Tetrahedron 67 (2011) 6673–6678

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Concise synthesis and structure–activity relationship of furospinosulin-1, a hypoxia-selective growth inhibitor from marine sponge

Naoyuki Kotoku *, Shinichi Fujioka, Chiaki Nakata, Masaki Yamada, Yuji Sumii, Takashi Kawachi, Masayoshi Arai, Motomasa Kobayashi *

Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan

A R T I C L E I N F O

Article history:
Received 9 April 2011
Received in revised form 2 May 2011
Accepted 3 May 2011
Available online 10 May 2011

Keywords:
Furospinosulin-1
Hypoxia-selective growth inhibitor
Marine sponge
Analogue compound

A B S T R A C T

Structure–activity relationship of furospinosulin-1 (**1**), a hypoxia-selective growth inhibitor isolated from marine sponge, was investigated. Concise synthetic method of **1** was developed, and some structurally modified analogues were prepared. Biological evaluation of them revealed that the whole chemical structure was important for the hypoxia-selective growth inhibitory activity of **1**. Among prepared, the desmethyl analogue **30** showed excellent hypoxia-selective inhibitory activity similar to that of **1** and also exhibited in vivo anti-tumor activity with oral administration.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Hypoxic environment in tumor is now recognized to be an important factor for tumor growth, angiogenesis, metastasis, and response to chemotherapy and irradiation.[1] Then, the compounds, which exhibit growth inhibitory activity against tumor cells under hypoxic environment selectively, are expected to be novel and promising lead for anti-cancer drugs and to be important bioprobe to find new responsible molecules for the adaptation of hypoxia in tumor cells.[2]

In the course of our search for hypoxia-selective growth inhibitors, we have isolated a furanosesterterpene, furospinosulin-1 (**1**),[3] from the Indonesian marine sponge of *Dactylospongia elegans*. It revealed that the compound **1** showed selective growth inhibitory activity against human prostate cancer cells DU145 under 1% of low oxygen atmosphere dose-dependently, while **1** did not inhibit production of Hypoxia Inducible Factor-1α (HIF-1α), which is considered to be a major transcription factor to adapt hypoxic environment in tumor. Moreover, orally administered furospinosulin-1 (**1**) suppressed tumor growth in the mice *s.c.*-inoculated mouse sarcoma S180 cells.[4] These intriguing bioactivities of furospinosulin-1 (**1**) prompted us to engage in synthetic study, in order to supply sufficient amount of the compound for further biological study and to develop a more promising drug lead for the treatment of cancer. Here we report the practical synthesis of furospinosulin-1 (**1**) and its analogues together with the biological evaluation of them (Fig. 1).

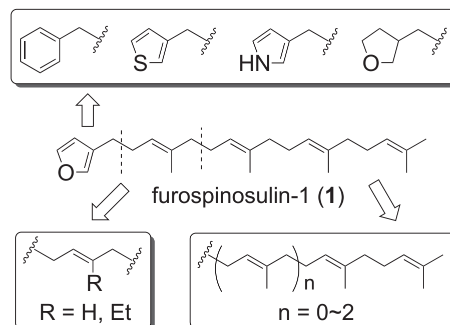


Fig. 1. Furospinosulin-1 (**1**).

2. Results and discussions

2.1. Synthesis of furospinosulin-1 and analogues

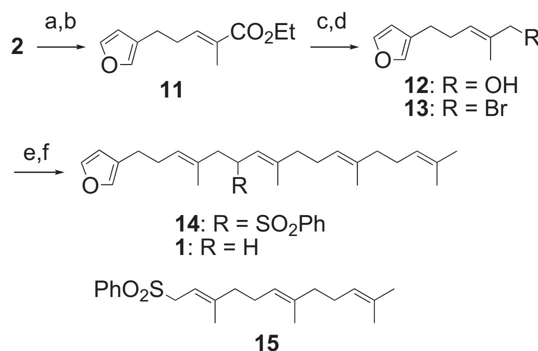
Furospinosulin-1 (**1**) has an aromatic furan ring and a long side chain consisting of four iterative isoprene units. As there has been no information about structure-requirement for the hypoxia-selective

* Corresponding authors. Tel.: +81 6 6879 8215; fax: +81 6 6879 8219; e-mail addresses: kotoku@phs.osaka-u.ac.jp (N. Kotoku), kobayasi@phs.osaka-u.ac.jp (M. Kobayashi).

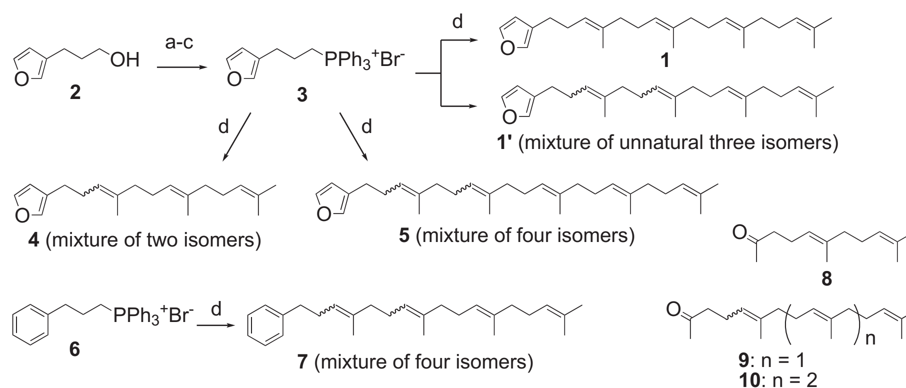
0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.05.009

growth inhibitory activity of **1**, we decided to elucidate structure–activity relationship of **1** through synthesis and biological evaluation of some structurally modified analogues.

Firstly, geometrically non-selective synthesis of **1** was examined, to analyze participation of the olefin geometry in the side chain of **1**. Wittig reaction between the phosphonium salt **3**,[5] which was prepared from 3-(3-furyl)propan-1-ol (**2**),[6] and commercially available farnesylacetone (**9**, a mixture of C-5 isomers) provided a mixture of four geometric isomers of **1** (Scheme 1). The natural type (*E*,*E*,*E*)-isomer and the other isomers (**1′**) were separated by using reversed-phase HPLC. Analogues **4** and **5** having a side chain of different lengths were also prepared as a mixture of isomers, through the coupling reaction between **3** and geranylacetone (**8**) or teplenone (**10**, a mixture of isomers), respectively. Furthermore coupling reaction between **9** and a known phosphonium salt **6**[7] gave a phenyl-analogue **7**.



Scheme 2. Reagents and conditions: (a) PCC, CH_2Cl_2, 81%; (b) $Ph_3P{=}C(Me)CO_2Et$, toluene, 60 °C, 80%; (c) DIBAL, CH_2Cl_2, 0 °C, quant.; (d) PPh_3, CBr_4, CH_2Cl_2, quant.; (e) **15**, *t*-BuOK, THF, −30 °C, 96%; (f) $LiBHEt_3$, $Pd(dppp)Cl_2$, THF, 0 °C, 96%.



Scheme 1. Reagents and conditions: (a) TsCl, pyridine, 89%; (b) LiBr, acetone, 94%; (c) PPh_3, toluene, 120 °C, 57%; (d) **8** or **9** or **10**, *n*-BuLi, THF, **1**: 23%; **4**: 26%; **5**: 31%.

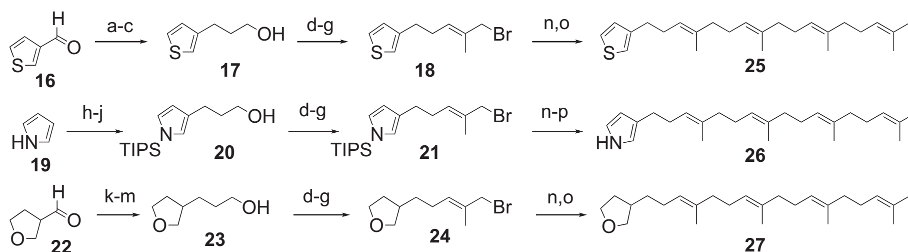
Secondly, geometry-selective total synthesis of furospinosulin-1 (**1**) was examined using sulfone-mediated coupling method.[8] In order to apply to the syntheses of various analogues, furospinosulin-1 (**1**) was divided into the two fragments of the allylic bromide **13** and the known farnesyl phenylsulfone **15**.[9] PCC oxidation of the alcohol **2** and subsequent Wittig olefination provided an enoate **11** (*E*/*Z*=20:1). The undesired *Z*-isomer could be removed by SiO_2 column chromatography easily. Then, DIBAL reduction of the ester moiety in **11** and following bromination of the hydroxyl group of **12** with CBr_4/PPh_3 gave a desired allylic bromide **13** in quantitative yield. Coupling reaction between compounds **13** and **15** proceeded smoothly using *t*-BuOK as a base[10] to yield a compound **14**, and final reductive desulfonylation[11] of **14** with $LiBHEt_3$ in the presence of $Pd(dppp)Cl_2$ afforded furospinosulin-1 (**1**), by 60% overall yield with six linear reaction steps from the known compound **2**. No olefin isomerization was observed throughout the synthesis.

We next executed the syntheses of some structurally modified analogues of **1** using the same synthetic method shown in Scheme 2. Various analogues could be prepared systematically by changing structure of the allylic bromide, to analyze the detailed structure–activity relationship around the aromatic ring. Scheme 3 shows the syntheses of the analogues having a different heterocycle. The thiophene, pyrrole, and tetrahydrofuran-containing allylic bromides (**18**, **21**, and **24**) were prepared in seven steps from the commercially available materials (**16**, **19**, and **22**), respectively.[12,13] Then, coupling reaction with the sulfone **15** and subsequent desulfonylation provided analogues **25**–**27** in good yield.

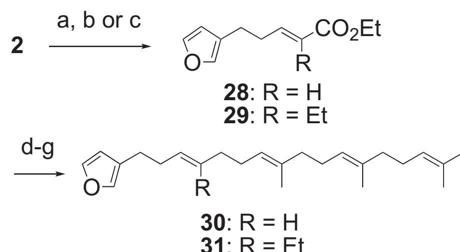
Two analogues (**30** and **31**) having a different substituent were also prepared, as depicted in Scheme 4. The use of the different Wittig or Horner–Emmons reagents as an elongation unit provided desired fragments **28** and **29**, and the following steps similar to those of the other analogues afforded compounds **30** and **31** having a proton or ethyl group in place of the methyl group nearest to the furan ring, respectively.

2.2. Biological evaluation of synthetic analogues

The growth inhibitory effects of the synthetic analogues against DU145 cells under normoxic or hypoxic conditions were evaluated, which were briefly summarized in Fig. 2. A mixture (**1′**) of three geometrical isomers of **1** showed very weak and non-selective growth inhibition. Analogue **4** having a shorter side chain showed non-selective toxicity at higher dose, and analogue **5** having a longer chain showed moderate hypoxia-selective activity similar to **1**. These results show that the both geometry and length of the side chain in **1** were important for exhibiting hypoxia-selective growth inhibitory activity. Phenyl analogue **7** showed only weak toxicity in normoxic condition. Thiophene analogue **25** showed weak but hypoxia-selective activity, and pyrrole analogue **26** and tetrahydrofuran analogue **27** were toxic even in lower dose. These evidences clearly indicate that the furan moiety might be the best structure in aromatic part. Ethyl analogue **31** showed no hypoxia-selective activity, maybe due to the steric repulsion, while desmethyl analogue **30** showed excellent hypoxia-selective activity both in lower and higher dose. These results revealed that the



Scheme 3. Reagents and conditions: (a)$Ph_3P{=}CHCO_2Et$, CH_2Cl_2, 95%; (b) DIBAL, CH_2Cl_2, 0 °C, 98%; (c) H_2/Pd–C, EtOH, quant.; (d) PCC, CH_2Cl_2; (e) $Ph_3P{=}C(Me)CO_2Et$, toluene, 60 °C; (f) DIBAL, CH_2Cl_2, 0 °C; (g) PPh_3, CBr_4, CH_2Cl_2, **18**: 49%; **21**: 66%; **24**: 41% (each for four steps); (h) TIPSCl, *n*-BuLi, THF, −78 °C, 98%; (i) NBS, THF, −78 °C, 93%; (j) *n*-BuLi, THF, −78 °C; trimethylene oxide, $BF_3 \cdot Et_2O$, 83%; (k) $(EtO)_2P(O)CH_2CO_2Et$, NaOH, CH_2Cl_2/H_2O, 51%; (l) H_2/Pd–C, AcOEt, quant.; (m) DIBAL, CH_2Cl_2, 0 °C, 75%; (n) **15**, *t*-BuOK, THF, −30 °C; (o) $LiBHEt_3$, Pd(dppp)Cl_2, THF, 0 °C, **25**: 56%; **27**: 50% (each for two steps); (p) TBAF, THF, **26**: 72% (three steps).



Scheme 4. Reagents and conditions: (a) PCC, CH_2Cl_2, 81%; (b) $Ph_3P{=}CHCO_2Et$, toluene, 55%; (c) $(EtO)_2P(O)CH(Et)CO_2Et$, NaH, THF, 50%; (d) DIBAL, CH_2Cl_2, 0 °C; (e) PPh_3, CBr_4, CH_2Cl_2; (f) **15**, *t*-BuOK, THF, −30 °C; (g) $LiBHEt_3$, Pd(dppp)Cl_2, THF, 0 °C, **30**: 75%; **31**: 69% (each for four steps).

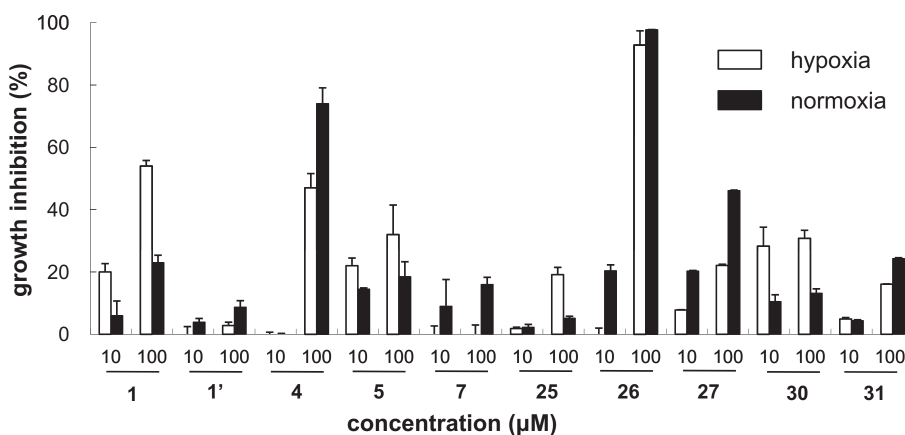


Fig. 2. Growth inhibition of synthetic analogues against DU145 cells.

whole chemical structure of **1** should be needed for its hypoxia-selective growth inhibitory activity. The binding molecule (protein) of **1** might recognize the whole structure of **1** strictly.

The detailed growth inhibition of desmethyl analogue **30** against DU145 cells was shown in Fig. 3. Comparing with that of **1**, analogue **30** exhibited greater growth inhibition at lower dose (1–10 μM) and lower toxicity in normoxic condition at higher dose (300 μM). These properties of **30** might be better than **1** for drug candidate.

Then, in vivo anti-tumor testing of analogue **30** was also examined. Murine sarcoma S180 cells were implanted subcutaneously, and the testing compound was administrated orally. The effectiveness of the compound was determined by weighing the surged tumor. It was found that the 10–50 mg/kg dose of analogue **30** inhibited growth of tumor, and 72% reduction of the tumor weight was observed at the dose of 50 mg/kg compared with that of the control (Fig. 4). Moreover, analogue **30** exhibited no significant acute toxicity, such as body weight loss or diarrhea, up to 100 mg/kg administration.

3. Conclusion

We investigated structure–activity relationship of furospinosulin-1 (**1**) through concise syntheses of some structurally modified analogues. It revealed that the whole chemical structure was important for the hypoxia-selective growth inhibition of **1**. We found that desmethyl analogue **30** showed excellent hypoxia-selective growth inhibitory activity similar to that of **1**, and also exhibited in vivo anti-tumor effect with oral administration. Development of more promising hypoxia-targeting anti-tumor drug candidate is now under investigation.

4. Experimental

4.1. General experimental

A JEOL JNM LA-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz) and a Varian NMR system (^{1}H: 600 MHz, ^{13}C: 150 MHz) spectrometer were used to obtain ^{1}H and ^{13}C NMR data using tetramethylsilane as an internal standard. Mass spectra were obtained with a Waters Q-Tof Ultima API using MeOH as a solvent. HPLC was performed using a Hitachi L-6000 pump equipped with Hitachi L-4000H UV detector. Silica gel (Kanto, 40–100 μm) and pre-coated thin layer chromatography (TLC) plates (Merck, $60F_{254}$) were used for column chromatography and TLC. Spots on TLC plates were detected by spraying phosphomolybdic acid solution (5 g phosphomolybdic

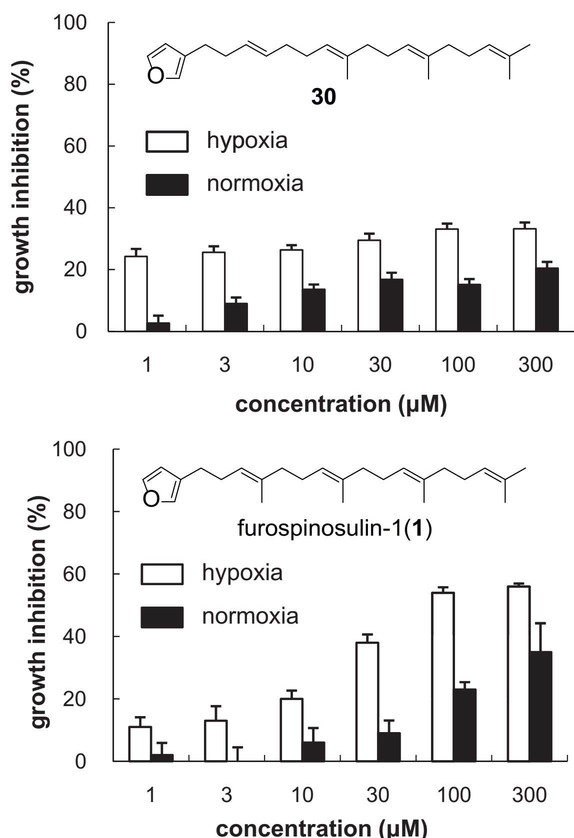


Fig. 3. Detailed growth inhibition of desmethyl analogue (**30**) and furospinosulin-1 (**1**).

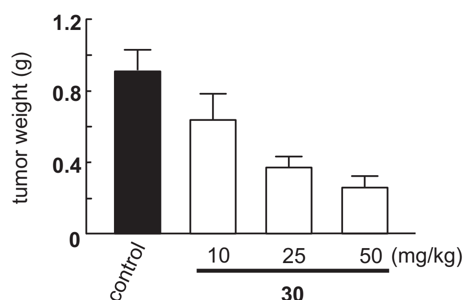


Fig. 4. anti-Tumor effect of desmethyl analogue (**30**).

acid in 100 mL of EtOH) and acidic *p*-anisaldehyde solution (*p*-anisaldehyde: 25 mL, *c*-H_2SO_4: 25 mL, AcOH: 5 mL, EtOH: 425 mL) with subsequent heating. Unless otherwise noted, all the reactions were performed under N_2 atmosphere using purchased reagents and solvents without further purification. After workup, the organic phases were dried over Na_2SO_4.

4.2. Geometry non-selective synthesis of furospinosulin-1 and analogues

*4.2.1. 3-(Furan-3-yl)propan-1-ol (**2**).* *n*-BuLi (1.65 M in *n*-hexane, 1.3 mL, 2.12 mmol) was added to a solution of 3-bromofuran (297 mg, 2.02 mmol) in THF (4.5 mL) at −78 °C, and the whole mixture was stirred for 30 min. Trimethylene oxide (0.15 mL, 2.32 mmol) and $BF_3 \cdot Et_2O$ (0.31 mL, 2.42 mmol) were added to the mixture, and the whole mixture was further stirred for 3 h at −78 °C. H_2O was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column chromatography (*n*-hexane/AcOEt=3:1) to give **2** (156 mg, 61%) as a colorless oil. IR (KBr): 3378, 2984, 1684, 1520, 1419, 1143 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.35 (1H, t, *J*=1.8 Hz), 7.23 (1H, s), 6.28 (1H, s), 3.68 (2H, t, *J*=6.4 Hz), 2.52 (2H, t, *J*=7.3 Hz), 1.83 (2H, dt, *J*=14.9, 6.7 Hz), 1.42 (1H, br s). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 142.8, 138.9, 124.4, 110.9, 62.2, 32.8, 21.0.

*4.2.2. [3-(Furan-3-yl)propyl]triphenylphosphonium bromide (**3**).* *p*-Toluenesulfonyl chloride (1.95 g, 10.2 mmol) was added to a solution of **2** (644 mg, 5.10 mmol) in pyridine (12 mL) at −20 °C, and the whole mixture was stirred for 6 h. H_2O was added to the mixture at 0 °C, and the whole mixture was extracted with Et_2O. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by SiO_2 column chromatography (*n*-hexane/AcOEt=5:1) to give 3-(furan-3-yl)propyl *p*-toluenesulfonate (1.27 g, 89%) as a colorless oil. IR (KBr): 2955, 1360, 1177 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.79 (2H, d, *J*=8.5 Hz), 7.35 (2H, d, *J*=7.9 Hz), 7.32 (1H, s), 7.11 (1H, s), 6.18 (1H, s), 4.04 (2H, t, *J*=6.4 Hz), 2.48 (2H, d, *J*=7.3 Hz), 2.46 (3H, s), 1.92–1.86 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 144.7, 143.0, 139.2, 133.1, 129.8 (2C), 127.9 (2C), 123.1, 110.6, 69.5, 29.2, 21.6, 20.5.

Lithium bromide (228 mg, 2.6 mmol) was added to a solution of the above product (147 mg, 0.53 mmol) in acetone (5.0 mL), and the whole mixture was stirred for 4 h at 60 °C. H_2O was added to the mixture at 0 °C, and the whole mixture was extracted with Et_2O. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by SiO_2 column chromatography (*n*-hexane/AcOEt=10:1) to give 3-(3-bromopropyl)furan (93.4 mg, 94%) as a colorless oil. IR (KBr): 2934, 1503, 1437, 1260 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.37 (1H, t, *J*=1.5 Hz), 7.26 (1H, s), 6.28 (1H, s), 3.41 (2H, t, *J*=6.7 Hz), 2.60 (2H, t, *J*=7.3 Hz), 2.12–2.07 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 143.0, 139.7, 123.1, 110.8, 33.0, 32.7, 23.0.

Triphenylphosphine (957 mg, 3.65 mmol) was added to a solution of the above product (276 mg, 1.46 mmol) in toluene (5.0 mL), and the whole mixture was stirred for 12 h at 120 °C. Removal of the solvent from the reaction mixture gave a crude product, which was purified by SiO_2 column (CH_2Cl_2/MeOH=10:1) to give **3** (307 mg, 57%) as a colorless amorphous solid. IR (KBr): 2899, 1439 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.85–7.75 (9H, m), 7.70–7.66 (6H, m), 7.31 (1H, d, *J*=1.2 Hz), 7.26 (1H, s), 6.18 (1H, d, *J*=1.2 Hz), 3.97–3.91 (2H, m), 2.87 (2H, t, *J*=7.0 Hz), 1.88 (2H, td, *J*=15.6, 7.5 Hz).

*4.2.3. Furospinosulin-1 (**1**) E,Z mixture.* *n*-BuLi (1.65 M in *n*-hexane, 0.17 mL, 0.39 mmol) was added dropwise to a solution of **3** (119 mg, 0.26 mmol) in THF (0.6 mL) at −78 °C, and the whole mixture was stirred for 30 min. A solution of farnesylacetone (**9**) (49.3 mg, 0.19 mmol) in THF (0.3 mL) was added to the solution via cannula, and the whole mixture was stirred for 3 h at 0 °C. Satd NH_4Cl was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt=30:1) to give 1 (15.4 mg, 23%) as a colorless oil (1:1:1:1 mixture of four geometrical isomers). ^1H NMR (500 MHz, $CDCl_3$) δ: 7.34 (1H, s), 7.21 (1H, s), 6.27 (1H, s), 5.17 (1H, t, *J*=7.3 Hz), 5.13–5.10 (3H, m), 2.45 (2H, q, *J*=7.1 Hz), 2.25 (2H, q, *J*=7.5 Hz), 2.06–2.00 (12H, m), 1.69–1.61 (15H, s). ESI-MS *m*/*z*: 377 $[M+Na]^+$. HR-ESI-MS: 377.2820, calcd for $C_{25}H_{38}ONa$. Found: 377.2836. Compound **1** ((*E*,*E*)-isomer) and other three-isomeric mixture were separated by reversed-phase HPLC (COSMOSIL $5C_{18}$ MS-II, MeOH/H_2O=9:1).

Analogues **4** and **5** were obtained from geranylacetone (**8**) or teplenone (**10**), respectively, using the same procedure as that for **1**. Analogue **7** was also obtained from known phosphonium salt **6**[7] and **9**.

4.3. Geometry-selective synthesis of furospinosulin-1 and analogues

*4.3.1. (E)-5-(Furan-3-yl)-2-methylpent-2-enoic acid ethyl ester (**11**).* A solution of **2** (460 mg, 3.64 mmol) in CH_2Cl_2 (6 mL) was added to the suspension of Celite (864 mg) and PCC (864 mg, 4.00 mmol) in CH_2Cl_2 (30 mL) via cannula, and the whole mixture was stirred for 3 h at rt. Then the mixture was diluted with Et_2O (50 mL), and the whole mixture was filtered through short SiO_2 pad. Removal of the solvent from the filtrate under reduced pressure to give a crude product, which was purified by SiO_2 column (*n*-hexane/Et_2O=1:1) to give 3-(furan-3-yl)propionaldehyde (365 mg, 81%) as a colorless oil. IR (KBr): 2926, 1726, 1502, 1389 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 9.81 (1H, s), 7.35 (1H, t, *J*=1.8 Hz), 7.23 (1H, s), 6.28–6.26 (1H, m), 2.78–2.77 (2H, m), 2.72–2.69 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 201.5, 143.1, 139.0, 123.3, 110.7, 44.0, 17.5.

Ph_3P=C(Me)CO_2Et (665 mg, 1.91 mmol) was added to the solution of the above product (198 mg, 1.59 mmol) in toluene (16 mL), and the whole mixture was stirred for 8 h at 60 °C. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt=5:1) to give **11** (247 mg, 80%) as a colorless oil. IR (KBr): 2982, 1711, 1447, 1368, 1275 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.35 (1H, dd, *J*=2.4, 1.2 Hz), 7.23 (1H, d, *J*=1.8 Hz), 6.79–6.75 (1H, m), 6.28 (1H, s), 4.19 (2H, q, *J*=7.1 Hz), 2.57 (2H, t, *J*=7.3 Hz), 2.43 (2H, q, *J*=7.5 Hz), 1.81 (3H, s), 1.29 (3H, t, *J*=7.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.1, 142.9, 140.9, 138.9, 128.5, 124.1, 110.8, 60.5, 29.2, 23.8, 14.3, 12.4. ESI-MS *m*/*z*: 231 [M+Na]$^+$. HR-ESI-MS: 231.0997, calcd for $C_{12}H_{16}O_3Na$. Found: 231.1005.

*4.3.2. (E)-5-(Furan-3-yl)-2-methylpent-2-en-1-ol (**12**).* DIBAL (1.0 M in *n*-hexane, 1.62 mL, 1.62 mmol) was added to a solution of **11** (96.0 mg, 0.46 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C, and the whole mixture was stirred for 20 min. 5% HCl was added to the mixture, and the whole mixture was extracted with CH_2Cl_2. Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt=5:1) to give **12** (77.4 mg, quant.) as a colorless oil. IR (KBr): 3333, 2929, 1503, 1449, 1383 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.35 (1H, s), 7.22 (1H, s), 6.28 (1H, s), 5.45 (1H, td, *J*=7.0, 1.4 Hz), 4.00 (2H, s), 2.49 (2H, t, *J*=7.6 Hz), 2.30 (2H, q, *J*=7.5 Hz), 1.66 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 142.7, 138.8, 135.5, 125.3, 124.7, 111.0, 68.8, 28.0, 24.7, 13.7. ESI-MS *m*/*z*: 189 [M+Na]$^+$. HR-ESI-MS: 189.0891, calcd for $C_{10}H_{14}O_2Na$. Found: 189.0917.

*4.3.3. (E)-3-(5-Bromo-4-methylpent-3-enyl)furan (**13**).* PPh_3 (147 mg, 0.55 mmol) and CBr_4 (186 mg, 0.55 mmol) were successively added to the solution of **12** (78.0 mg, 0.46 mmol) in CH_2Cl_2 (2.3 mL) at 0 °C, and the whole mixture was stirred for 30 min. H_2O was added to the mixture, and the whole mixture was extracted with CH_2Cl_2. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt=30:1) to give **13** (97.3 mg, quant.) as a colorless oil. IR (KBr): 2920, 1501, 1437, 1206 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.35 (1H, s), 7.21 (1H, s), 6.27 (1H, s), 5.63 (1H, td, *J*=7.0, 1.0 Hz), 3.97 (2H, s), 2.49 (2H, t, *J*=7.6 Hz), 2.29 (2H, q, *J*=7.3 Hz), 1.74 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 142.7, 138.9, 132.7, 130.4, 124.3, 110.9, 41.5, 28.7, 24.2, 14.7.

*4.3.4. (2E, 6E)-(3,7,11-Trimethyldodeca-2,6,10-triene-1-sulfonyl)benzene (**15**).* $PhSO_2Na$ (411 mg, 2.51 mmol) was added to the solution of farnesyl bromide (549 mg, 1.93 mmol) in DMF (10 mL) at 0 °C, and the whole mixture was stirred for 1 h. Satd NH_4Cl aq was added to the mixture, and the whole mixture was extracted with Et_2O. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt=10:1) to give **15** (464 mg, 70%) as a colorless oil. IR (KBr): 2920, 1447, 1306, 1146 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.88–7.86 (2H, m), 7.64–7.63 (1H, m), 7.55–7.52 (2H, m), 5.19 (1H, td, *J*=8.1, 1.4 Hz), 5.08–5.05 (2H, m), 3.81 (2H, d, *J*=7.9 Hz), 2.05–1.99 (8H, m), 1.68 (3H, s), 1.60 (3H, s), 1.58 (3H, s), 1.31 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 146.4, 138.7, 135.7, 133.5, 131.4, 128.9 (2C), 128.6 (2C), 124.2, 123.3, 110.3, 56.1, 39.7, 26.7, 26.2, 25.7, 17.7, 16.2, 16.0.

*4.3.5. ((3E,7E,11E)-4,8,12,16-Tetramethyl-6-(phenylsulfonyl)heptadeca-3,7,11,15-tetraen-1-yl)furan (**14**).* *t*-BuOK (1.0 M in THF, 0.16 mL, 0.16 mmol) was added to a solution of **13** (30.1 mg, 0.13 mmol) and **15** (41.2 mg, 0.12 mmol) in THF (0.6 mL) at −30 °C, and the whole mixture was stirred for 1 h. Satd NH_4Cl aq was added to the mixture at 0 °C, and the whole mixture was extracted with Et_2O. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt=20:1) to give **14** (62.4 mg, 96%) as a colorless oil.

IR (KBr): 2920, 1447, 1306, 1146 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.84 (2H, d, *J*=6.7 Hz), 7.62 (1H, t, *J*=7.6 Hz), 7.51 (2H, t, *J*=7.6 Hz), 7.31 (1H, t, *J*=1.5 Hz), 7.15 (1H, s), 6.22 (1H, s), 5.19 (1H, t, *J*=7.0 Hz), 5.08–5.04 (2H, m), 4.91 (1H, d, *J*=10.4 Hz), 3.89–3.87 (1H, m), 2.89 (1H, d, *J*=12.2 Hz), 2.39 (2H, t, *J*=7.6 Hz), 2.29 (1H, dd, *J*=13.7, 6.9 Hz), 2.20 (2H, q, *J*=7.3 Hz), 2.04–1.97 (8H, m), 1.68 (3H, s), 1.60 (3H, s), 1.58 (3H, s), 1.52 (3H, s), 1.15 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 145.1, 142.5, 138.7, 137.9, 135.5, 133.3, 131.3, 130.6, 129.2 (2C), 128.6 (2C), 127.6, 124.5, 124.1, 123.4, 117.2, 110.8, 63.4, 39.63, 39.61, 37.3, 28.3, 26.6, 26.2, 25.6, 24.7, 17.6, 16.3, 15.92, 15.88. ESI-MS *m*/*z*: 517 [M+Na]$^+$. HR-ESI-MS: 517.2752, calcd for $C_{31}H_{42}O_3NaS$. Found: 517.2727.

*4.3.6. Furospinosulin-1 (**1**).* $LiBHEt_3$ (1.0 M in THF, 0.34 mL, 0.34 mmol) was added dropwise to a solution of **14** (49.3 mg, 0.097 mmol) and Pd(dppp)Cl_2 (11.4 mg, 0.019 mmol) in THF (0.48 mL) at 0 °C, and the whole mixture was stirred for 30 min. Satd NH_4Cl aq was added to the mixture at 0 °C, and the whole mixture was extracted with AcOEt. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt=10:1) to give **1** (33.0 mg, 96%) as a colorless oil. IR (KBr): 2920, 1447, 1306, 1146 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.33 (1H, s), 7.21 (1H, s), 6.27 (1H, s), 5.17 (1H, t, *J*=6.5 Hz), 5.11 (3H, s), 2.45 (2H, t, *J*=7.3 Hz), 2.25 (2H, q, *J*=7.3 Hz), 2.07 (6H, m), 2.00 (6H, m), 1.68 (3H, s), 1.60 (12H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 142.5, 138.8, 135.8, 135.0, 134.9, 131.3, 125.0, 124.4, 124.3, 124.2, 123.7, 110.1, 39.7, 28.4, 26.8, 26.6, 26.5, 25.7, 25.0, 17.7, 16.04, 16.00. ESI-MS *m*/*z*: 377 [M+Na]$^+$. HR-ESI-MS: 377.2820, calcd for $C_{25}H_{38}ONa$. Found: 377.2801.

Analogues **25**–**27**, **30**, and **31** were prepared using the same procedure as that for **1**, using compounds **17**, **20**, **23**, **28**, and **29** as substrates.

4.4. Biological evaluation of furospinosulin-1 analogues

4.4.1. Cell cultures. Human prostate cancer cell line DU145 and mouse sarcoma cell S180 were cultured in RPMI 1640 supplemented with heat-inactivated 10% fetal bovine serum (FBS) and kanamycin (50 μg/mL) in a humidified atmosphere of 5% CO_2 at 37 °C.

4.4.2. Assay for anti-proliferative activity under hypoxic condition. The cells in the culture medium was plated into each well of 96-well plates (1×10^4 cells/well/200 μL) for 4 h in a humidified atmosphere of 5% CO_2 at 37 °C (normoxic condition). Then, the plates were incubated for 12 h in the 94% nitrogen, 5% CO_2, and 1% O_2 (hypoxic condition) inducing hypoxia related genes, such

as HIF-1α. After 12 h incubation, testing compounds were added, and then the plates were incubated for an additional 24 h in the hypoxic condition. The cell proliferation was detected according to established MTT method as previously described.[4] The growth inhibition rate was calculated as percentage of parallel negative controls. The anti-proliferative activity of testing compounds under normoxic condition was also evaluated by colorimetric reagent of MTT.

*4.4.3. In vivo anti-tumor effect of analogue **30***. All animal procedures were approved by the Committee on Animal Experimentation of Osaka University. Mouse sarcoma S180 cells (1×10^6 cells/body) were implanted subcutaneously into the right ventral flank of female ddY mice (6 weeks old). After one week from implantation, analogue **30** was administered on every other day for 2 week (total seven times) by oral administration as a suspension in 1% sodium carboxymethyl cellulose (CMC–Na). Then, tumor was isolated and weighed to calculate the inhibition ratio. The control group and the group treated with analogue **30** consisted of six mice each in this study.

Acknowledgements

This study was financially supported by the Uehara Memorial Foundation, and by the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Supplementary data

The experimental procedures and physical data for compounds **4**–**31**, together with the ^{1}H and ^{13}C NMR spectra of compounds **1**–**31**, are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.05.009.

References and notes

1. Dewhirst, M. W.; Cao, Y.; Moeller, B. *Nat. Rev. Cancer* **2008**, *8*, 425.
2. Wouters, B. G.; Koritzinsky, M. *Nat. Rev. Cancer* **2008**, *8*, 851.
3. Cimino, G.; De Stefano, S.; Minale, L. *Tetrahedron* **1972**, *28*, 1315.
4. Arai, M.; Kawachi, T.; Setiawan, A.; Kobayashi, M. *ChemMedChem* **2010**, *5*, 1919.
5. Magatti, C. V.; Kaminski, J. J.; Rothberg, I. *J. Org. Chem.* **1991**, *56*, 3102.
6. Sperry, J. B.; Wright, D. L. *Tetrahedron* **2006**, *62*, 6551.
7. Kaiser, S.; Smidt, S. P.; Pfaltz, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 5194.
8. Grieco, P. A.; Masaki, Y. *J. Org. Chem.* **1974**, *39*, 2135.
9. Robustell, B.; Abe, I.; Prestwich, G. D. *Tetrahedron Lett.* **1998**, *39*, 957.
10. Zheng, Y.; Lin, J.; Li, C.; Li, J. *J. Chem. Res.* **2007**, 686.
11. Mohri, M.; Kinoshita, H.; Inomata, K.; Kotake, H. *Chem. Lett.* **1985**, 451.
12. Martins, A.; Lautens, M. *J. Org. Chem.* **2008**, *73*, 8705.
13. Morrison, M. D.; Hanthorn, J. J.; Pratt, D. A. *Org. Lett.* **2009**, *11*, 1051.