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Structural and stereochemical requirements of the spiroketal group of hippuristanol for antiproliferative activity

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ABSTRACT

Hippuristanol is a natural product that has recently been shown to inhibit eukaryotic translation initiation and tumor cell proliferation. To investigate the structure and activity relationship of hippuristanol, we synthesized a series of analogs by expanding the size of its F ring and determined their effects on the proliferation of cancer cell lines. All changes to the F-ring of hippuristanol resulted in 3-fold to >100-fold decrease in activity.

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Hippuristanol, a polyoxygenated steroid with potent cytotoxicities against tumor cell lines, was discovered from the gorgonian *Isis hippuris.*¹ Recently, it was reported that this natural product appeared to work through eukaryotic initiation factor (eIF)4A I and II, an ATP-dependent RNA helicase, blocking its binding to mRNA hence inhibiting translational initiation, making it the second small molecule, after the marine natural product pateamine A, that modulate the function of eIF4A protein family.² Given the critical role of translation in cell proliferation, hippuristanol is considered a promising lead for the development of anti-tumor agents. Moreover, translation is also necessary for viral infection, suggesting that hippuristanol has potential as an antiviral agent.

Recently, we disclosed the first synthesis of hippuristanol using the commercially available hydrocortisone as a starting material.³ A key step in the synthesis entailed the addition of 2,3-dihydrofuran to a β -hydroxymethylketone precursor to construct the spiroketal functionality (Fig. 1). This chemistry rendered it possible to attain several analogs with structural alteration at the F-ring of hippuristanol. The preliminary structure and activity relationship data suggested that besides the three hydroxyls at C3, C11 and C20, the '*R*' configuration at C22 and the three methyl groups on the F ring were critical for activity. Herein, we report the synthesis of a new series of analogs of hippuristanol with altered structures and stereochemistry of the E and F rings and the determination of their antiproliferative activity.

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In our previous preliminary SAR study, we adopted a structural 'contraction' strategy by making analogs with the removal of a subset or all of the methyl groups on the F-ring of hippuristanol in addition to altering the stereochemistry at C22, C20 and C24.³ In the present work, we decided to proceed, for most analogs, with structural 'expansion', that is, increasing the ring size from five to six, adding an extra methyl group or enlarging the methyl groups to ethyl groups.

For the F-ring building blocks, we began with two commercially available α , α -dimethyl- γ -butyrolactone **2** and γ -butyrolactone **3** (Scheme 1). Nucleophilic attack of the γ -butyrolactone derivatives with the Grignard reagents (prepared from magnesium with iodomethane or bromoethane) in Et₂O at 30 °C led to the corresponding diols, which were then oxidized to 3,3,4,4-tetramethylbutyrolactone **4** or 4,4-diethylbutyrolactone **5** with the required methyl or ethyl groups by PCC, in 83% and 86% yield, respectively.^{3,4} Treatment of the resulting lactones with DIBAL-H at -68 °C in CH₂Cl₂ offered pairs of racemic acetals that were stable below 20 °C. Finally, the acetals were dehydrated at high temperature (250 °C) without any solvent to provide the desired 2,2,3,3-tetramethyl-2,3-dihydrofuran **6** and 2,2-diethyl-2,3-dihydrofuran **7** in 62% and 65% yield, respectively.^{3,5}

To prepare an F-ring analog lacking one of the methyl groups, the commercial (–)-p-pantolactone **8** was chosen as the starting precursor (Scheme 2). Activation of the α -hydroxyl group at pantolactone **8** with an Ms group in CH₂Cl₂, followed by reduction in the presence of Zn and Nal, provided 3,3-dimethylbutyrolactone **9** in 86% yield.⁶ Subsequently, reduction and dehydration of **9** gave rise

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Figure 1. Hippuristanol and its synthetic strategy.



Scheme 1. Reagents and conditions: (a) MeMgI or EtMgBr, Et_2O , 30 °C; (b) PCC, CH₂Cl₂, rt, 83% (for **4**, two steps) and 86% (for **5**, two steps); (c) DIBAL-H, CH₂Cl₂, -68 °C; (d) Al₂O₃, 250 °C, 62% (for **6**, two steps) and 65% (for **7**, two steps).



Scheme 2. Reagents and conditions: (a) MsCl, Et₃N, DMAP, CH₂Cl₂, rt; (b) Zn, Nal, DME, reflux, 86% (for two steps); (c) DIBAL-H, CH₂Cl₂, -68 °C; (d) Al₂O₃, 200 °C, 60% (for two steps).

to the corresponding 3,3-dimethyl-2,3-dihydrofuran **10** in 60% yield.

With the F-ring precursors in hand, we synthesized their corresponding hippuristanol analogs using similar chemistry as that for the synthesis of hippuristanol (Scheme 3).³ Treatment of the three 2,3-dihydrofuran derivatives prepared above with *t*-BuLi in THF re-

sulted in the corresponding 5-lithio-2,3-dihydrofuran, which were then added to ketone **1** at 0 °C to provide mainly the required 20β -OH adducts. Otherwise, low temperature (-68 °C) led mainly to the adducts with 20α -OH configuration, which we previously found dramatically decreased the antiproliferative activity. The crude products with 20β-OH were acidified directly with the addition of 5% HCl to open the 2,3-dihydrofuran ring to introduce the side chain with a 22-carbonyl and a 25-hydroxyl groups.⁷ Cyclization of the resulting intermediate containing 16-hydroxyl, 22-carbonyl and 25-hydroxyl substituents was then achieved to provide the desired 22-spiro-ketal functionalities. Subsequent deprotection of the remaining Bz groups with LiAlH₄ furnished the desired analogs 11a/11b, 12a/12b, 13a/13b in satisfactory yields.^{3,8} Analog 14 with 20α -OH were also found in a 17% yield. We note that the epimerization of 22-spiro-ketal could still be observed under acid conditions, suggesting that substitutions in the F ring did not influence the epimerization significantly.

Generally, ketals with six-membered rings are more difficult to epimerize, compared to five-membered rings. Thus, the same procedure used for the preparation of analogs with five-membered F rings was employed on the commercial 3,4-dihydropyran **15** with **1** to synthesize analogs **19a/19b** with six-membered F rings in good yields (Scheme 4).^{3,7} To obtain ring-expansion analogs with methyl substitutions, the commercially available ketone **16** was converted to lactone **17** using Baeyer–Villiger rearrangement in 67% yield.⁹ Subsequent reduction and dehydration of lactone **17**



Scheme 3. Reagents and conditions: (a) t-BuLi, THF, 0 °C; (b) 0.1 M HCl, THF, rt; (c) LiAlH₄, THF, 40 °C.



Scheme 4. Reagents and conditions: (a) *t*-BuLi, THF, 0 °C; (b) 0.1 M HCl, THF, rt; (c) LiAlH₄, THF, 40 °C; (d) 30% H₂O₂, (CF₃CO)₂O, rt, 67%; (e) DIBAL-H, CH₂Cl₂, -68 °C; (f) Al₂O₃, 200 °C, 37% (two steps).

provided 2,2-dimethyl-3,4-dihydropyran precursor **18**. However, the same procedure employed on 2,2-dimethyl-3,4-dihydropyran with ketone **1** offered the desired **20a/20b** in only 3% and 7% yield, respectively, for the 6-lithio-2,2-dimethyl-3,4-dihydropyran (prepared from 2,2-dimethyl-3,4-dihydropyran with *t*-BuLi in THF) mainly caused the elimination of 16β-OH to result in a α ,β-unsaturated ketone, instead of the expected 20β-OH adduct. Despite the low yields, sufficient amount of each analog was obtained upon scaling up the reaction to enable their evaluation in cell proliferation assays. However, the final congeners with the six-membered F rings (**19** and **20**) could still epimerize at 22-spiro-ketal upon acidic treatment.

We determined the activity of the new analogs in HeLa cell proliferation assay using incorporation of $[^{3}H]$ thymidine as a readout (Table 1).³ In the previous study, it was found that *R* stereochem-

Table 1

	Anti	proli	ferative	activity	of	hippu	ristanol	analogs ¹⁰
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Table 1	(continu	(ed
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istry at C22 is essential for activity. The same stereochemical requirement at C22 is seen in the new series of analogs among several pairs of diastereomers that differ only in the stereochemistry at C22. Thus, analogs 11a and 12a are both significantly more active than **11b** and **12b**. The same stereochemical requirement also applies to the analogs with six-membered F rings with analogs 19a and 20a being much more active than 19b and 20b. For the substituents on the F ring, replacement of the gem-dimethyl³ with gemdiethyl substituents led to a 10-fold decrease in activity (12a). Together with the previous observation that an analog that lacks the two methyl group is inactive,³ these results suggest that the gemdimethyl substitution is optimal for activity. It is interesting to note that the analogs with expanded six-membered F rings retained antiproliferative activity. In particular, 19a whose counterpart with five-membered F ring is inactive,³ is moderately active. This may be due to the potential compensation of the missing gem-dimethyl groups by the methylene carbon inserted into the F ring, partially compensating for the lost hydrophobic interaction with the binding pocket in eIF4A. Restoration of the gem-dimethyl group in 19a led to further enhancement of the activity (20a). Together, these results helped to further define the boundary for future design and synthesis of analogs of hippuristanol with improved stability and high biological activity as leads for developing anticancer and antiviral agents.

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- Procedure for the synthesis of analogs 12a and 12b: To a solution of 2,2diethyl-2,3-dihydrofuran 7 (200 µL, 1.46 mmol) in anhydrous THF (3 mL) was added t-BuLi (1.5 M in pentane, 1.3 mL, 1.95 mmol) at -68 °C. The reaction was allowed to warm to 0 °C and the stirring continued for another 15 min at rt. The mixture was cooled to 0 °C again and ketone 1 (91 mg, 0.16 mmol) in anhydrous THF (1 mL) was added. After stirring for 1 h, the reaction was quenched with H₂O. The resulting mixture was diluted with EtOAc (30 mL), and washed with brine. The organic layer was dried, filtered, and concentrated. The residue was dissolve in THF (4 mL) and 0.1 M HCl (1 mL). After stirring for 2 h, the mixture was diluted with EtOAc (30 mL), washed with brine, dried, and concentrated. The crude product was dissolved in anhydrous THF (2 mL), and LiAlH₄ (33 mg, 0.87 mmol) was added. After stirring for 20 min at 40 °C, the reaction was quenched with H₂O slowly. To the resulting mixture was added EtOAc (10 mL), NaHCO₃ (1 g) and Na₂SO₄ (1 g), and the stirring continued for another 3 h. The mixture was then filtered and concentrated to give a residue, which was purified by silica gel column chromatography (petroleum ether-EtOAc, 2:1) to afford analogs 12a (34 mg, 44%) and 12b (10 mg, 13%).
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- Analytical data for the analogs **11a/11b**, **12a/12b**, **13a/13b**, **14**, **19a/19b**, **20a/20b**. *Compound* **11a**: $[\alpha]_D^{28}$ +39.9 (*c* 0.87, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 10. 4.32-4.27 (m, 2H), 4.05 (br s, 1H), 3.10 (s, 1H), 2.21-2.17 (m, 1H), 2.16-2.12 (m, 1H), 2.06–2.00 (m, 1H), 1.39 (s, 3H), 1.29 (s, 3H), 1.21 (s, 3H), 1.12 (s, 3H), 1.08 (s, 3H), 1.03(s, 3H), 0.94 (s, 3H), 0.80–0.76 (m, 1H); ¹³C NMR (100 MHz, CDCl3): 8 113.7, 86.2, 79.8, 78.8, 67.7, 66.0, 65.4, 57.8, 56.7, 49.3, 47.4, 41.7, 41.6, 39.5, 35.9, 34.9, 33.8, 32.1, 31.2, 29.8, 28.4, 28.2, 27.5, 25.2, 24.1, 23.9, 23.8, 18.2, 13.6; HR-ESIMS calcd for C₂₉H₄₈O₅Na [M+Na]⁺ 499.33940, found

499.33924. *Compound* **11b**: $[\alpha]_D^{27}$ -16.1 (*c* 0.31, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 4.44–4.39 (m, 1H), 4.30 (br s, 1H), 4.05 (br s, 1H), 2.44 (d, *J* = 14.4 Hz, 1H), 2.16–2.12 (m, 1H), 2.07–2.01 (m, 1H), 1.37 (s, 3H), 1.31 (s, 3H), 1.20 (s, 3H), 1.08 (s, 3H), 1.04 (s, 3H), 1.03 (s, 3H), 0.93 (s, 3H), 0.81–0.77 (m, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 117.4, 85.5, 82.7, 78.7, 67.7, 66.2, 66.0, 57.77, 57.75, 48.5, 46.9, 41.8, 41.5, 39.5, 35.8, 34.9, 31.9, 31.8, 31.3, 29.9, 28.2, 27.4, 27.2, 24.6, 24.3, 23.8, 23.7, 18.5, 13.7; HR-ESIMS calcd for $C_{29}H_{48}O_5Na$ [M+Na]⁺ 499.33940, found 499.33975. *Compound* **12a**: $[\alpha]_D^{28}$ +38.0 (*c* 0.52, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 4.36–4.30 (m, 2H), 4.05 (br s, 1H), 3.08 (s, 1H), 2.23–2.19 (m, 1H), 2.08–2.01 (m, 1H), 1.42 (s, 3H), 1.31 (s, 3H), 1.04 (s, 3H), 0.89 (t, J = 7.6 Hz, 3H), 0.84 (t, J = 7.6 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 116.1, 88.5, 80.6, 78.9, 68.3, 66.6, 66.4, 58.4, 57.4, 49.9, 42.3, 40.1, 36.5, 35.5, 34.3, 33.5, 33.3, 32.7, 32.1, 31.8, 30.4, 29.8, 28.8, 28.6, 28.1, 18.5, 14.2, 8.9, 8.8; HR-ESIMS calcd for $C_{29}H_{48}O_5$ Na [M+Na]⁺ 499.33940, found 499.34072. Compound **12b**: $[\alpha]_D^{28} - 17.7$ (c 0.29, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 4.42 (dd, J = 12,8, 7,2 Hz, 1H), 4,31 (br s, 1H), 4,05 (br s, 1H), 2,18–2,14 (m, 1H), 1,35 (s, 3H), 1.32 (s, 3H), 1.03 (s, 3H), 0.84 (t, J = 7.6 Hz, 6H); ¹³C NMR (100 MHz, CDCl3): 8 120.6, 87.9, 82.7, 78.9, 68.1, 66.4, 66.2, 58.4, 58.1, 48.9, 42.1, 39.9, 36.3, 35.3, 32.7, 32.4, 31.9, 31.8, 31.4, 30.3, 30.2, 28.7, 27.9, 26.8, 19.7, 14.2, 8.7, 8.4; HR-ESIMS calcd for $C_{29}H_{48}O_5Na$ [M+Na]⁺ 499.33940, found 499.34087. *Compound* **13a**: $[\alpha]_D^{27}$ +42.8 (*c* 0.44, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 4.38–4.30 (m, 2H), 4.05 (br s, 1H), 3.78 (d, *J* = 8.0 Hz, 1H), 3.53 (d, *J* = 8.0 Hz, 1H), 3.10 (s, 1H), 2.22–2.18 (m, 1H), 2.07–2.01 (m, 1H), 1.38 (s, 3H), 1.30 (s, 3H), 1.21 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H), 0.80–0.76 (m, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 117.3, 80.7, 80.5, 79.8, 68.1, 66.4, 65.6, 58.3, 57.1, 49.8, 47.5, 42.1, 39.9, 37.9, 36.3, 35.2, 34.1, 32.5, 31.6, 30.1, 29.1, 28.6, 27.9, 27.1, 26.3, 18.4, 14.1; HR-ESIMS calcd for C27H44O5Na [M+Na]+ 471.30810, found 471.30893. Compound **13b**: $[\alpha]_{27}^{27}$ -42.0 (c 0.83, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 4.43-4.38 (m, 1H), 4.31 (br s, 1H), 4.05 (br s, 1H), 3.64 (d, *J* = 7.2 Hz, 1H), 3.51 (d, J = 8.0 Hz, 1H), 2.20-2.14 (m, 2H), 2.09-2.02 (m, 1H), 1.98-1.88 (m, 1H), 1.37 (s, 3H), 1.32 (s, 3H), 1.14 (s, 3H), 1.05 (s, 3H), 1.03 (s, 3H), 0.81–0.78 (m, 1H); ¹³C NMR (100 MHz, CDCl₃); δ 121.1, 82.7, 79.8, 79.4, 68.0, 66.6, 66.3, 58.3, 58.1, 48.7, 46.4, 42.0, 39.8, 37.9, 36.2, 35.2, 32.3, 31.76, 31.75, 30.3, 28.6, 27.8, 27.7, 26.9, 26.0, 19.0, 14.1; HR-ESIMS calcd for C₂₇H₄₄O₅Na $[M+Na]^+$ 471.30810, found 471.30901. Compound 14: $[\alpha]_D^{27}$ -36.8 (c 0.32, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 4.60-4.55 (m, 1H), 4.42 (s, 1H), 4.31 (br s, 1H), 4.04 (br s, 1H), 2.22-2.18 (m, 1H), 2.12-2.08 (m, 1H), 1.43 (s, 3H), 1.24 (s, 3H), 1.12 (s, 3H), 1.11 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H), 0.95 (s, 3H), 0.82-0.78 (m, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 114.9, 85.6, 80.1, 79.7, 69.7, 67.7, 65.9, 58.2, 57.6, 47.8, 47.6, 42.8, 39.9, 39.4, 35.8, 34.8, 31.7, 31.4, 30.2, 28.2, 27.4, 24.3, 24.0, 23.8, 23.7, 23.0, 17.8, 13.9; HR-ESIMS calcd for C₂₉H₄₈O₅Na [M+Na]⁺ 499.33940, found 499.33963. *Compound* **19a**: $[a]_D^{27}$ +11.4 (c 0.70, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 4.4 (dd, J = 15.2, 7.6 Hz, 1H), 4.30 (br s, 1H), 4.05 (br s, 1H), 3.98–3.92 (m, 1H), 3.76–3.72 (m, 1H), 3.29 (s, 1H), 2.22–2.18 (m, 1H), 2.09–2.03 (m, 1H), 1.32 (s, 3H), 1.25 (s, 3H), 1.03 (s, 3H), 0.80–0.77 (m, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 105.0, 81.4, 81.0, 68.1, 66.5, 66.4, 61.8, 58.3, 57.1, 50.0, 42.4, 40.0, 36.3, 35.3, 33.8, 32.6, 31.6, 30.21, 30.18, 28.7, 27.9, 27.4, 24.9, 20.1, 18.5, 14.1; HR-MALDI calcd for $C_{26}H_{42}O_5Na$ [M+Na]⁺ 457.2924, found 457.2933. *Compound* **19b**: $[\alpha]_{27}^{27}$ -34.3 (*c* 0.94, CHCl₃); ¹H NMR (400 MHz, CDCl₃₀): δ 4.38–4.33 (m, 1H), 4.30 (br s, 1H), 4.05 (br s, 1H), 3.79–3.72 (m, 1H), 3.60–3.56 (m, 1H), 2.18–2.14 (m, 1H), 2.09–2.03 (m, 1H), 1.34 (s, 3H), 1.11 (s, 3H), 1.03 (s, 3H), 0.81–0.77 (m, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 110.2, 83.5, 79.2, 68.0, 66.8, 66.4, 61.1, 58.5, 58.0, 48.7, 42.0, 39.9, 36.3, 35.3, 32.3, 31.8, S_{12} , 30.2, 28.6, 28.1, 27.9, 26.5, 25.1, 19.9, 19.7, 14.1; HR-MALDI calcd for $C_{26}H_{42}O_5Na$ [M+Na]⁺ 457.2924, found 457.2928. *Compound* 20a: ¹H NMR (400 MHz, CDCl₃): δ 4.35–4.29 (m, 2H), 4.05 (br s, 1H), 3.50 (s, 1H), 2.23–2.19 (m, 1H), 1.39 (s, 3H), 1.38 (s, 3H), 1.24 (s, 3H), 1.20 (s, 3H), 1.04 (s, 3H), 0.80–0.77 (m, 1H). *Compound* **20b**: ¹H NMR (300 MHz, CDCl₃): δ 4.36 (dd,