH_2Cl_2-i-PrOH (90-10) afforded the argentatin analogues **13** (6.5 mg, 58%), **14** (6.0 mg, 53%), and **15** (5.2 mg, 46%).

(16β,20S,24R)-20,24-epoxy-16,25-dihydroxy-2'-amino-1,3-thiazolo-[3,2]cycloartane (**13**) White solid; $[\alpha]_D^{25}$ +112 (c 0.1, MeOH); IR (KBr) v_{max} 3371 (O-H), 3209 (N-H), 2939 (C-H), 1624, 1531, 1377, 1184, 1053, 732 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.48 (d, J = 4.6 Hz, 1H, H-19a), 0.74 (d, J = 4.6 Hz, 1H, H-19b), 0.88 (m, 1H, H-6a), 0.90 (s, 3H, H_3-28), 1.06 (m, 1H, H-7a), 1.10 (s, 3H, H_3-30), 1.12

(s, 3H, H$_3$-26), 1.23 (s, 3H, H$_3$-27), 1.24 (s, 3H, H$_3$-29), 1.27 (s, 3H, H$_3$-18), 1.37 (m, 1H, H-7b), 1.44 (s, 3H, H$_3$-21), 1.51 (m, 1H, H-15a), 1.62 (m, 1H, H-5), 1.67 (m, 1H, H-22a), 1.72 (m, 1H, H-6b), 1.78 (m, 1H, H-8), 1.82 (m, 2H, H$_2$-12), 1.93 (m, 2H, H$_2$-23), 2.01 (d, J = 15.8 Hz, 1H, H-1a), 2.04 (m, 1H, H-15b), 2.12 (m, 2H, H$_2$-11), 2.14 (d, J = 7.5 Hz, 1H, H-17), 2.24 (m, 1H, H-22b), 2.81 (d, J = 15.8 Hz, 1H, H-1b), 3.84 (t, J = 7.6 Hz, 1H, H-24), 4.60 (ddd, J = 12.7, 7.7, 4.9 Hz, 1H, H-16), 4.78 (s, 2H, NH$_2$-2'); ^{13}C NMR (100 MHz, CDCl$_3$): δ 164.6 (C-2'), 154.6 (C-3), 117.0 (C-2), 87.2 (C-20), 84.5 (C-24), 73.4 (C-16), 70.9 (C-25), 55.6 (C-17), 48.8 (C-15), 48.5 (C-5), 46.3 (C-8), 46.6 (C-14), 46.1 (C-13), 39.3 (C-4), 37.3 (C-22), 33.2 (C-12), 32.0 (C-1), 30.4 (C-19), 27.3 (C-27, C-29), 26.2 (C-26), 26.1 (C-7, C-11), 25.7 (C-18), 24.9 (C-10), 23.8 (C-23), 23.0 (C-30), 21.5 (C-21), 21.4 (C-6), 20.6 (C-28), 19.3 (C-9); HRESIMS m/z calcd for C$_{31}$H$_{49}$N$_2$O$_3$S 529.3464, found 529.3465 [M+H]$^+$.

(16β,20R,24R)-16,24-epoxy-25-hydroxy-2'-amino-1,3-thiazolo-[3,2]cycloartane (**14**) White solid; $[\alpha]_D^{25}$ +54 (c 0.1, CHCl$_3$); IR (KBr) ν_{max} 3375 (N-H), 2943 (C-H), 1620, 1531, 1461, 1373, 1110, 1060, 736 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.48 (d, J = 4.4 Hz, 1H, H-19a), 0.74 (d, J = 4.4 Hz, 1H, H-19b), 0.90 (s, 3H, H$_3$-28), 0.92 (d, J = 6.6 Hz, 3H, H$_3$-21), 1.06 (m, 1H, H-7a), 1.07 (s, 6H, H$_3$-26, H$_3$-27), 1.11 (s, 3H, H$_3$-30), 1.16 (s, 3H, H$_3$-18), 1.24 (s, 3H, H$_3$-29), 1.36 (m, 1H, H-7b), 1.38 (m, 1H, H-22a), 1.50 (m, 1H, H-15a), 1.56 (m, 1H, H-23a), 1.57 (m, 1H, H-5), 1.60 (m, 1H, H-17), 1.65 (m, 2H, H$_2$-12), 1.74 (m, 1H, H-22b), 1.78 (m, 2H, H$_2$-6), 1.90 (m, 1H, H-15b), 1.92 (m, 1H, H-23b), 1.80 (m, 1H, H-8), 2.02 (d, J = 15.4 Hz, 1H, H-1a), 2.07 (m, 1H, H-20), 2.10 (m, 2H, H$_2$-11), 2.81 (d, J = 15.4 Hz, 1H, H-1b), 3.57 (dd, J = 12.4, 1.2 Hz, 1H, H-24), 4.60 (m, 1H, H-16), 4.72 (s, 2H, NH$_2$-2'); ^{13}C NMR (100 MHz, CDCl$_3$): δ 164.4 (C-2'), 154.6 (C-3), 117.1 (C-2), 82.5 (C-24), 74.9 (C-16), 73.2 (C-25), 57.4 (C-17), 48.3 (C-5), 46.0 (C-8), 45.8 (C-14), 45.6 (C-13), 45.1 (C-15), 39.3 (C-4), 35.4 (C-22), 32.8 (C-12), 32.0 (C-1), 30.1 (C-19), 28.9 (C-20), 27.3 (C-29), 25.7 (C-11), 25.6 (C-7, C-27), 24.8 (C-10), 23.5 (C-23), 23.4 (C-26), 22.9 (C-30), 21.6 (C-6), 20.9 (C-21), 19.8 (C-28), 19.4 (C-9), 19.1 (C-18); HRESIMS m/z calcd for C$_{31}$H$_{49}$N$_2$O$_2$S, 513.3515, found 513.3514 [M+H]$^+$.

(16β,20R,24R)-16,24,25-trihydroxy-2'-amino-1,3-thiazolo-[3,2] cycloartane (**15**) White solid; $[\alpha]_D^{25}$ +137

(c 0.1, MeOH); IR (KBr) ν_{max} 3421 (O-H), 3340 (N-H), 2931 (C-H), 1627, 1531, 1461, 1380, 1185,

1083 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$+CD$_3$OD): δ 0.52 (d, J = 4.3 Hz, 1H, H-19a), 0.74 (d, J = 4.3

Hz, 1H, H-19b), 0.93 (m, 1H, H-6a), 0.96 (s, 3H, H$_3$-28), 0.97 (d, J = 6.0 Hz, 3H, H$_3$-21), 1.11 (m, 1H,

H-7a), 1.12 (s, 3H, H$_3$-26), 1.17 (m, 1H, H-22a), 1.18 (s, 3H, H$_3$-30), 1.19 (s, 3H, H$_3$-27), 1.22 (s, 3H,

H$_3$-18), 1.23 (s, 3H, H$_3$-29), 1.40 (m, 1H, H-7b), 1.42 (m, 1H, H-15a), 1.60 (m, 2H, H$_2$-23), 1.62 (m, 1H,

H-5), 1.68 (m, 1H, H-17), 1.72 (m, 2H, H$_2$-12), 1.77 (m, 1H, H-6b), 1.80 (m, 1H, H-8), 1.82 (m, 1H, H-

22b), 1.91 (m, 1H, H-20), 2.05 (d, J = 16.0 Hz, 1H, H-1a), 2.08 (m, 1H, H-15b), 2.13 (m, 2H, H$_2$-11),

2.80 (d, J = 16.0 Hz, 1H, H-1b), 3.38 (dd, J = 10.4, 2.2 Hz, 1H, H-24), 4.46 (m, 1H, H-16), 7.82 (s, 1H,

NH-1'); ^{13}C NMR (100 MHz, CDCl$_3$+CD$_3$OD): δ 168.9 (C-2'), 154.4 (C-3), 116.6 (C-2), 78.6 (C-24),

73.9 (C-16), 73.4 (C-25), 58.2 (C-17), 50.6 (C-5), 48.8 (C-15), 47.8 (C-14), 47.7 (C-13), 46.6 (C-8),

40.3 (C-4), 33.8 (C-12), 32.9 (C-1), 31.2 (C-22), 30.0 (C-19), 28.5 (C-20), 27.7 (C-29), 26.1 (C-27),

27.0 (C-23), 25.6 (C-11), 25.4 (C-7, C-10), 23.4 (C-30), 22.8 (C-26), 20.9 (C-6), 20.8 (C-9), 20.7 (C-

28), 19.9 (C- 18), 18.5 (C-21); HRESIMS m/z calcd for C$_{31}$H$_{51}$N$_2$O$_3$S 513.3620, found 531.3607

[M+H]$^+$.

Synthesis of argentatin analogues 16, 17 and 18

Phenylhydrazine hydrochloride (1.5 eq) was added to stirred solutions of **1** (10.0 mg, 1.0 eq), **2** (15.0

mg, 1.0 eq) and **3** (10.0 mg, 1.0 eq) in CH$_3$CO$_2$H (1.0 mL) at 25 °C, warmed up to 60 °C and stirred at

this temperature for 5 h (TLC control). The reaction mixtures were then quenched with 10% aq.

NaHCO$_3$ solution and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried

(anhydrous Na$_2$SO$_4$) and concentrated under reduced pressure to give crude products, which were

purified by column chromatography using silica gel and eluting with toluene-EtOAc (90:10) and

CH$_2$Cl$_2$-i-PrOH (96:4) to afford the argentatin analogues **16** (8.6 mg, 74%), **17** (10.0 mg, 57%), and **18**

(7.8 mg, 68%).

(16β,20*S*,24*R*)-20,24-epoxy-16,25-dihydroxy-1'H-indolo [3,2-b]cycloartane (**16**) White solid; $[\alpha]_D^{25}$ +134

(*c* 0.1, MeOH); IR (KBr) ν_{max} 3417 (O-H), 2958 (C-H), 1461, 1384, 1180, 1103, 740 cm^{-1}; ^{1}H NMR

(400 MHz, CDCl$_3$): δ 0.50 (d, *J* = 4.4 Hz, 1H, H-19a), 0.72 (d, *J* = 4.4 Hz, 1H, H-19b), 0.91 (m, 1H, H-

6a), 0.96 (s, 3H, H$_3$-28), 1.10 (m, 1H, H-7a), 1.13 (s, 3H, H$_3$-26), 1.23 (s, 3H, H$_3$-30), 1.24 (s, 3H, H$_3$-

27), 1.30 (s, 3H, H$_3$-18), 1.32 (s, 3H, H$_3$-29), 1.43 (m, 1H, H-7b), 1.47 (s, 3H, H$_3$-21), 1.57 (m, 1H, H-

15a), 1.74 (m, 1H, H-22a), 1.77 (m, 1H, H-6b), 1.78 (m, 1H, H-5), 1.84 (m, 2H, H$_2$-12), 1.96 (m, 2H,

H$_2$-23), 1.98 (m, 1H, H-8), 2.08 (m, 1H, H-15b), 2.18 (d, *J* = 7.5 Hz, 1H, H-17), 2.26 (d, *J* = 15.2 Hz,

1H, H-1a), 2.28 (m, 1H, H-22b), 2.31 (m, 2H, H$_2$-11), 2.90 (d, *J* = 15.2 Hz, 1H, H-1b), 3.86 (t, *J* = 7.7

Hz, 1H, H-24), 4.62 (ddd, *J* = 12.7, 7.7, 5.0 Hz, 1H, H-16), 7.04 (t, *J* = 7.0 Hz, 1H, H-4'), 7.10 (t, *J* =

7.0 Hz, 1H, H-3'), 7.29 (d, *J* = 7.6 Hz, 1H, H-2'), 7.38 (d, *J* = 7.6 Hz, 1H, H-5'), 7.79 (s, 1H, NH-1');

^{13}C NMR (100 MHz, CDCl$_3$): δ 142.9 (C-3), 135.8 (C-6'), 127.6 (C-7'), 121.0 (C-3'), 118.9 (C-4'), 117.8

(C-5'), 110.5 (C-2'), 108.1 (C-2), 87.3 (C-20), 84.5 (C-24), 73.5 (C-16), 70.9 (C-25), 55.6 (C-17), 48.8

(C-15), 48.7 (C-5), 46.7 (C-8), 46.6 (C-14), 46.3 (C-13), 36.5 (C-4), 37.4 (C-22), 33.3 (C-12), 30.6 (C-

19), 29.9 (C-1), 27.5 (C-29), 27.3 (C-27), 26.2 (C-26), 26.1 (C-7, C-11), 25.7 (C-18), 25.3 (C-10), 24.1

(C-30), 23.8 (C-23), 21.2 (C-6), 21.3 (C-21), 20.6 (C-28), 19.3 (C-9); HRESIMS *m/z* calcd for

C$_{36}$H$_{52}$NO$_3$ 546.3947, found 546.3940 [M+H]$^+$.

(16β,20*R*,24*R*)-16,24-epoxy-25-hydroxy-1'H-indolo[3,2-b]cycloartane (**17**) White solid; $[\alpha]_D^{25}$ +28 (*c* 0.1,

CHCl$_3$); IR (KBr) ν_{max} 3317 (N-H), 2954 (C-H), 1465, 1384, 1157, 1056, 964, 732 cm^{-1}; ^{1}H NMR (400

MHz, CDCl$_3$): δ 0.50 (d, *J* = 4.2 Hz, 1H, H-19a), 0.70 (d, *J* = 4.2 Hz, 1H, H-19b), 0.94 (s, 3H, H$_3$-28),

0.95 (d, *J* = 6.2 Hz, 3H, H$_3$-21), 1.08 (s, 6H, H$_3$-26, H$_3$-27), 1.19 (s, 3H, H$_3$-18), 1.23 (s, 3H, H$_3$-30),

1.24 (m, 1H, H-7a), 1.32 (s, 3H, H$_3$-29), 1.41 (m, 1H, H-22a), 1.43 (m, 1H, H-7b), 1.52 (m, 1H, H-15a),

1.59 (m, 1H, H-23a), 1.61 (m, 1H, H-5), 1.65 (m, 1H, H-17), 1.73 (m, 2H, H$_2$-12), 1.76 (m, 1H, H-22b),

1.78 (m, 2H, H$_2$-6), 1.91 (m, 1H, H-15b), 1.93 (m, 1H, H-23b), 1.97 (m, 1H, H-8), 2.08 (m, 1H, H-20),

2.25 (d, *J* = 15.4 Hz, 1H, H-1a), 2.26 (m, 2H, H$_2$-11), 2.90 (d, *J* = 15.4 Hz, 1H, H-1b), 3.58 (dd, *J* =

12.6, 2.0 Hz, 1H, H-24), 4.61 (m, 1H, H-16), 7.04 (t, J = 7.1 Hz, 1H, H-4'), 7.09 (t, J = 7.0 Hz, 1H, H-3'), 7.29 (d, J = 7.8 Hz, 1H, H-2'), 7.38 (d, J = 7.4 Hz, 1H, H-5'), 7.76 (s, 1H, NH-1'); ^{13}C NMR (100 MHz, CDCl$_3$): δ 142.9 (C-3), 135.8 (C-6'), 127.6 (C-7'), 120.9 (C-3'), 118.9 (C-4'), 117.8 (C-5'), 110.5 (C-2'), 108.2 (C-2), 82.5 (C-24), 74.9 (C-16), 73.2 (C-25), 57.4 (C-17), 48.5 (C-5), 46.5 (C-8), 45.8 (C-14), 45.7 (C-13), 45.2 (C-15), 36.4 (C-4), 35.4 (C-22), 32.8 (C-12), 30.4 (C-19), 29.9 (C-1), 28.9 (C-20), 27.5 (C-29), 25.8 (C-11, C-27), 25.7 (C-7), 24.6 (C-10), 24.0 (C-30), 23.8 (C-26), 23.5 (C-23), 21.3 (C-6), 20.9 (C-21), 19.8 (C-28), 19.5 (C-9), 19.1 (C-18); HRESIMS m/z calcd for $C_{36}H_{52}NO_2$ 530.3998, found 530.3978 $[M+H]^+$.

(16β,20R,24R)-16,24,25-trihydroxy-1'H-indolo[3,2-b] cycloartane (**18**) White solid; yield 68% (7.8 mg); $[\alpha]_D^{25}$ +111.0° (c 0.1, MeOH); IR (KBr) ν_{max} 3417 (O-H), 2939 (C-H), 1465, 1380, 1180, 1053, 740 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.50 (d, J = 4.2 Hz, 1H, H-19a), 0.71 (d, J = 4.2 Hz, 1H, H-19b), 0.95 (d, J = 6.1 Hz, 3H, H$_3$-21), 0.96 (s, 3H, H$_3$-28), 0.98 (m, 1H, H-6a), 1.13 (m, 1H, H-22a), 1.16 (s, 3H, H$_3$-26), 1.20 (s, 3H, H$_3$-18), 1.21 (s, 3H, H$_3$-27), 1.23 (s, 3H, H$_3$-30), 1.26 (m, 1H, H-7a), 1.32 (s, 3H, H$_3$-29), 1.38 (m, 1H, H-15a), 1.44 (m, 1H, H-7b), 1.60 (m, 2H, H$_2$-23), 1.64 (m, 1H, H-5), 1.70 (m, 1H, H-17), 1.74 (m, 2H, H$_2$-12), 1.80 (m, 1H, H-6b), 1.90 (m, 1H, H-22b), 1.95 (m, 1H, H-20), 1.98 (m, 1H, H-8), 2.14 (m, 1H, H-15b), 2.26 (d, J = 15.5 Hz, 1H, H-1a), 2.28 (m, 2H, H$_2$-11), 2.90 (d, J = 15.5 Hz, 1H, H-1b), 3.58 (dd, J = 11.7, 2.3 Hz, 1H, H-24), 4.50 (m, 1H, H-16), 7.03 (t, J = 6.8 Hz, 1H, H-4'), 7.10 (t, J = 7.2 Hz, 1H, H-3'), 7.29 (d, J = 7.2 Hz, 1H, H-2'), 7.38 (d, J = 7.2 Hz, 1H, H-5'), 7.76 (s, 1H, NH-1'); ^{13}C NMR (100 MHz, CDCl$_3$): δ 142.8 (C-3), 135.8 (C-6'), 127.6 (C-7'), 120.9 (C-3'), 118.9 (C-4'), 117.8 (C-5'), 110.5 (C-2'), 108.1 (C-2), 75.2 (C-24), 73.0 (C-16, C-25), 56.8 (C-17), 48.9 (C-5), 48.1 (C-15), 46.7 (C-14), 46.6 (C-8), 45.3 (C-13), 36.6 (C-4), 32.8 (C-12), 31.1 (C-22), 30.4 (C-19), 29.9 (C-1), 27.5 (C-29), 26.8 (C-27), 26.6 (C-20), 26.2 (C-23), 25.9 (C-7), 25.8 (C-11), 24.7 (C-10), 24.1 (C-30), 23.2 (C-26), 21.3 (C-6), 20.2 (C-28), 19.6 (C-18), 19.5 (C-9), 17.7 (C-21); HRESIMS m/z calcd for $C_{36}H_{54}NO_3$ 548.4104, found 548.4103 $[M+H]^+$.

Cytotoxicity evaluation

Cytotoxic activity of test compounds was determined in a panel of human non-small cell lung (NCI-H460), human CNS glioma (SF-268), human breast (MCF-7) and normal cells (WI 38) using the dye-reduction (MTT) assay as described previously [32]. Doxorubicin and DMSO were used as positive and negative controls, respectively. The test compounds were dissolved in DMSO and diluted to the desired concentrations in culture medium and were added to 96-well plates. After 72 h, incubation at 37 °C in a CO_2 incubator, dye solution (MTT [3-(4, 5-dimethylthiazol-2-yl)- 2,5- diphenyl tetrazolium bromide]) was added to each well (1:10 dilution). After agitation, incubation was continued for 4 h at 37 °C in a CO_2 incubator. The supernatant liquid from each well was carefully removed, and the resulting formazan crystals were dissolved in DMSO. The absorbance in each well was read using a microplate fluorometer (Ex/Em: 570/650).

Compliance with ethical standards

Conflict of interest I. M. has disclosed financial interests in TEVA Pharmaceuticals Hungary and the University of Debrecen, Hungary, which are unrelated to the subject of the research presented here. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1

Cytotoxicity (IC_{50}) data of argentatins A–C (**1**–**3**) and their semi-synthetic analogues (**7**–**12**) against selected cancer cell lines and human normal cells[a]

Compound	Cell lines[b]			
	NCI-H460	SF-268	MCF-7	WI-38
1	31.2±0.0	35.0±0.2	32.8±1.2	41.4±2.8
2	17.5±0.9	31.2±1.7	23.1±0.4	21.0±2.9
3	>35.0	>35.0	>35.0	>35.0
7	4.0±0.8	>10.0	3.8±1.0	9.8±1.0
8	4.4±0.7	>10.0	4.0±0.4	>10.0
9	3.8±0.8	>10.0	5.7±0.6	8.1±1.1
10	3.9±0.3	7.6 ± 0.7	3.6±0.1	5.9±0.5
12	3.4±0.7	>10	4.0±0.8	>10.0
Doxorubicin	0.1±0.0	0.4±0.1	0.3±0.1	0.8±0.1

[a]Results are expressed as Inhibition Concentration (IC_{50}) value in μM; Doxorubicin and DMSO were used as positive and negative controls, respectively; Analogues **11** and **13**–**17** were inactive against all 3 cancer cell lines and normal cells up to 10.0 μM concentration. [b]Key: NCI-H460 = human non-small cell lung cancer; SF-268 = human CNS cancer (glioma); MCF-7 = human breast cancer; WI-38 = normal human primary fibroblast cells.
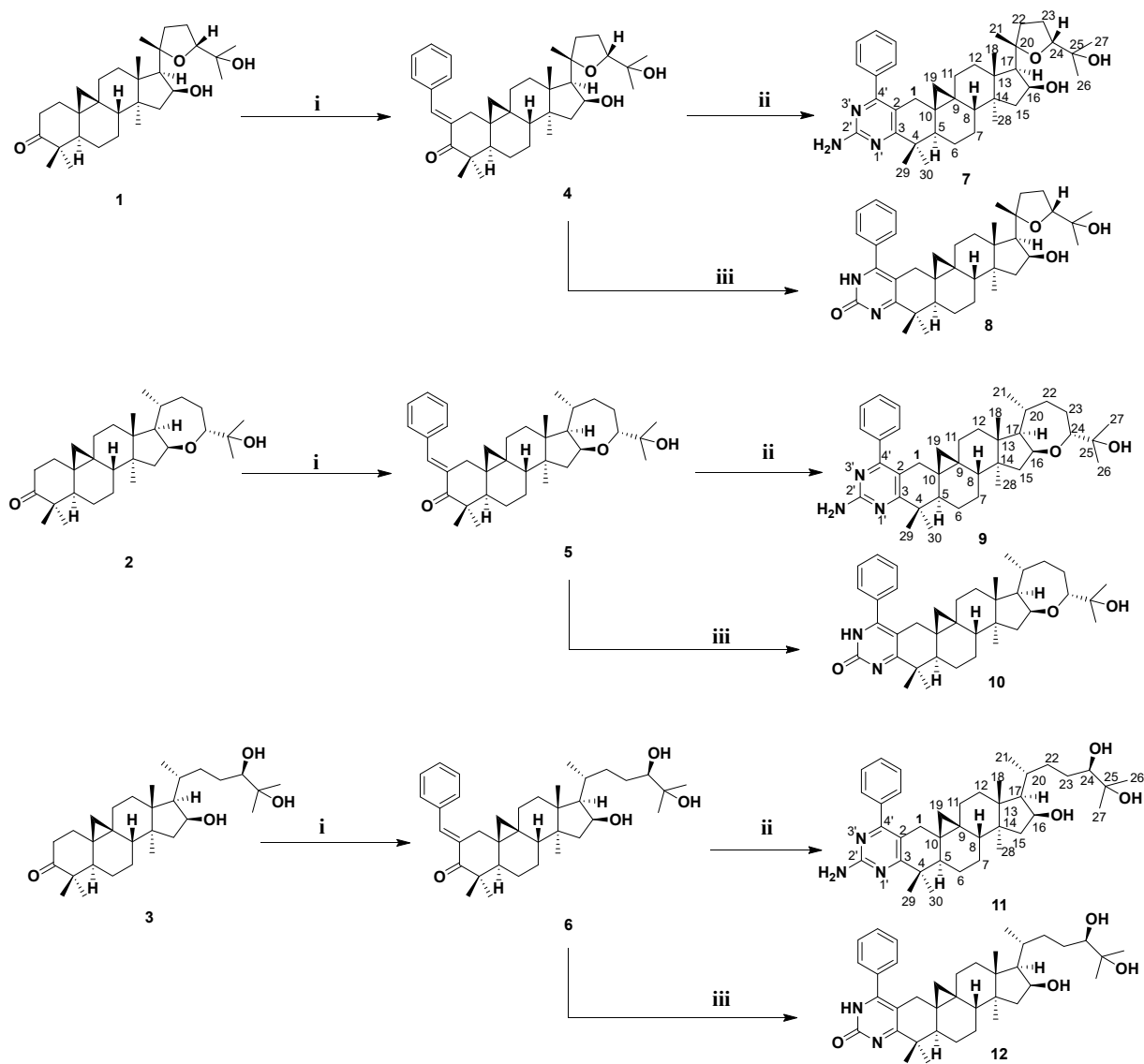
Scheme 1. Synthesis of pyrimidine analogues of argentatins A–C (**1**–**3**). *Reagents and conditions*: i) EtOH, KOH, benzaldehyde, 45 °C, 12 h; ii) a. EtOH, KOH, NH₂C(=NH)NH₂.HCl, reflux, 12 h; b. 1,4 dioxane, DDQ, 25 °C, 1 h; iii) a. EtOH, KOH, NH₂C(=O)NH₂ reflux, 12 h; b. 1,4 dioxane, DDQ, 25 °C, 1 h.
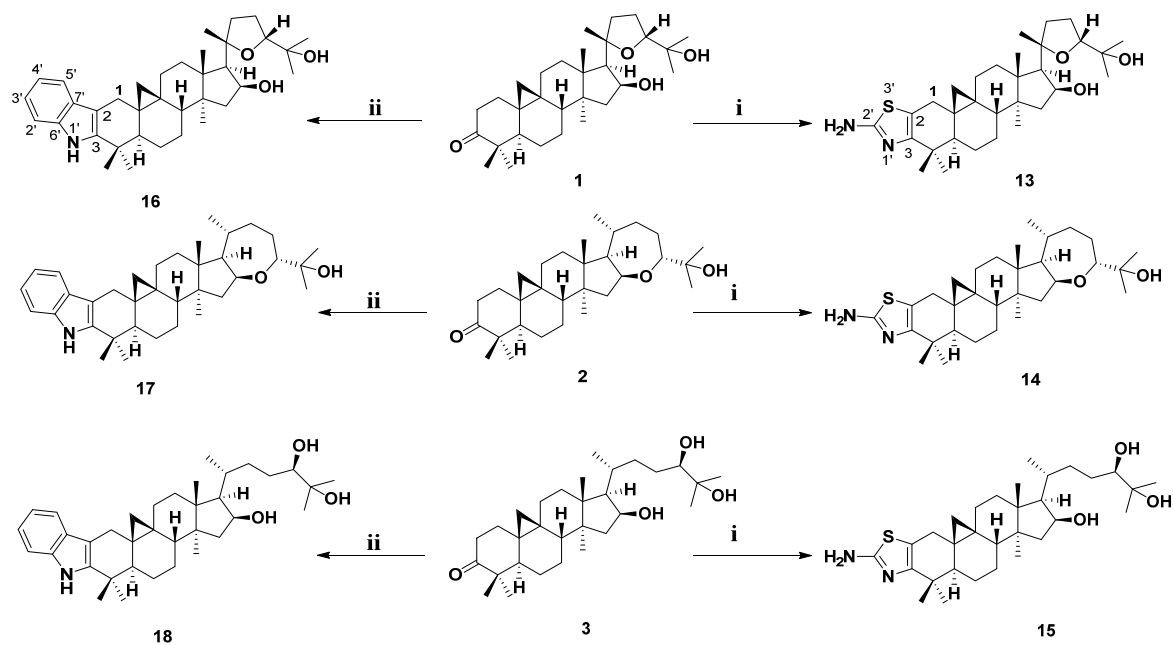
Scheme 2. Synthetic routes to thiazole and indole analogues of argentatins A–C. *Reagents and conditions*: i. a. CH₃COOH (1.0 mL), Br₂ (1.5 eq), 0 °C–25 °C, 1 h; b. Ethanol (0.5 mL), NH₂C(=S)NH₂ (1.5 eq), reflux, 12 h; ii. CH₃COOH (1.0 mL), PhNHNH₂.HCl (1.5 eq), 50 °C, 5 h.

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