SYNTHESIS OF SUNITINIB–METASTIN CONJUGATE, A NOVEL ESTERASE-SENSITIVE PRODRUG SYSTEM BASED ON LACTONIZATION REACTION

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Abstract – We describe a strategy for preparing sunitinib–metastin conjugate, a prodrug composed of the anticancer agent sunitinib for renal cell carcinoma and the carrier protein metastin, which are conjugated to each other by a linker. We designed a modified L-homoserine linker, which is composed of an acyl group that acts as the masking group for hydrolysis with an esterase, as well as a carbon chain of appropriate length between sunitinib and metastin. The sunitinib–metastin conjugate was converted into a hydrolyte by hydrolysis of the acyl group with an esterase, and sunitinib was released by intramolecular lactonization. Sunitinib–metastin conjugate, an esterase-sensitive amide prodrug that has a modified L-homoserine linker that participates in the intramolecular lactonization, was synthesized.

INTRODUCTION

The KISS-1 gene is located on human chromosome lq32.12 KISS-1 encodes a C-terminus peptide with 54 amino acid residues called metastin (kisspeptin), which is the ligand of the G-protein-coupled metastin
Both metastin and the metastin receptor inhibit tumor invasion or migration through focal adhesion kinase, paxillin, MAP kinase or Rho A, and have been implicated in melanoma, thyroid cancer, esophageal squamous cell carcinoma, hepatocellular carcinoma, pancreatic carcinoma, breast cancer, ovarian cancer, renal cell carcinoma (RCC), upper tract urothelial carcinoma, bladder cancer, and prostate cancer.\textsuperscript{4-6} Metastin and the metastin receptor are expressed in the hypothalamus, brainstem, spinal cord, pituitary, ovary, prostate, and placenta in normal human tissue, and play a pivotal role in the control of the hypothalamic-pituitary-gonadal axis via regulation of gonadotropin-releasing hormone secretion.\textsuperscript{7} Moreover, it is reported that the metastin receptor is overexpressed in human RCC, and that metastin and the metastin receptor are probable targets for suppressing RCC metastasis.\textsuperscript{8}

Drug delivery systems (DDSs), which are engineered technologies for the targeted delivery and controlled release of a therapeutic agent, have been the focus of intensive studies. The delivery of an anticancer agent directly and locally to an affected part of the body is expected to yield high curative effects without producing any side effects. Generally, anticancer agents show low selectivity for tumor sites, the development of anticancer agents having a protein or a monoclonal antibody that shows affinity for tumor sites, and the control of their pharmacokinetics in vivo have been carried out. As the interaction between a ligand and a receptor has high specificity, and the development of a prodrug that uses metastin as the carrier protein would be a valuable contribution to the treatment of RCC. Although sunitinib is the active drug for RCC treatment as tyrosine kinase inhibitor, there are many systemic side effects. As far as we know, there is no report of a prodrug that uses metastin as the carrier protein and sunitinib. In order to reduce the risk of systemic side effects of sunitinib, we decided to synthesize sunitinib–metastin conjugate with metastin as the carrier protein. To this end, we embarked on the synthesis of the sunitinib–metastin conjugate and examined its potential for use as a prodrug for RCC treatment.

The design concept of the linker between sunitinib and metastin was considered as follows. Sunitinib should be released upon lactonization of the hydrolyte produced by the esterase hydrolysis of the sunitinib–metastin conjugate (Scheme 1).\textsuperscript{9} Therefore, the linker should have an acyl group as the masking group, which should be removed by esterase hydrolysis in vivo, and should form a \( \gamma \)-lactone by intramolecular lactonization. Moreover, if the linker has a short carbon chain between sunitinib and metastin, the proximity of metastin to sunitinib would inhibit the binding of metastin to the metastin receptor. Accordingly, we decided to conjugate metastin to the linker terminus with an extended carbon chain. Finally, we designed a modified L-homoserine linker, which is composed of an acyl group that acts as the masking group for hydrolysis with an esterase, as well as a carbon chain of appropriate length between sunitinib and metastin.
We initially investigated the binding position of sunitinib to the modified \(\text{L-homoserine linker}\) and the optimization of the acyl group as the masking group. Because the structure of sunitinib made it difficult to bind to the linker, we decided to connect the linker to the nitrogen atom of the oxyindole ring of sunitinib. Furthermore, the linker terminus, which was connected to metastin, had to be a carboxylic acid. Therefore, the linker was synthesized by using an acetyl or an isobutyryl group as the acyl group, and then connected to sunitinib. Compounds having an acetyl or an isobutyryl group as the masking group were evaluated for the release of sunitinib by enzyme hydrolysis under physiological conditions \textit{in vitro}. An aliquot of the reaction mixture was subjected to HPLC to monitor the reaction. The synthesis of the amide prodrug, sunitinib–metastin conjugate, which has a modified \(\text{L-homoserine linker}\), is described in detail below.

**RESULTS AND DISCUSSION**

The linker having an acetyl group or an isobutyryl group as the masking group was synthesized by the following procedure (Scheme 2). Compounds \(\text{6 and 7}\) were synthesized from commercially available Boc-\(\text{L-homoserine}\). Boc-\(\text{L-homoserine}\) was converted into benzyl ester \(\text{1}\), which was then treated with acetic anhydride in pyridine to provide acetate \(\text{2}\). The benzyl group of \(\text{2}\) was removed with hydrogen gas and 10% \(\text{Pd/C}\), and the resultant carboxylic acid was treated with pentafluorophenol in the presence of \(\text{N,N'-dicyclohexylcarbodiimide (DCC)}\) in \(\text{CH}_2\text{Cl}_2\) to give activated ester \(\text{4}\). Treatment of \(\text{4}\) with sunitinib in \(\text{N,N-dimethylformamide (DMF)}\) afforded compound \(\text{6}\). Compound \(\text{7}\), which possesses an isobutyryl...
group as the masking group, was synthesized in a similar way from compound 1 via compound 3 using isobutyryl chloride instead of acetic anhydride.

![Scheme 2](image)

Compounds 6 and 7 were evaluated to determine whether sunitinib is released by the intramolecular lactonization of the hydrolyte obtained, by hydrolysis with porcine liver esterase (PLE) (Scheme 3).

![Scheme 3](image)
First, compound 6 was converted into the malic acid salt, and this was dissolved in phosphate-buffered saline (PBS, pH 7.4) and left to stand at 36.6 °C with or without PLE. The reaction was monitored by HPLC equipped with a photodiode array detector to measure the absorption spectrum of sunitinib at the maximum absorption wavelength of 431 nm (Figures 1 and 2).

**Figure 1.** HPLC chromatographic profiles of compound 6 (A: without esterase as a blank, B: with esterase)

HPLC conditions: column, NUCLEODUR 3CN column (3 μm, 4.6 x 150 mm, Chemco Inc.); mobile phase, MeCN/aqueous NH₄OAc (20 mmol/L, pH 6.8)=55:45; flow rate, 1 mL/min; temperature, 40 °C; wavelength, 431 nm.
The $t_R$ values for sunitinib, compound 6, and compound 7 were 8, 20, and 16 min, respectively. It was observed that compound 6 having an acetyl group released sunitinib via the hydrolyte in the absence of
PLE at 3 h after the start of the reaction (Figure 1A). In the case of compound 7 having an isobutyryl group, sunitinib was released slightly in the absence of PLE at 8 h after the start of the reaction, and was released in the presence of PLE at 3 h after the start of the reaction (Figure 2). The results indicated that the isobutyryl group was a more suitable masking group than the acetyl group.

Scheme 4
Sunitinib–metastin conjugate 14 was synthesized by the following procedure (Scheme 4).
10-Undecenoic acid was treated with tert-butanol, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl), and 4-(N,N-dimethylamino)pyridine (DMAP) in THF to provide compound 9. The Boc group of compound 3 was removed by treating 3 with CHCl₃/trifluoroacetic acid (TFA) (2.5:1), and the product was reacted with allyl chloroformate in 1,4-dioxane/saturated aqueous NaHCO₃ (3:1) to afford compound 8. Compound 8 was reacted with compound 9 in the presence of 0.2 molar equiv of Grubbs 2nd generation catalyst in CH₂Cl₂ to yield compound 10. The benzyl group of compound 10 was removed by treating 10 under an atmosphere of hydrogen with 10% Pd/C, and the product was then treated with pentafluorophenol and DCC in CH₂Cl₂ to provide compound 11. Compound 11 was mixed with sunitinib in DMF at room temperature for two days to obtain compound 12. The t-butyl group of compound 12 was removed by treating 12 with CHCl₃/TFA (4:1), and the product was then treated with pentafluorophenol, EDC-HCl, and DMAP in CH₂Cl₂ to provide compound 13. Compound 13 was mixed with metastin in DMF at room temperature for five days to furnish sunitinib–metastin conjugate 14.

The properties of conjugate 14 were determined by ESI-TOF-MS analysis. Figure 3 shows the ESI-TOF-MS spectrum of conjugate 14.

![Figure 3. ESI-TOF-MS spectrum of compound 14](image-url)
The theoretical average $m/z$ values for the individual charge states are given in the table. The ESI-TOF-MS spectrum of conjugate 14 showed multiply charged ions at $m/z$ 1031.2981, 1178.1360, and 1374.0596. When the conjugate has three sunitinib-linker molecules per unit molecule of metastin, the values for $m/z$ 1031.2981, 1178.1360, and 1374.0596 were [M+8H]$^8+$, [M+7H]$^7+$, and [M+6H]$^6+$, respectively. The results suggest that conjugate 14 is composed of about three sunitinib-linker molecules per unit molecule of metastin.

### Table. Theoretical average $m/z$ values for charge states of conjugate 14

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<th>Charge</th>
<th>Sunitinib-linker (mol)/metastin (1 mol)</th>
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EXPERIMENTAL

General Experimental Procedures

Thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F$_{254}$ plates. Optical rotations were measured on a JASCO P-1030 digital polarimeter. IR spectra were recorded on a JASCO FT/IR 620 spectrophotometer, and NMR spectra, on a Bruker AV-600 spectrometer (600 MHz for $^1$H NMR, 150 MHz for $^{13}$C NMR). $^1$H chemical shifts in CDCl$_3$ were referenced to residual CHCl$_3$ (7.26 ppm), and $^{13}$C chemical shifts, to CDCl$_3$ (77.03 ppm). Mass spectra were recorded on a Waters Micromass LCT spectrometer. Analytical HPLC was carried out on a PU-980 pump unit (JASCO) equipped with a multiwavelength detector MD-910, and a NUCLEODUR 3CN column (3 μm, 4.6 × 150 mm, Chemco Inc.). Preparative HPLC was carried out on a PU-986 pump unit (JASCO) equipped with a UV-970 detector (254 nm), and a CAPCELL PAK TYPE UG80 column (5 μm, 20 × 250 mm, Shiseido Co., Ltd.).

(S)-Benzyl 2-((tert-butoxycarbonyl)amino)-4-hydroxybutanoate (1): Boc-L-homoserine (300 mg, 1.3 mmol) was dissolved in EtOH (2.0 mL) to prepare a solution, to which 2.0 mol/L aqueous NaOH (690 μL,
1.4 mmol) was added. The mixture was stirred at room temperature for 24 h. The solvent was removed in vacuo, and the residue was dissolved in DMF (400 µL) together with benzyl bromide (327 µL, 2.7 mmol). The mixture was stirred at room temperature for 46 h. The solvent was removed in vacuo and the residue was dissolved in CHCl$_3$ (10 mL). Saturated aqueous NaHCO$_3$ (10 mL) was added to the solution, and the whole was extracted with CHCl$_3$ (3 × 10 mL). The combined CHCl$_3$ extracts were washed with brine (10 mL), dried over Na$_2$SO$_4$, and filtered, and the solvent was removed in vacuo. The residue was subjected to column chromatography (silica gel, 2:1 hexane/EtOAc) to afford 1 (345 mg, 86%) as a colorless amorphous gum: $[\alpha]_{D}^{25}$ -38.5 (c 0.21, MeOH); IR (film) $\nu$ 3356, 1777, 1685, 1532, 1163 cm$^{-1}$. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.39–7.36 (4H, m), 7.32–7.29 (1H, m), 5.10 (1H, br s), 4.71 (2H, s), 4.45 (1H, t, $J$ = 8.8 Hz), 4.36 (1H, br s), 4.25 (1H, ddd, $J$ = 11.3, 9.4, 5.8 Hz), 2.67–2.62 (1H, m), 2.11–2.06 (1H, m), 1.47 (9H, s); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 175.3 (s), 155.5 (s), 140.9 (s), 128.6 × 2 (d), 127.7 (d), 127.0 × 2 (d), 80.7 (s), 65.8 (t), 65.4 (t), 50.2 (d), 30.7 (t), 28.3 × 3 (q); HR-ESIMS $m/z$ calcd for C$_{16}$H$_{24}$NO$_5$ [M+H]$^+$ 310.1654, found 310.1635.

(S)-Benzyl 4-acetoxy-2-((tert-butoxycarbonyl)amino)butanoate (2): A solution of 1 (23.8 mg, 0.077 mmol) in pyridine (2.0 mL) was treated with acetic anhydride (1.0 mL). The mixture was stirred at room temperature for 12 h. The solvent was removed in vacuo and the residue was dissolved in CHCl$_3$ (10 mL). Saturated aqueous NaHCO$_3$ (10 mL) was added to the solution, and the whole was extracted with CHCl$_3$ (3 × 10 mL). The combined CHCl$_3$ extracts were washed with brine (10 mL), dried over Na$_2$SO$_4$, and filtered, and the solvent was removed in vacuo. The residue was subjected to column chromatography (silica gel, 3:1 hexane/EtOAc) to afford 2 (22.7 mg, 84%) as a colorless amorphous gum: $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.42–7.33 (5H, m), 5.19 (2H, s), 5.15 (1H, d, $J$ = 11.8 Hz), 4.48–4.44 (1H, m), 4.19 (1H, dq, $J$ = 12.3, 6.8 Hz), 4.12 (1H, dq, $J$ = 12.3, 6.8 Hz), 2.23–2.17 (1H, m), 2.09–2.02 (1H, m), 2.01 (3H, s), 1.45 (9H, s); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 172.1 (s), 170.8 (s), 155.3 (s), 135.3 (s), 128.7 × 2 (d), 128.5 (d), 128.3 × 2 (d), 80.1 (s), 67.3 (t), 60.4 (t), 51.0 (d), 31.3 (t), 28.3 × 3 (q), 20.8 (q); HR-ESIMS $m/z$ calcd for C$_{18}$H$_{26}$NO$_6$ [M+H]$^+$ 352.1760, found 352.1763.

(S)-Benzyl 2-((tert-butoxycarbonyl)amino)-4-(isobutyryloxy)butanoate (3): To a solution of 1 (413 mg, 1.34 mmol) in pyridine (400 µL) was added isobutyryl chloride (141 µL, 1.34 mmol), and the mixture was stirred at room temperature for 9 h. The solvent was removed in vacuo and the residue was dissolved in CHCl$_3$ (10 mL). Saturated aqueous NaHCO$_3$ (20 mL) was added to the solution, and the whole was extracted with CHCl$_3$ (3 × 10 mL). The combined CHCl$_3$ extracts were washed with brine (20 mL), dried over Na$_2$SO$_4$, and filtered, and the solvent was removed in vacuo. The residue was subjected to column chromatography (silica gel, 3:1 hexane/EtOAc) to afford 3 (413 mg, 81%) as a colorless amorphous gum: $[\alpha]_{D}^{25}$ -26.6 (c 0.26, MeOH); IR (film) $\nu$ 3369, 2975, 1715, 1516, 1366, 1159 cm$^{-1}$. $^1$H
NMR (600 MHz, CDCl$_3$) $\delta$ 7.40–7.35 (5H, m), 5.19 (2H, s), 5.16–5.13 (1H, m), 4.48 (1H, $q$, $J$ = 6.2 Hz), 4.19–4.14 (1H, m), 4.11 (1H, $dd$, $J$ = 12.4, 5.3 Hz), 2.51 (1H, quint, $J$ = 6.8 Hz), 2.22–2.17 (1H, m), 2.06–1.99 (1H, m), 1.45 (9H, s), 1.16 (6H, $d$, $J$ = 6.8 Hz); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 176.9 (s), 172.1 (s), 155.2 (s), 135.2 (s), 128.6 × 2 (d), 128.5 (d), 128.3 × 2 (d), 80.1 (s), 67.3 (t), 60.4 (t), 51.1 (d), 33.9 (d), 31.2 (t), 28.3 × 3 (q), 18.9 × 2 (q); HR-ESIMS $m/z$ calcd for C$_{20}$H$_{30}$NO$_6$ [M+H]$^+$ 380.2073, found 380.2074.

(S)-Pentafluorophenyl 4-acetoxy-2-((tert-butoxycarbonyl)amino)butanoate (4): 10% Pd/C (44.0 mg) was added to a solution of 2 (44.0 mg, 0.13 mmol) in MeOH (2.0 mL), and the mixture was stirred at room temperature under an atmosphere of hydrogen for 30 min. The catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in CH$_2$Cl$_2$ (500 $\mu$L) together with pentafluorophenol (116 mg, 0.63 mmol) and DCC (36.4 mg, 0.18 mmol). The mixture was stirred at room temperature for 23 h. Saturated aqueous NaHCO$_3$ (30 mL) was added, and the whole was extracted with CHCl$_3$ (3 × 10 mL). The combined CHCl$_3$ extracts were washed with brine (30 mL), dried over Na$_2$SO$_4$, and filtered, and the solvent was removed in vacuo. The residue was subjected to column chromatography (silica gel, 4:1 hexane/EtOAc) to afford 4 (42.3 mg, 76%) as a colorless amorphous gum: $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 5.19 (1H, $d$, $J$ = 7.3 Hz), 4.78–4.77 (1H, m), 4.31–4.26 (1H, m), 4.21 (1H, $dq$, $J$ = 12.3, 7.3 Hz), 2.41–2.33 (1H, m), 2.24–2.16 (1H, m), 2.01 (3H, s), 1.46 (9 H, s); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 170.8 (s), 168.7 (s), 155.1 (s), 141.8 (d), 140.6 (d), 140.2 (d), 138.8 (d), 137.1 (d), 124.7 (d), 80.8 (s), 60.0 (t), 50.9 (d), 30.9 (t), 28.2 × 3 (q), 20.8 (q); HR-ESIMS $m/z$ calcd for C$_{17}$H$_{18}$F$_5$NO$_6$Na [M+Na]$^+$ 450.0952, found 450.0942.

(S)-Pentafluorophenyl 2-((tert-butoxycarbonyl)amino)-4-(isobutyryloxy)butanoate (5): 10% Pd/C (102 mg) was added to a solution of 3 (151 mg, 0.40 mmol) in MeOH (6.1 mL), and the mixture was stirred at room temperature under an atmosphere of hydrogen for 30 min. The catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in CH$_2$Cl$_2$ (1.5 mL) together with pentafluorophenol (355 mg, 1.93 mmol) and DCC (172 mg, 0.83 mmol). The mixture was stirred at room temperature for 20 h. Saturated aqueous NaHCO$_3$ (30 mL) was added, and the whole was extracted with CHCl$_3$ (3 × 10 mL). The combined CHCl$_3$ extracts were washed with brine (30 mL), dried over Na$_2$SO$_4$, and filtered, and the solvent was removed in vacuo. The residue was subjected to column chromatography (silica gel, 4:1 hexane/EtOAc) and then HPLC (60:40 MeCN/H$_2$O) to afford 5 (87.7 mg, 48%) as a colorless amorphous gum: $[\alpha]_D^{25}$ -20.3 (c 0.55, CHCl$_3$); IR (film) $\nu$ 3359, 2978, 1794, 1718, 1521, 1157 cm$^{-1}$. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 5.21 (1H, br d, $J$ = 8.0 Hz), 4.77 (1H, $dd$, $J$ = 11.5, 6.4 Hz), 4.28 (1H, quint, $J$ = 5.6 Hz), 4.22–4.19 (1H, m), 2.56 (1H, sept, $J$ = 7.0 Hz), 2.38–2.36 (1H, m), 2.20–2.17 (1H, m), 1.45 (9H, s), 1.176 (3H, d, $J$ = 7.0 Hz), 1.175 (3H, d, $J$ = 7.0 Hz); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 176.9
(S,Z)-3-((tert-Butoxycarbonyl)amino)-4-(3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-5-fluoro-2-oxoindolin-1-yl)-4-oxobutyl acetate (6): Free sunitinib was obtained by the following operations. A solution of sunitinib malate in CHCl₃ (30 mL) was treated with aqueous NaOH (1.0 mol/L, 30 mL), washed with brine (30 mL), dried over Na₂SO₄, and filtered, and the solvent was removed in vacuo. Compound 4 (10.2 mg, 0.024 mmol) and sunitinib (5.4 mg, 0.014 mmol) were dissolved in DMF (200 μL), and the mixture was stirred at room temperature for five days. The solvent was removed in vacuo, and the residue was subjected to column chromatography (silica gel, 7:1 CHCl₃/MeOH) to afford 6 (2.2 mg, 25%) as an orange amorphous solid: ¹H NMR (600 MHz, CDCl₃) δ 12.74 (1H, br s), 8.18 (1H, dd, J = 8.9, 4.7 Hz), 7.40 (1H, s), 7.19 (1H, dd, J = 8.4, 2.6 Hz), 6.92 (1H, td, J = 8.9, 2.6 Hz), 6.65 (1H, br s), 5.92 (1H, br s), 5.57 (1H, br d, J = 7.6 Hz), 4.28 (2H, dq, J = 26.0, 5.9 Hz), 3.54-3.49 (2H, m), 2.73 (2H, br s), 2.63-2.59 (4H, m), 2.53 (3H, s), 2.42 (3H, s), 2.36-2.31 (1H, m), 2.09-1.97 (1H, m), 1.94 (3H, s), 1.56 (9H, s), 1.08 (6H, t, J = 7.0 Hz); ¹³C NMR (150 MHz, CDCl₃) δ 173.3 (s), 170.8 × 2 (s), 168.2 (s), 160.1 (s), 159.6 (s), 155.5 (s), 155.1 (s), 151.4 (t), 140.1 (s), 133.8 (d), 128.3 (s), 126.5 (s), 125.2 (s), 120.8 (s), 117.8 (d), 113.2 (d), 111.1 (s), 103.9 (d), 80.2 (s), 60.9 (t), 52.6 (d), 51.4 (t), 46.6 × 2 (t), 36.7 (t), 32.0 (t), 28.4 × 3 (q), 20.9 (q), 14.4 (q), 11.5 × 3 (q); HR-ESIMS m/z calcd for C₁₉H₂₂F₅NO₆Na [M+Na]⁺ 478.1265, found 478.1257.

(S,Z)-3-((tert-Butoxycarbonyl)amino)-4-3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-5-fluoro-2-oxoindolin-1-yl)-4-oxobutyl isobutyrate (7): Compound 5 (14.6 mg, 0.032 mmol) and sunitinib (7.9 mg, 0.020 mmol) were dissolved in DMF (300 μL), and the mixture was stirred at room temperature for five days. The solvent was removed in vacuo, and the residue was subjected to column chromatography (silica gel, 7:1 CHCl₃/MeOH) to afford 7 (6.0 mg, 45%) as an orange amorphous solid: [α]²⁵_D +42.8 (c 0.23, CHCl₃); IR (film) ν 2974, 1698, 1570, 1521, 1474 cm⁻¹. ¹H NMR (600 MHz, CDCl₃) δ 12.74 (1H, br s), 7.93 (1H, br t, J = 5.7 Hz), 7.31 (1H, s), 7.15 (1H, dd, J = 8.7, 2.5 Hz), 6.83 (1H, td, J = 8.5, 2.5 Hz), 6.79 (1H, dd, J = 8.5, 4.4 Hz), 5.95–5.85 (1H, m), 5.33 (1H, br d, J = 6.8 Hz), 4.17–4.15 (1H, m), 4.12 (2H, t, J = 6.7 Hz), 3.82 (2H, t, J = 5.7 Hz), 3.24 (1H, t, J = 5.7 Hz), 3.12 (4H, q, J = 7.3 Hz), 2.55 (3H, s), 2.51–2.49 (1H, m), 2.49 (3H, s), 2.17–2.11 (1H, m), 1.94–1.91 (1H, m), 1.41 (9H, s), 1.36 (3H, t, J = 7.3 Hz), 1.35 (3H, t, J = 7.3 Hz), 1.13 (3H, d, J = 6.8 Hz), 1.12 (3H, d, J = 6.8 Hz); ¹³C NMR (150 MHz, CDCl₃) δ 177.1 (s), 176.5 (s), 166.5 (s), 159.9 (s), 158.3 (s), 155.6 (s), 138.9 (s), 133.3 (s), 131.0 (s), 128.8 (s), 124.2 (d), 120.1 (s), 114.3 (s), 112.8 (s), 112.7 (d), 109.9 (d), 105.1 (d), 79.2 (s), 61.4 (t), 52.7 (t), 52.4 (d), 48.0 × 2 (d), 35.8 (t), 35.6 (t), 33.9 (d), 28.4 × 3 (q), 19.9 ×
2 (q), 14.2 (q), 11.3 (q), 8.6 × 2 (q); HR-ESIMS m/z calcd for C_{35}H_{49}FN_{5}O_{7} [M+H]^+ 670.3616, found 670.3611.

(S)-Benzy1 2-((allyloxy)carbonyl)amino)-4-(isobutyryloxy)butanoate (8): A solution of 3 (284 mg, 0.75 mmol) in CHCl\textsubscript{3}/TFA (2.5:1, 3.5 mL) was stirred at room temperature for 30 min. The solvent was removed \textit{in vacuo}, and the residue was dissolved in 1,4-dioxane (2.1 mL) together with allyl chloroformate (159 µL, 1.50 mmol). The mixture was stirred at room temperature for 5 min, and saturated NaHCO\textsubscript{3} solution (0.70 mL) was added. Then, the mixture was stirred at room temperature for 10 h. Aqueous HCl (1 M, 20 mL) was added, and the whole was extracted with CHCl\textsubscript{3} (3 × 10 mL). The combined CHCl\textsubscript{3} extracts were washed with brine (10 mL), dried over Na\textsubscript{2}SO\textsubscript{4}, and filtered, and the solvent was removed \textit{in vacuo}. The residue was subjected to column chromatography (silica gel, 5:1:1 CHCl\textsubscript{3}/hexane/EtOAc) to afford 8 (216 mg, 80%) as a colorless oil: [α]_{25}^{D} \text{+0.4 (c 0.24, CHCl}_{3}); IR (film) \nu 3350, 2974, 1730, 1528, 1157 cm\textsuperscript{-1}. \textsuperscript{1}H NMR (600 MHz, CDCl\textsubscript{3}) δ 7.38−7.32 (5H, m), 5.89 (1H, octet, J = 5.5 Hz), 5.30 (1H, d, J = 17.3 Hz), 5.21 (1H, d, J = 8.7 Hz), 5.18 (2H, d, J = 4.2 Hz), 4.56 (2H, d, J = 5.5 Hz), 4.53 (1H, dd, J = 12.8, 7.6 Hz), 4.17 (1H, dd, J = 12.8, 7.0 Hz), 4.09 (1H, dd, J = 11.7, 7.0 Hz), 2.48 (1H, sept, J = 7.0 Hz), 2.22 (1H, sept, J = 7.0 Hz), 2.06 (1H, sept, J = 7.0 Hz), 1.136 (3H, d, J = 7.0 Hz), 1.134 (3H, d, J = 7.0 Hz); \textsuperscript{13}C NMR (150 MHz, CDCl\textsubscript{3}) δ 176.8 (s), 171.7 (s), 155.7 (s), 135.1 (s), 123.5 (d), 128.6 × 2 (d), 128.5 (d), 128.2 × 2 (d), 117.9 (t), 67.4 (t), 65.9 (t), 60.2 (t), 51.5 (d), 33.8 (d), 31.2 (d), 18.9 × 2 (q); HR-ESIMS m/z calcd for C_{19}H_{25}NO_{6}Na [M+Na]^+ 386.1580, found 386.1576.

tert-Butyl undec-10-enoate (9): 10-Undecenoic acid (500 mg, 2.7 mmol) was dissolved in THF (0.5 mL) together with tert-butanol (1.1 mL, 11.6 mmol) and DMAP (33.2 mg, 0.27 mmol), and EDC-HCl (522 mg, 2.7 mmol) was added at 0 °C. The mixture was stirred at this temperature for 1 h, and then at room temperature for 20 h. Saturated aqueous NaHCO\textsubscript{3} (10 mL) was added, and the mixture was extracted with CHCl\textsubscript{3} (3 × 10 mL). The combined CHCl\textsubscript{3} extracts were washed with brine (10 mL), dried over Na\textsubscript{2}SO\textsubscript{4}, and filtered, and the solvent was removed \textit{in vacuo}. The residue was subjected to column chromatography (silica gel, 20:1 hexane/EtOAc) to afford 9 (355 mg, 55%) as a colorless oil: [α]_{25}^{D} -3.6 (c 0.18, CHCl\textsubscript{3}); IR (film) ν 3077, 2978, 2927, 2855, 1732, 1640 cm\textsuperscript{-1}. \textsuperscript{1}H NMR (600 MHz, CDCl\textsubscript{3}) δ 5.80 (1H, ddt, J = 17.1, 10.2, 6.7 Hz), 4.98 (1H, dd, J = 17.1, 3.7 Hz), 4.91 (1H, d quint, J = 10.2, 1.2 Hz), 2.19 (2H, t, J = 7.5 Hz), 2.03 (2H, qt, J = 6.7, 1.5 Hz), 1.56 (2H, quint, J = 7.5 Hz), 1.44 (9H, s), 1.36 (2H, quint, J = 7.5 Hz), 1.28 (8H, br s); \textsuperscript{13}C NMR (150 MHz, CDCl\textsubscript{3}) δ 173.3 (s), 139.1 (d), 114.1 (t), 79.9 (s), 35.6 (t), 33.8 (t), 29.3 × 2 (t), 29.2 (t), 29.0 (t), 28.9 (t), 28.1 × 3 (q), 25.1 (t); HR-ESIMS m/z calcd for C_{19}H_{25}O_{2} [M+H]^+ 241.2168, found 241.2168.
(S,E)-tert-Butyl 12-(((1-(benzyloxy)-4-(isobutyryloxy)-1-oxobutan-2-yl)carbamoyl)oxy)dodec-10-enoate (10): Compounds 8 (122 mg, 0.33 mmol) and 9 (161 mg, 0.67 mmol) were dissolved in CH₂Cl₂ (434 μL) together with Grubbs 2nd generation catalyst (28.4 mg, 0.033 mmol), and the mixture was stirred at 55 °C under an atmosphere of argon for 45 h. The solvent was removed in vacuo, and the residue was dissolved in CH₂Cl₂. Chromatorex® NH silica gel (500 mg) was added to the solution, and the mixture was stirred at room temperature for 1 h. Insoluble material was filtered off, and the filtrate was concentrated to dryness. The residue was subjected to HPLC (85:15 MeOH/H₂O) to provide 10 (54.9 mg, 29%) as a colorless oil: [α]₂₅D +2.3 (c 0.29, CHCl₃); IR (film) ν 3354, 2974, 2929, 2857, 1730, 1154 cm⁻¹. ¹H NMR (600 MHz, CDCl₃) δ 7.38–7.31 (5H, m), 5.74 (1H, quint, J = 7.0 Hz), 5.53 (1H, quint, J = 7.0 Hz), 5.37 (1H, d, J = 7.9 Hz), 5.17 (2H, dd, J = 12.5, 4.5 Hz), 4.53–4.51 (1H, m), 4.49 (2H, d, J = 7.0 Hz), 4.16 (1H, dq, J = 11.7, 6.9 Hz), 4.08 (1H, dq, J = 12.1, 6.9 Hz), 2.47 (1H, sept, J = 6.8 Hz), 2.23–2.21 (1H, m), 2.18 (2H, t, J = 7.6 Hz), 2.068–2.006 (1H, m), 2.02 (2H, q, J = 6.9 Hz), 1.56 (2H, quint, J = 7.0 Hz), 1.43 (9H, s), 1.36–1.34 (2H, m), 1.27 (8H, br s), 1.129 (3H, d, J = 7.0 Hz), 1.128 (3H, d, J = 7.0 Hz); ¹³C NMR (150 MHz, CDCl₃) δ 176.8 (s), 173.3 (s), 171.8 (s), 155.8 (s), 136.4 (d), 135.1 (s), 128.6 × 2 (d), 128.5 (d), 128.2 × 2 (d), 123.9 (d), 79.8 (s), 67.3 (t), 66.1 (t), 60.2 (t), 51.4 (d), 35.6 (t), 33.8 (d), 32.2 (t), 31.2 (t), 29.25 (t), 29.20 (t), 29.08 (t), 29.04 (t), 28.1 × 3 (q), 25.1 (t), 18.88 (q), 18.79 (q); HR-ESIMS m/z calcd for C₃₂H₄₉NO₈Na [M+Na]⁺ 598.3356, found 598.3350.

(S)-tert-Butyl 12-(((4-(isobutyryloxy)-1-oxo-1-(pentafluorophenoxy)butan-2-yl)carbamoyl)oxy)dodecanoate (11): 10% Pd/C (5.0 mg) was added to a solution of 10 (36.5 mg, 0.063 mmol) in CH₂Cl₂/EtOAc (2:1, 3.0 mL), and the mixture was stirred at room temperature under an atmosphere of hydrogen for 30 min. The catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in CH₂Cl₂ (200 μL) together with pentafluorophenol (35.0 mg, 0.19 mmol) and DCC (13.1 mg, 0.063 mmol). The mixture was stirred at room temperature for 4 h. Saturated aqueous NaHCO₃ (10 mL) was added, and the mixture was extracted with CHCl₃ (3 × 10 mL). The combined CHCl₃ extracts were washed with brine (10 mL), dried over Na₂SO₄, and filtered, and the solvent was removed in vacuo. The residue was subjected to column chromatography (silica gel, 6:1 hexane/EtOAc) to afford 11 (33.2 mg, 81%) as a colorless oil: [α]₂₅D -19.7 (c 0.33, MeOH); IR (film) ν 3342, 2975, 2931, 2857, 1730, 1523, 1155, 999 cm⁻¹. ¹H NMR (600 MHz, CDCl₃) δ 5.42 (1H, d, J = 8.0 Hz), 4.83 (1H, td, J = 8.0, 5.2 Hz), 4.30 (1H, quint, J = 5.0 Hz), 4.20 (1H, dq, J = 12.8, 5.0 Hz), 4.08 (2H, t, J = 6.2 Hz), 2.55 (1H, sept, J = 7.0 Hz), 2.42–2.37 (1H, m), 2.24–2.22 (1H, m), 2.19 (2H, t, J = 7.6 Hz), 1.62–1.60 (2H, m), 1.57–1.53 (2H, m), 1.43 (9H, s), 1.32–1.25 (12H, m), 1.17 (3H, d, J = 7.0 Hz), 1.16 (3H, d, J = 7.0 Hz); ¹³C NMR
(150 MHz, CDCl$_3$) δ 176.9 (s), 173.5 (s), 168.5 (s), 156.1 (s), 141.83 (s), 141.78 (s), 140.1 (s), 137.12 (s), 137.06 × 2 (s), 80.1 (s), 65.9 (t), 60.1 (t), 51.2 (d), 35.6 (t), 33.9 (d), 31.0 (t), 29.5 × 2 (t), 29.4 (t), 29.3 (t), 29.2 (t), 28.9 (t), 28.1 × 3 (q), 25.8 (t), 25.1 (t), 18.9 × 2 (q); HR-ESIMS m/z calcd for C$_{31}$H$_{44}$F$_5$NO$_8$Na [M+Na]$^+$ 676.2885, found 676.2906.

(\textit{S,Z})-\textit{tert}-Butyl

12-(((1-(3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-5-fluoro-2-oxoindolin-1-yl)-4-(isobutyryloxy)-1-oxobutan-2-yl)carbamoyl)oxy)dodecanoate (12): Compound 11 (16.9 mg, 0.026 mmol) and sunitinib (6.9 mg, 0.017 mmol) were dissolved in DMF (200 μL), and the mixture was stirred at room temperature for two days. The solvent was removed in vacuo, and the residue was subjected to column chromatography (silica gel, 20:1 CHCl$_3$/MeOH) to afford 12 (13.7 mg, 61%) as an orange amorphous solid: [\(\alpha\)]$^{25}_D$ +36.0 (c 0.38, MeOH); IR (film) \(\nu\) 3411, 2975, 2928, 2855, 1728, 1154 cm$^{-1}$. $^1$H NMR (600 MHz, CDCl$_3$) δ 12.68 (1H, s), 8.16 (1H, q, \(J = 4.5\) Hz), 8.14–8.11 (1H, m), 7.38 (1H, s), 7.20 (1H, dd, \(J = 8.1, 2.0\) Hz), 6.90 (1H, td, \(J = 8.7, 2.0\) Hz), 5.95–5.91 (1H, m), 5.74 (1H, d, \(J = 8.7\) Hz), 4.27 (2H, t, \(J = 6.4\) Hz), 4.06 (2H, t, \(J = 6.8\) Hz), 3.71–3.69 (2H, m), 3.07–3.04 (2H, m), 2.94 (4H, quint, \(J = 6.6\) Hz), 2.60 (3H, s), 2.51 (3H, s), 2.49–2.43 (1H, m), 2.39–2.34 (1H, m), 2.19 (2H, t, \(J = 7.7\) Hz), 2.11–2.06 (1H, m), 1.56–1.52 (2H, m), 1.43 (9H, s), 1.29–1.24 (16H, m), 1.22 (6H, m), 1.12 (3H, d, \(J = 7.0\) Hz), 1.11 (3H, d, \(J = 7.0\) Hz); $^{13}$C NMR (150 MHz, CDCl$_3$) δ 176.8 (s), 173.4 (s), 169.9 (s), 168.2 (s), 165.8 (s), 159.7 (s), 156.4 (s), 140.5 (s), 133.4 (s), 132.0 (s), 128.3 (s), 126.4 (s), 125.3 (d), 120.7 (s), 117.8 (d), 113.0 (d), 111.1 (s), 104.1 (d), 79.9 (s), 65.5 (t), 60.8 (t), 53.2 (d), 51.5 (t), 47.1 (t), 47.0 (t), 35.9 (t), 35.6 (t), 33.9 (d), 31.8 (t), 29.53 (t), 29.46 (t), 29.33 (t), 29.31 (t), 29.11 (t), 29.06 (t), 29.0 (t), 28.1 × 3 (q), 25.9 (t), 25.1 (t), 18.94 (q), 18.91 (q), 14.4 (q), 11.3 (q), 9.5 (q), 9.4 (q); HR-ESIMS m/z calcd for C$_{47}$H$_{71}$F$_5$O$_9$Na [M+H]$^+$ 868.5236, found 868.5256.

(\textit{S,Z})-Pentafluorophenyl

12-(((1-(3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-5-fluoro-2-oxoindolin-1-yl)-4-(isobutyryloxy)-1-oxobutan-2-yl)carbamoyl)oxy)dodecanoate (13): A solution of 12 (74.0 mg, 0.085 mmol) in CHCl$_3$/TFA (4:1, 2.0 mL) was stirred at room temperature for 3 h, and the solvent was removed in vacuo. The residue was dissolved in CH$_2$Cl$_2$ (200 μL) together with pentafluorophenol (49.2 mg, 0.27 mmol) and DMAP (0.14 mg, 0.0011 mmol), and EDC·HCl (37.5 mg, 0.20 mmol) was added at 0 °C. The mixture was stirred at this temperature for 1 h, and then at room temperature for 21 h. Saturated aqueous NaHCO$_3$ (10 mL) was added, and the mixture was extracted with CHCl$_3$ (3 × 10 mL). The combined CHCl$_3$ extracts were washed with brine (10 mL), dried over Na$_2$SO$_4$, and filtered, and the solvent was removed in vacuo. The residue was subjected to column chromatography (silica gel, 20:1 CHCl$_3$/MeOH) to afford 13 (60 mg, 72%) as a yellow amorphous solid: [\(\alpha\)]$^{25}_D$ +43.8 (c
0.23, CHCl$_3$); IR (film) v 3364, 2927, 2855, 2360, 1791, 1698, 1520 cm$^{-1}$. $^1$H NMR (600 MHz, CDCl$_3$) δ 11.62 (1H, br s), 8.16 (1H, q, $J$ = 4.5 Hz), 8.14–8.12 (1H, m), 7.39 (1H, s), 7.17 (1H, dd, $J$ = 8.3, 2.6 Hz), 6.91 (1H, td, $J$ = 8.7, 2.6 Hz), 5.95–5.91 (1H, m), 5.72 (1H, d, $J$ = 8.3 Hz), 4.27 (2H, t, $J$ = 5.8 Hz), 4.07 (2H, t, $J$ = 6.4 Hz), 3.89 (2H, br t, $J$ = 4.5 Hz), 3.31–3.29 (2H, m), 3.20–3.16 (4H, m), 2.67 (3H, s), 2.65 (2H, t, $J$ = 7.5 Hz), 2.60 (3H, s), 2.46–2.41 (1H, m), 2.37–2.31 (1H, m), 2.09–2.06 (1H, m), 1.76 (2H, quint, $J$ = 7.5 Hz), 1.639–1.580 (2H, m), 1.444 (3H, d, $J$ = 7.2 Hz), 1.441 (3H, t, $J$ = 7.2 Hz), 1.33–1.32 (2H, m), 1.32–1.24 (12H, m), 1.13 (3H, d, $J$ = 7.2 Hz), 1.09 (3H, d, $J$ = 7.2 Hz); $^{13}$C NMR (150 MHz, CDCl$_3$) δ 176.9 (s), 173.0 (s), 169.8 (s), 168.2 (s), 166.0 (s), 159.7 (s), 156.3 (s), 142.0 × 2 (s), 141.0 (s), 140.3 (s), 138.7 × 2 (s), 137.7 (s), 134.1 (s), 132.0 (s), 128.3 (s), 126.5 (s), 125.4 (d), 119.6 (s), 117.9 (d), 113.1 (d), 111.3 (s), 104.1 (d), 65.5 (t), 60.8 (t), 53.6 (t), 53.1 (d), 48.86 (t), 48.83 (t), 35.5 (t), 33.9 (d), 33.4 (t), 31.8 (t), 29.5 (t), 29.4 (t), 29.28 (t), 29.25 (t), 29.1 (t), 29.0 (t), 28.9 (t), 25.8 (t), 24.8 (t), 18.92 (q), 18.90 (q), 14.7 (q), 11.7 (q), 8.7 × 2 (q); HR-ESIMS m/z calcd for C$_{49}$H$_{61}$F$_{6}$N$_5$O$_9$ [M+H]$^+$ 978.4452, found 978.4474.

**Sunitinib–metastin conjugate (14):** Compound 13 (3.7 mg, 0.0038 mmol) and metastin (1.1 mg, 0.00018 mmol) were dissolved in DMF (600 μL), and the mixture was stirred at room temperature for five days. The mixture was passed through a PD-10 desalting column (GE Healthcare Life Sciences) with H$_2$O as eluent to give a fraction containing the conjugate. The solution was lyophilized by using FDU-2200 (EYELA) to provide conjugate 14 (0.96 mg, 64%); ESI-TOF-MS m/z 1031.2981 [M+8H]$^{8+}$, m/z 1374.0596 [M+7H]$^{7+}$, m/z 1778.1360 [M+6H]$^{6+}$.

**Enzymatic Hydrolysis of Compounds 6 and 7**
An equivalent molar amount of malic acid was added to compound 6 or 7. The mixture was dissolved in methanol, and the solution was concentrated to dryness. The residue (each 290 μg) was dissolved in PBS (450 μL, pH 7.4) and the solution was left to stand at 36.6 °C with or without PLE (100 μg, 17 units/mg, SIGMA). The reaction was monitored by injecting a 10 μL aliquot of the reaction mixture into an analytical HPLC equipped with DP-L910W software, using the following conditions: mobile phase, 55:45 MeCN/20 mM sodium acetate buffer; flow rate, 1 mL/min; λ = 431 nm. The $t_R$ values for sunitinib, compound 6, and compound 7 were 8, 20, and 16 min, respectively.

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**REFERENCES**