JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Antineoplastic Agents. 603. Quinstatins: Exceptional Cancer Cell Growth Inhibitors

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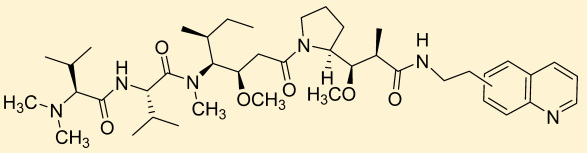
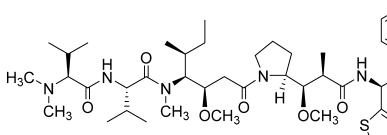
Supporting Information

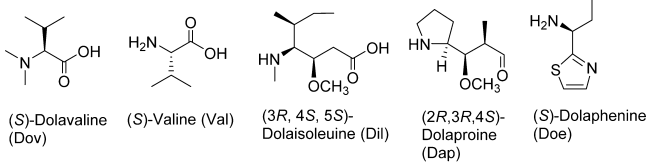
ABSTRACT: Discovery of the exceptionally powerful anticancer drug dolastatin 10 (**1**), contained in the sea hare *Dolabella auricularia*, opened a new frontier needed for improving human cancer treatment. Subsequently, major advances have been achieved based on results of structurally modifying this unusual natural peptide while maintaining the remarkable anticancer activity necessary for preparation of successful monoclonal antibody drug conjugates (ADC). Among the first several hundred SAR products based on dolastatin 10 our group synthesized and termed auristatins was auristatin E (**2a**). An anticancer activity-equivalent, desmethylaurisatin E (**2b**), linked to a CD30 monoclonal antibody is the very successful anticancer drug Adcetris, now approved for use in 65 countries. In the present investigation, we discovered a new subset of auristatins designated quinstatins derived from dolastatin 10 by replacing the C-terminal Doe unit with a carefully designed quinoline, which led to low or subnanomolar levels of cancer cell growth inhibition required for construction of chemically unique ADC drugs. The synthesis of quinstatins 2−8 is presented along with their cancer cell line biological data.



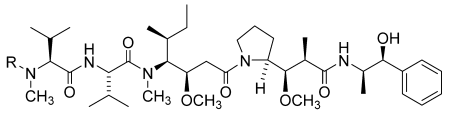
Our discovery[1a,b] of the dolastatin series[1c,d] of cancer cell growth inhibitors contained in the wide ranging sea hare *Dolabella auricularia* revealed a new and very promising vista for anticancer drug discovery. This proved to be broadly confirmed by the clinical development[2] of dolastatins 10 (**1**) and 15[2c] and structural modifications we designated auristatins.[3] By 2001, auristatin E (**2a**)[3b] began preclinical development as a very promising antibody drug conjugate (ADC) by linkage to a CD30 monoclonal antibody. Soon, the development continued, employing the cancer biology equivalent desmethylauristatin E (**2b**).[4,5]



1, Dolastatin 10



(*S*)-Dolavaline (Dov) | (*S*)-Valine (Val) | (3*R*, 4*S*, 5*S*)-Dolaisoleuine (Dil) | (2*R*,3*R*,4*S*)-Dolaproine (Dap) | (*S*)-Dolaphenine (Doe)



2a, R = CH_3, Auristatin E
b, R = H, Desmethyl-auristatin E

Presently, the resulting ADC anticancer drug[4a−e,5] designated Adcetris has been approved for use in 65 countries. For example, treatment of patients with relapsed or refractory Hodgkin's lymphoma has resulted in a remarkable level of complete remissions.[4d] Those early clinical results have inspired a major current research effort to link auristatin (**2b**) to other monoclonal antibodies[4a,b] representing a spectrum of cancer types. In parallel, considerable research has been continued to discover structural modifications of dolastatin 10 that would provide such powerful cancer cell growth inhibition without a high toxic level and hence provide a much better therapeutic range for inspired ADC use. Toward this objective we have investigated a new series of dolasatatin 10 modified structures termed quinstatins that involve a quinoline foundation. This research has resulted in a special subset of the auristatins with generally very low to subnanomolar cancer cell growth inhibitory activities, namely, quinstatins 2−8 (Figure 1).

RESULTS AND DISCUSSION

The synthesis of quinstatins 2 (**3**) and 6 (**4**) and the cytotoxic activity of quinstatins 2 (**3**) 3−5, 6 (**4**), 7, and 8, described as follows, serves as a useful illustration of this new quinoline approach to reaching the dolasatin 10 level of potential anticancer activity. Extensive experience from our prior structure−activity relationship (SAR) studies[3] of dolastatin 10 (**1**) suggested that employing a quinoline ring base[3a] replacement for the C-terminal Doe unit of **1** separated by a two-carbon linker from the peptide could be very productive. This prediction proved to be on target and was implemented in the manner following the use of 2′-aminoethylquinolines to provide the necessary spacing

Special Issue: Special Issue in Honor of Phil Crews

Received: November 3, 2016

DOI: 10.1021/acs.jnatprod.6b01006
J. Nat. Prod. XXXX, XXX, XXX−XXX

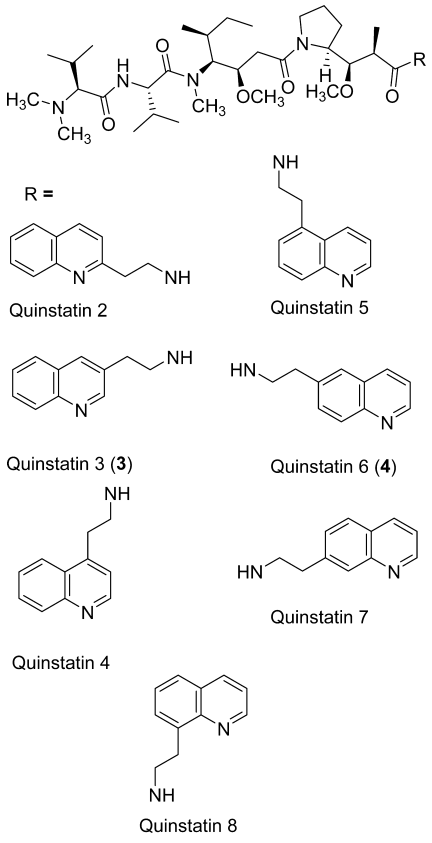
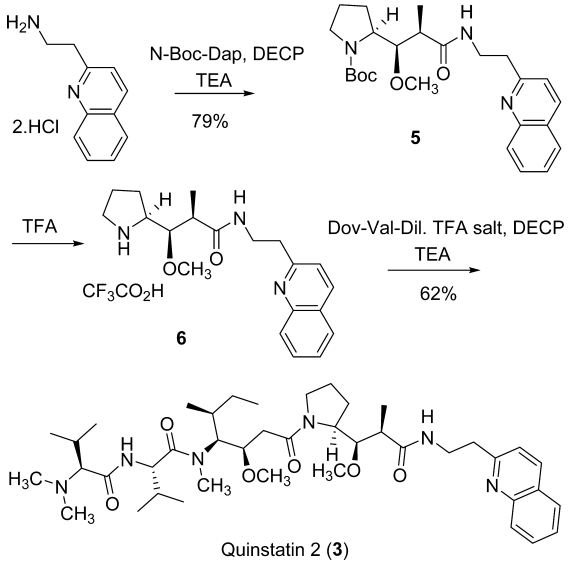


Figure 1. Quinstatins 2−8.

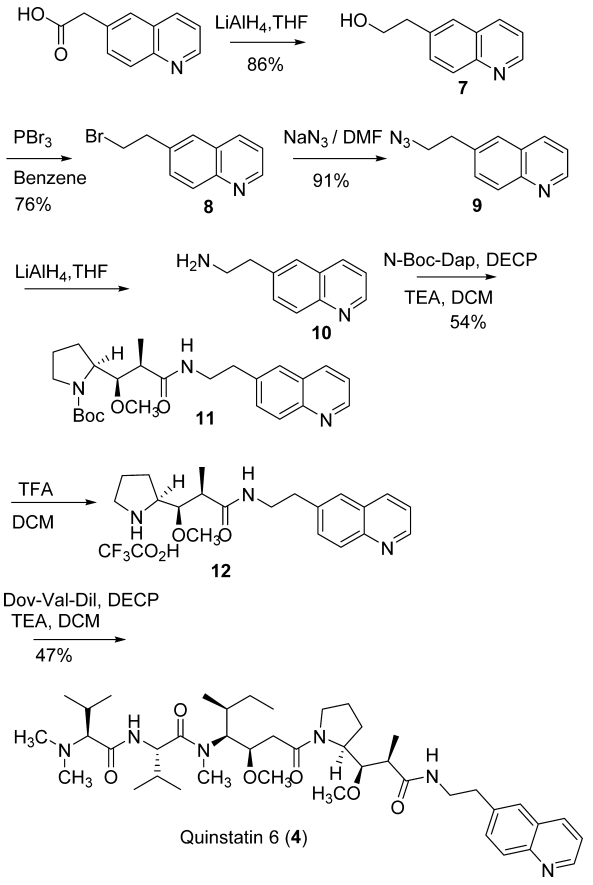
from the peptide unit. Illustrated is the convenient synthesis of quinstatins 2 (**3**), 4, 5, and 8, as summarized in Scheme 1, for quinstatin 2 (**3**).

In contrast, synthesis of quinstatins 3, 6 (**4**), and 7 required an alternative route. By way of an example, the synthesis of quinstatin 6 (**4**) is presented in Scheme 2. Converting the available quinoline 6-acetic acid to an amide followed by reduction to the corresponding amine resulted in complex mixtures of reduction products. This was surprising and not corrected by employing a

Scheme 1. Synthesis of Quinstatin 2 (3)



Scheme 2. Synthesis of Quinstatin 6 (4)



series of amide reduction methods. However, reduction of the carboxylic acid to the alcohol and proceeding via the bromide to an azide to an amine (Scheme 2) proved to be very effective. Doubling of signals in the ^{1}H and ^{13}C NMR spectra for the quinstatins was attributed to the presence of conformational isomers,[3a,7] with quinstatin 5 having one major conformation upon examination of its NMR spectra.

Adoption of Scheme 1 or 2 led to expansion of the quinstatin series of important cytotoxic agents with the exceptionally strong potency (Table 1) necessary for preparation of successful cancer monoclonal drug conjugates. Table 1 summarizes a series of cancer cell line growth inhibition experiments performed in parallel with very revealing and important results. These results are also in sharp contrast to our previous study using direct attachment of the peptide to 2- and 6-aminoquinoline,[3a] where the cytotoxic potency was far weaker. Very noteworthy is the usual cancer biology equivalence of auristatin E (**2a**) and desmethylauristatin E (**2b**) as well as the 10 to 100 times increase in inhibition provided by quinstatin 2 as compared to auristatin 2-AQ.[3a] In these experiments quinstatin 8 delivered cancer inhibitions close to the remarkably powerful dolastatin 10 level (0.006 nM). Presently our group is continuing research directed at further probing requirements for increasing the therapeutic range of dolastatin 10 while retaining its powerful anticancer activity and a more favorable therapeutic index.

EXPERIMENTAL SECTION

General Experimental Procedures. Both *N*-Boc-dolaproine and Dov-Val-Dil·TFA were synthesized as described earlier.[6,7] 2-(1′-Ethyl-2′-amino)quinoline dihydrochloride and 6-(1′-acetic acid)quinoline were purchased from J&W Pharmlab LLC and Astatech, Inc.,

DOI: 10.1021/acs.jnatprod.6b01006
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. Human Cancer Cell Line (GI_{50} μg/mL [nM]) Growth Inhibition Results from Comparison Experiments

	cell line[a]					
compound	BXPC-3	MCF-7	SF-268	NCI-H460	KM20L2	DU-145
dolastatin 10[1a] (**1**)	0.000040 [0.051]	0.0000042 [0.005]	0.0000043 [0.006]	0.00018 [0.229]	0.0000080 [0.010]	0.0000052 [0.007]
quinstatin 2 (**3**)	0.0093 [12.36]	0.00031 [0.412]	0.00023 [0.31]	0.0051 [6.77]	0.00033 [0.438]	0.00031 [0.412]
quinstatin 3	0.021 [27.9]	0.00023 [0.305]	0.00029 [0.385]	0.031 [41.2]	0.00040 [0.532]	0.00020 [0.266]
quinstatin 4	0.0044 [5.84]	0.00030 [0.398]	0.00019 [0.252]	0.0043 [5.71]	0.00032 [0.425]	0.00023 [0.305]
quinstatin 5	0.0021 [2.79]	0.000073 [0.097]	0.00011 [0.146]	0.0048 [6.37]	0.00023 [0.305]	0.00013 [0.172]
quinstatin 6 (**4**)	0.0020 [2.66]	0.00040 [0.532]	0.00030 [0.399]	0.0043 [5.71]	0.00039 [0.518]	0.00030 [0.399]
quinstatin 7	0.0031 [4.12]	0.000030 [0.039]	0.000024 [0.032]	0.011 [14.61]	0.000041 [0.054]	0.000030 [0.040]
quinstatin 8	0.00050 [0.425]	0.000040 [0.053]	0.000023 [0.030]	0.00090 [1.19]	0.000040 [0.053]	0.000040 [0.053]
auristatin E (**2a**)	>0.001 [>1.37]	0.00017 [0.232]	0.00036 [0.492]	0.00039 [0.533]	0.00036 [0.492]	0.00031 [0.424]
desmethylauristatin E (**2b**)	>0.001 [>1.39]	0.00029 [0.404]	0.00031 [0.432]	0.00049 [0.683]	0.00043 [0.599]	0.00030 [0.418]

[a]Cancer cell lines in order: pancreas (BXPC-3); breast (MCF-7); CNS (SF-268); lung (NCI-H460); colon (KM20L2); prostate (DU-145).

respectively, and were used as received. 3-(1′-Acetic acid)quinoline, 4-(1′-ethyl-2′-amino)quinoline, 5-(1′-ethyl-2′-amino)quinoline, and 7-(1′-acetic acid)quinoline were purchased from Princeton Bimolecular Research, Inc., and 8-(1′-ethyl-2′-amino)quinoline was purchased from Fischer Scientific; all were used as received. Other reagents including diethyl cyanophosphonate (DEPC) and anhydrous solvents were purchased from Acros Organics (Fisher Scientific) and Sigma–Aldrich Chemical Co. and were used as received.

Melting points are uncorrected and were determined with a Fischer–Johns melting point apparatus. Optical rotations were measured by use of a Perkin–Elmer 241 polarimeter, and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. The ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity INOVA 400 and 500 and Bruker 400 instruments with deuterated solvents. High-resolution mass spectra were obtained using a JEOL LCMate instrument in the Arizona State University CLAS High Resolution Mass Spectroscopy Laboratory and provided by Dr. John C. Knight and Natalya Zolotova. For thin-layer chromatography, Analtech silica gel GHLF Uniplates were used and visualized with short-wave UV irradiation and an iodine chamber. For column chromatography, silica gel (230–400 mesh ASTM) from E. Merck (Darmstadt, Germany) was employed.

Quinstatin 2 (3). *2-(1′- Ethyl-2′-amido-Boc-Dap)quinoline (**5**).* To a stirred solution of Boc-Dap[6] (0.06 g, 0.21 mmol) and 2-(1′-ethyl- 2′-amino)quinoline dihydrochloride (0.08 g, 0.32 mmol) in anhydrous CH_2Cl_2 (1 mL) at 0 °C under N_2 were added triethylamine (TEA) (0.2 mL, 1.43 mmol) and DEPC (0.1 mL, 0.6 mmol). The reaction mixture was stirred at 0 °C with warming to rt for 6 h and then concentrated under reduced pressure to an orange-colored residue that was purified by chromatography on a silica gel column. Gradient elution with hexanes 100% to 7:2 hexanes–acetone gave the product as an off-white waxy solid, 112 mg (79%): TLC R_f 0.5 (3:4 hexanes–acetone); doubling of signals observed in the ^{1}H and ^{13} C NMR data indicating conformational isomers in almost 1:1 ratio; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (1H, d, J = 8 Hz), 7.95 (1H, d, J = 8 Hz), 7.72 (1H, d, J = 8 Hz), 7.63 (1H, t, J = 8 Hz), 7.43 (1H, t, J = 8 Hz), 7.23 (1H, d, J = 8 Hz), 6.99, 6.85 (1H, br s), 3.83–3.56 (4H, m), 3.46–3.34 (1H, m), 3.28 (4H, m), 3.18–3.01 (2H, m), 2.36–2.15 (1H, m), 1.83–1.47 (4H, m), 1.37, 1.41 (9H, s, *t*-Bu), 1.12–1.09 (3H, m); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 174.1, 173.6, 160.1 154.6, 154.3, 147.6, 136.5, 129.6, 128.7, 127.6, 126.8, 126.1, 121.7, 83.9, 82.3, 79.6, 79.0, 60.7, 60.5, 58.7, 46.8, 46.5, 44.2, 43.9, 38.3, 38.1, 37.5, 37.3, 28.5, 25.7, 25.3, 252, 24.4, 23.9, 14.1, 14.05; (+)-HRAPCIMS m/z 442.2707 $[M + H]^+$ (calcd for $C_{25}H_{36}N_3O_4$ 442.2706).

*2-(1′-Ethyl-2′-amido-Dap)quinoline trifluoroacetate salt (**6**).* To a stirred solution of compound **5** (0.070 g, 0.16 mmol) in CH_2Cl_2 (4 mL) at 0 °C under N_2 was added trifluoroacetic acid (TFA) (1 mL), and stirring was continued for 1.5 h at 0 °C. The reaction was stopped and concentrated under reduced pressure to remove CH_2Cl_2 and excess TFA to yield a yellow oil. This material was used without further purification for the next reaction.

*2-(1′-Ethyl-2′-amido-Dap-Dil-Val-Dov)quinoline [quinstatin 2 (**3**)].* Amide **6** and Dov-Val-Dil·TFA[7] (90 mg, 0.16 mmol) were dissolved in anhydrous CH_2Cl_2 (5 mL), and the solution was stirred under N_2 and cooled to 0 °C. TEA (0.12 mL, 0.86 mmol) and DEPC (0.035 mL, 0.21 mmol) were added, and the mixture was stirred under N_2 for 18 h with warming to room temperature (rt). The reaction mixture was concentrated under reduced pressure and separated on a silica gel column. Elution with CH_2Cl_2–MeOH (95:5) gave the product as a colorless foam (74 mg, 62%): TLC R_f 0.3 (CH_2Cl_2–CH_3OH 7%); $[\alpha]^{22}_D$ −8.2 (c 0.27, $CHCl_3$); conformational isomers are present[3a,7] (~2:1), and doubling of signals was observed in both the proton and carbon NMR spectra for **3**; ^{1}H NMR ($CDCl_3$, 400 MHz) major conformer δ 8.08 (1H, d, J = 8.8 Hz), 8.02 (1H, d, J = 8.4 Hz), 7.79 (1H, d, J = 7.0 Hz), 7.70 (1H, t, J = 8.0 Hz), 7.51 (1H, t, J = 6.8 Hz), 7.33 (1H, d, J = 9.0 Hz), 7.16 (1H, m), 6.88 (1H, m), 4.80, 4.78 (1H, d, J = 6.9, 9 Hz), 4.12–4.04 (2H, m), 3.89–3.74 (3H, m), 3.43–3.25 (8H, m), 3.33, 3.27 (s, OCH_3), 3.23–3.15 (3H, m), 3.00 (3H, s), 2.48–2.22 (9H, m), 2.20–1.84 (5H, m), 1.73 (1H, m), 1.54 (1H, m) 1.43–1.32 (1H, m), 1.25–1.20 (3H, m), 1.06–0.88 (16 H, m), 0.85–0.79 (3H, m); ^{13}C NMR ($CDCl_3$, 400 MHz) two conformers, δ 174.1, 173.7, 173.4, 171.8, 170.2, 170.16, 160.5, 160.1, 147.9, 147.8, 136.9, 136.5, 129.9, 129.6, 129.0, 128.9, 127.8, 127.7, 127.0, 126.9, 126.4, 126.1, 121.9, 121.8, 86.2, 82.2, 78.4, 76.6, 61.7, 60.5, 59.2, 58.3, 58.1, 53.9, 53.7, 47.7, 46.6, 45.2, 44.2, 43.0, 38.7, 38.0, 37.9, 37.6, 37.3, 33.3, 31.1, 27.8, 26.1, 25.8, 25.1, 24.9, 23.5, 20.3, 20.0, 17.9, 17.8, 14.4, 10.9, 10.5 ppm; (+)-HRAPCIMS m/z 753.5292 $[M + H]^+$ (calcd for $C_{42}H_{69}N_6O_6$, 753.5279).

Quinstatin 3. *3-(1′-Ethyl-2′-hydroxy)quinoline.* To a stirred solution containing 3-(1′-acetic acid)quinoline (0.03 g, 0.16 mmol) in anhydrous tetrahydrofuran (THF) (2 mL) at 0 °C was added dropwise $LiAlH_4$ (1 M solution in THF, 0.2 mL, 0.3 mmol, 1.25 equiv). The reaction mixture was stirred at 0 °C for 1 h and dampened with the successive addition of water (8 μL), 15% NaOH (8 μL), and again water (24 μL), prior to being stirred for 45 min at 0–5 °C and drying (Na_2SO_4). The solution was filtered to remove the precipitated solids, and the filtrate was concentrated under reduced pressure to give a yellow oil, which solidified on standing to a waxen solid (15.8 mg, 57%): TLC R_f 0.17 (CH_2Cl_2–MeOH 3%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.71 (1H, s), 8.01 (1H, d, J = 7.5 Hz), 7.96 (1H, s), 7.70 (1H, d, J = 8.4 Hz), 7.62 (1H, t, J = 7.5 Hz), 7.48 (1H, t, J = 7.5 Hz), 3.97 (2H, t, J = 6.4 Hz), 3.01 (2H, t, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.9, 146.6, 135.5, 131.8, 128.9, 128.8, 128.0, 127.4, 126.7, 62.9, 36.5.

3-(1′-Ethyl-2′-bromo)quinoline. A stirred solution of 3-(1′-ethyl-2′-hydroxy)quinoline (0.15 g, 0.86 mmol) in anhydrous benzene (20.0 mL) was heated gently to effect a solution and then cooled to 0 °C. Phosphorus tribromide (0.2 mL, 2.4 g, 2.1 mmol, 2.4 equiv) in benzene (1.5 mL) was added, and the mixture heated to 60 °C for 45 min, then cooled to 0 °C. Saturated aqueous $NaHCO_3$ (6 mL) was added until neutral pH. The mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic extracts were washed with water, dried (Na_2SO_4), and concentrated to give a yellow residue, which was separated using silica gel column chromatography by gradient elution of CH_2Cl_2 (100%) → CH_2Cl_2 (96%)–CH_3OH (4%), to yield the product as a yellow oil (68 mg, 34% yield): TLC R_f 0.54

DOI: 10.1021/acs.jnatprod.6b01006
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(CH_2Cl_2 (96%)−CH_3OH (4%); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.79 (1H, s), 8.09 (1H, d, J = 8.8 Hz), 8.00 (1H, s), 7.80 (1H, d, J = 8.0 Hz) 7.63 (1H, t, J = 7.8 Hz) 7.54 (1H, t, J = 8 Hz), 3.66 (2H, t, J = 7.8 Hz), 3.36 (2H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.6 (x2), 135.4, 131.7, 129.5, 129.4, 128.1, 127.7, 127.1, 36.6, 32.5.

3-(1′-Ethyl-2′-azido)quinoline. To a stirred solution containing 3-(1′-ethyl-2′-bromo)quinoline (0.023 g, 0.1 mmol) in anhydrous dimethylformamide (DMF) (0.5 mL) was added NaN_3 (15 mg, 0.23 mmol, 2.3 equiv). The reaction mixture was heated to 90 °C for 30 min, cooled, diluted with ethyl acetate (3 mL), washed with water (3 mL) and brine (3 mL), dried ($MgSO_4$), and concentrated to give an azide as a brown oil, 15.5 mg, 79% yield: TLC R_f 0.50 (CH_2Cl_2 (97%)−CH_3OH (3%)); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.79 (1H, s), 8.13 (1H, d, J = 8 Hz), 8.07 (1H, d, J = 8 Hz), 7.67 (1H, s), 7.59 (1H, d, J = 8.8 Hz), 7.41 (1H, dd, J = 8, 4 Hz), 3.63 (2H, t, J = 7 Hz), 3.09 (1H, t, J = 7 Hz); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 150.2, 147.4, 137.8, 136.4, 135.7, 130.6, 129.8, 127.1, 121.3, 52.2, 35.3.

3-(1′-Ethyl-2′-amino)quinoline. To a stirred solution of the preceding azide (0.015 g, 0.075 mmol) in anhydrous THF (1 mL) cooled to 0 °C under N_2 was added dropwise $LiAlH_4$ (1 M solution in THF, 0.1 mL, 0.1 mmol, 1.3 equiv). Stirring at 0 °C was continued for 45 min. The reaction was completed by the dropwise addition of H_2O (4 μL), 15% NaOH (4 μL), and H_2O (12 μL) successively, and stirring was continued for 30 min. The mixture was diluted with ethyl acetate (5 mL) and dried (Na_2SO_4), and the product solution filtered. The filter cake was washed with ethyl acetate (5 mL), and the organic layers were combined, concentrated, and dried under reduced pressure to give a yellow oil (11 mg, 84%): ^1H NMR ($CDCl_3$, 400 MHz) indicated a mixture of the desired amine along with a dihydroquinoline based on the observed upfield shift of the aromatic ring signals in the proton spectrum. The material was carried forward as this mixture to coupling with Boc-Dap.

3-(1′-Ethyl-2′-amido-Boc-Dap)quinoline. To a solution of the preceding amine mixture (0.04 g, 0.23 mmol) in anhydrous CH_2Cl_2 (1 mL) was added a solution of Boc-Dap[6] (0.06 g, 0.22 mmol) in anhydrous CH_2Cl_2 (1 mL), and the resulting mixture cooled to 0 °C. Triethylamine (0.2 mL, 1.43 mmol) and DEPC (0.12 mL, 0.8 mmol) were added, and the solution was stirred at 0 °C with warming to rt for 6 h. Concentration under reduced pressure led to a dark orange oil, which was separated by silica gel chromatography. Elution with the gradient 100% hexanes → 1:1 → 2:3 hexanes−acetone yielded a dark orange oil, 100 mg. Purification was achieved using flash silica gel chromatography by gradient elution of hexanes 100% → hexanes−acetone (1:4) to give a frothy product (20 mg, 21% yield): ^1H NMR ($CDCl_3$, 400 MHz) δ 8.77 (1H, s), 8.06 (1H, d, J = 8.6 Hz), 7.98 (1H, br s), 7.74 (1H, d, J = 8 Hz), 7.66 (1H, t, J = 7.4 Hz), 7.52 (1H, t, J = 7.4 Hz), 6.59 (1H, br s), 5.85 (1H, br s), 3.81−3.41 (4H, m), 3.34 (3H, s), 3.20−3.08 (2H, m), 3.06−2.99 (2H, m), 2.28 (1H, m), 1.83−1.70 (2H, m), 1.64−1.54 (2H, m), 1.50−1.38 (9H, m), 1.22−1.13 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 174.2, 155.1, 151.9, 147.1, 135.4, 132.2, 129.3, 128.2, 127.6, 127.0, 84.1, 82.2, 80.0, 79.7, 61.0, 59.4, 58.7, 53.7, 47.1, 46.7, 44.7, 44.2, 33.0, 28.8, 26.0, 25.2, 24.8, 24.3, 15.1, 14.3; (+)-HRAPCIMS m/z 442.2707 $[M + H]^+$ (calcd for $C_{25}H_{36}N_3O_4$, 442.2706).

3-(1′-Ethyl-2′-amido-Dap)quinoline trifluoroacetate salt. To a stirred solution of the preceding amide (0.02 g, 0.04 mmol) in anhydrous CH_2Cl_2 (1.5 mL) at 0 °C under N_2 was added TFA (0.3 mL, 0.2 g, 1.76 mmol, 44 equiv). The solution was stirred for 1.5 h at 0 °C and then concentrated and dried under reduced pressure to give a residue, which was used immediately in the next reaction.

3-(1′-Ethyl-2′-amido-Dap-Dil-Val-Dov)quinoline (quinstatin 3). To a stirred solution of the preceding TFA salt (0.015 g, 0.04 mmol) and Dov-Val-Dil TFA[7] (0.025 g, 0.04 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C under N_2 was added TEA (0.03 mL) followed by DEPC (0.011 mL), and the solution was stirred at 0 °C for 3 h. The solvent was removed at room temperature under reduced pressure to give a yellow oil, which was separated by chromatography on a silica gel column eluting with a gradient of CH_2Cl_2−MeOH 4% to CH_2Cl_2−MeOH 12%, which gave the product in the final fractions as an oil, 30 mg, containing contaminants. These were removed by extracting the product into dichloromethane and washing with water, drying (Na_2SO_4), and concentrating to an off-white glass solid (powder when scratched), 10 mg (29% yield): TLC R_f 0.14 (hexanes−acetone, 1:1); $[\alpha]^{22}_D$ −22.6 (c 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.75 (1H, br s), 8.03 (1H, d, J = 8 Hz), 7.98 (1H, s), 7.74 (1H, d, J = 8 Hz), 7.63 (1H, t, J = 8 Hz), 7.50 (1H, t, J = 7.6 Hz), 7.27 (1H, m), 6.90 (1H, nm), 4.73 (1H, t, J = 8 Hz), 3.95 (1H, m), 3.84 (1H, m), 3.78 (1H, d, J = 9 Hz), 3.75−3.47 (2H, m), 3.38−3.14 (8H, m), 3.38, 3.21 (s, OCH_3), 3.13−2.91 (6H, m), 2.64−2.47 (6H, br s), 2.28 (1H, m), 2.17−1.70 (6H, m), 1.64 (2H, m), 1.35−1.11 (6H, m), 1.07−0.81 (16H, m), 0.81−0.68 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.7, 173.0, 170.6, 152.2, 147.0, 135.5, 135.47, 132.4, 131.l, 129.2, 129.1, 128.3, 127.7, 127.3, 127.2, 127.0, 81.8, 70.0, 77.43, 61.0, 59.9, 58.5, 58.1, 54.5, 47.9, 46.0, 45.0, 40.5, 37.5, 33.2, 32.9, 31.2, 30.6, 29.9, 28.2, 26.0, 25.2, 24.9, 19.9, 19.6, 18.6, 18.4, 16.1, 15.5, 11.0, 8.8; (+)-HRAPCIMS mlz 753.5282 $[M + H]^+$ (calcd for $C_{42}H_{69}N_6O_6$, 753.5279).

Quinstatin 4. *4-(1′-Ethyl-2′-amido-Boc-Dap)quinoline.* To a stirred solution of Boc-Dap[6] (0.1 g, 0.35 mmol) and 4-(1′-ethyl-2′-amino)quinoline (0.06 mL, 0.38 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0 °C under N_2 was added TEA (0.15 mL, 1.08 mmol) and DEPC (0.06 mL, 0.38 mmol, l. l equiv). The reaction mixture was stirred at 0 °C with warming to rt for 18 h and then concentrated under reduced pressure to a brown oil, which was purified by chromatography on silica gel (eluents: CH_2Cl_2−MeOH 2−4%) to provide the desired product as a colorless, waxy solid (0.096 g, 62%): TLC R_f 0.2 (CH_2Cl_2−MeOH 4%); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.14 (1H, s), 7.89 (1H, d, J = 8.4 Hz), 7.69 (1H, d, J = 8.4 Hz), 7.62 (1H, t, J = 6.4 Hz), 7.50 (1H, t, J = 7.6 Hz), 7.47 (1H, s), 6.76, 6.54 (1H, br s, NH), 3.86−3.57 (4H, m), 3.45−3.22 (4H, m), 3.17−3.02 (3.5H, m), 2.77 (0.5 H, br s), 2.33−2.14 (l H, m), 1.83−1.33 (13H, m), 1.15 (3H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.2, 173.6, 154.8, 154.5, 152.8, 152.2, 136.5, 130.8, 130.6, 127.6, 127.4, 127.1, 126.9,126.3, 119.3, 84.0, 82.3, 79.9, 79.3, 60.9, 60.7, 59.1, 58.8, 47.0, 46.6, 44.3, 39.5, 39.4, 36.9, 36.7, 28.8, 28.7, 25.8, 25.4, 24.6, 24.1, 14.4; (+)-HRAPCIMS m/z 442.2716 $[M + H]^+$ (calcd for $C_{25}H_{36}N_3O_4$, 442 0.2706).

4-(1′-Ethyl-2′-amido-Dap)quinoline trifluoroacetate salt. To a stirred solution of the preceding amide (0.070 g, 0.16 mmol) in CH_2Cl_2 (2 mL) at 0 °C under N_2 was added TFA (0.5 mL), and stirring was continued for 2 h at 0 °C. The reaction mixture was concentrated under reduced pressure to yield a yellow oil. This was dried under high vacuum for a further 18 h and used without further purification for the next reaction.

4-(1′-Ethyl-2′-amido-Dap-Dil-Val-Dov)quinoline (quinstatin 4). The preceding TFA salt and Dov-Val-Dil.TFA[7] (83 mg, 0.16 mmol) were dissolved in anhydrous CH_2Cl_2 (2 mL), and the solution was stirred under N_2 and cooled to 0 °C. TEA (0.12 mL, 0.86 mmol) and DEPC (0.04 mL, 0.26 mmol, 1.7 equiv) were added, and the mixture was stirred under N_2 at 0 °C for 8 h. The reaction mixture was concentrated under reduced pressure and separated by column chromatography (eluent: gradient elution CH_2Cl_2−MeOH 5−10%) to give the product as a colorless glass, 48 mg (42%): TLC R_f 0.075 (CH_2Cl_2−CH_3OH 5%); $[\alpha]^{22}_D$ −6.9 (c 0.36, $CHCl_3$); ^1H NMR ($CDC1_3$, 400 MHz) δ 9.15 (1H, br s), 7.90 (1H, d, J = 8 Hz), 7.70 (1H, d, J = 8 Hz), 7.63 (1H, m), 7.55−7.48 (2H, m), 6.95−6.88 (2H, m), 4.88, 4.74 (1H, dd, J = 8.4, 6 Hz), 4.02 (1H, m), 3.82 (1H, dd, J = 8, 2 Hz), 3.74−3.60 (3H, m), 3.32 (2H, d, J = 4 Hz), 3.29 (3H, OCH_3), 3.23 (3H, OCH_3), 3.15−3.10 (3H, m), 2.96 (3H, br s), 2.43−2.30 (3H, m), 2.23 (6H, s), 2.16−1.86 (5H, m), 1.64 (2H, m), 1.43−1.25 (2H, m), 1.21−1.03 (4H, m), 1.05−0.82 (16 H, m), 0.81−0.76 (3H, m); ^{13}C ($CDCl_3$, 100 MHz), δ 174.3, 173.9, 170.3, 170.2, 153.0, 152.7, 152.4, 152.3, 136.6, 130.9, 130.6, 127.6, 127.5, 127.4, 127.2, 126.9, 126.3, 119.4, 119.3, 86.2, 82.1, 76.6, 61.8, 60.6, 59.4, 59.2, 58.3, 58.1, 53.9, 47.8, 46.7, 45.2, 44.4, 42.9, 39.6, 39.2, 37.6, 37.2, 36.5 36.0, 33.3, 31.1, 31.06, 29.8, 27.8, 26.0, 25.9, 25.1, 24.9, 23.5, 20.3, 20.0, 17.97, 17.94, 16.0, 15.5, 14.7, 10.9, 10.5; (+)-HRAPCIMS m/z 753.5275 $[M + H]^+$ (calcd for $C_{42}H_{69}N_{60}O_6$, 753.5279).

Quinstatin 5. *5-(1′-Ethyl-2′-amido-Boc-Dap)quinoline.* To a stirred solution of Boc-Dap[6] (0.1 g, 0.35 mmol) and 5-(1′-ethyl-2′-amino)quinoline (0.06 mL, 0.38 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0 °C under N_2 were added TEA (0.15 mL, 1.08 mmol) and DEPC (0.06 mL, 0.38 mmol, 1.1 equiv). The reaction mixture was stirred at

DOI: 10.1021/acs.jnatprod.6b01006
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0 °C with warming to rt for 18 h and then concentrated under reduced pressure to a light brown oil, which was purified by chromatography on silica gel (eluent: CH_2Cl_2−CH_3OH 2−4%) to provide the desired product as a colorless waxen solid (0.101 g, 66% yield): $[\alpha]^{22}_D$ −3.5 (*c* 4.5, CH_2Cl_2); TLC R_f 0.38 (CH_2Cl_2−MeOH 5%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.81 (1H, s), 8.50 (1H, d, *J* = 9.2 Hz), 7.91(1H, d, *J* = 8.4 Hz), 7.54 (1H, t, *J* = 7.1 Hz), 7.39−7.29 (2H, m), 6.79, 6.28 (0.5 H, br s, NH), 3.79−3.65 (2H, m), 3.6−3.4 (2.5H, m), 3.34−3.09 (7H, m), 2.69 (1.5H, m), 2.3 (1H, m), 1.89−1.52 (4.5H, m), 1.50−1.33 (10H, m), 1.25−1.09 (3.5H); ^{13}C NMR ($CDCl_3$, 100 MHz) confomers observed, δ 174.8, 174.3, 155.0, 150.1, 148.7, 136.0, 135.8, 132.7, 129.1, 128.6, 128.4, 127.3, 127.2, 121.2, 84.1, 82.2, 79.9, 79.6, 60.9, 59.4, 59.35, 58.7, 47.0, 46.6, 44.6, 43.9, 40.7, 32.3, 32.2, 28.7, 28.6, 26.0, 25.3, 24.7, 24.2, 14.8, 14.4; (+)-HRAPCIMS *m*/*z* 442.2701 $[M + H]^+$ (calcd for $C_{25}H_{36}N_3O_4$, 442.2706).

5-(1′-Ethyl-2′-amido-Dap)quinoline trifluoroacetate salt. To a stirred solution of the preceding amide (0.070 g, 0.16 mmol) in CH_2Cl_2 (2 mL) at 0 °C under N_2 was added TFA (0.5 mL), and stirring was continued for 2 h at 0 °C. The reaction was stopped and concentrated under reduced pressure to yield a yellow oil. After drying under high vacuum for a further 18 h the TFA salt was used without further purification in the next reaction.

5-(1′-Ethyl-2′-amido-Dap-Dil-Val-Dov)quinoline (quinstatin 5). The above TFA salt and Dov-Val-Dil.TFA[7] (83 mg, 0.16 mmol) were dissolved in anhydrous CH_2Cl_2 (2 mL), and the solution was stirred under N_2 and cooled to 0 °C. TEA (0.12 mL, 0.86 mmol) and DEPC (0.04 mL, 0.26 mmol, 1.7 equiv) were added, and the mixture was stirred under N_2 at 0 °C for 7 h. The reaction mixture was concentrated under reduced pressure and separated by column chromatography (eluent: gradient elution CH_2Cl_2−MeOH 5−10%) to give the product as a colorless glass, 41 mg (35%): TLC R_f 0.075 (CH_2Cl_2−CH_3OH 5%); $[\alpha]^{20}_D$ −34 (*c* 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.86 (1H, m), 8.55 (1H, d, *J* = 8.4 Hz), 7.94 (1H, d, *J* = 8 Hz), 7.57 (1H, t, *J* = 8 Hz), 7.43−7.37 (2H, m), 6.90 (2H, m), 4.73 (1H, dd, *J* = 8, 6.4 Hz), 4.06−3.94 (1H, m), 3.79 (1H, d, *J* = 8.6 Hz), 3.66−3.54 (1H, m), 3.53−3.36 (2H, m), 3.35−3.21 (10H, m), 3.32, 3.25 (s, OCH_3), 2.98 (3H, s), 2.47−2.29 (5H, m), 2.21 (6H, s), 2.09−1.83 (5H, m), 1.78 (3H, m), 1.37−1.26 (1H, m), 1.20 (3H, m), 1.01−0.82 (18H, m), 0.78 (3H, t, *J* = 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.7, 171.9, 170.7, 150.2, 148.8, 136. l, 132.8, 129.1, 128.9, 128.6, 127.5, 127.3, 121.2, 82.0, 76.6, 60.9, 59.7, 58.1, 54.0, 47.9, 44.8, 43.0, 40.8, 37.5, 33.1, 32.3, 31.1, 27.8, 25.8, 25.1, 20.3, 17.9, 16.0, 15.1, 10.8; (+)-HRAPCIMS *m*/*z* 753.5274 $[M + H]^+$ (calcd for $C_{42}H_{69}N_6O_6$, 753.5279).

Quinstatin 6 (4). *6-(1′-Ethyl-2′-hydroxy)quinoline (7).* To a stirred solution containing 6-(1′-acetic acid)quinoline (0.09 g, 0.48 mmol) in anhydrous THF (9 mL) at 0 °C was added dropwise $LiAlH_4$ (1 M solution in THF, 0.6 mL, 0.6 mmol, 1.25 equiv).[8] The reaction mixture was stirred at 0 °C for 1 h and terminated by addition of H_2O (0.02 mL), 15% NaOH (0.02 mL), and again H_2O (0.06 mL), prior to stirring for 45 min at 0 °C and drying (Na_2SO_4). The solids were removed by filtration, and the organic filtrate was concentrated and dried further under reduced pressure to produce alcohol **7**, a yellow oil, 72 mg, 86% yield: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.82 (1H, dd *J* = 1.6, 4.3 Hz), 8.08 (1H, d, *J* = 8 Hz), 8.00 (1H, d, *J* = 8 Hz), 7.64 (1H, br s), 7.58 (1H, dd, *J* = 8, 1.8 Hz), 7.36 (1H, dd, *J* = 8.8, 4.4 Hz), 3.98 (2H, t, *J* = 6.5 Hz), 3.06 (1H, t, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.3, 147.4, 137.5, 135.9, 131.3, 129.7, 127.4, 125.7, 121.4, 63.4, 39.4.

6-(1′-Ethyl-2′-bromo)quinoline (8).[9] A solution of phosphorus tribromide (0.9 mL, 5.6 mmol, 8 equiv) anhydrous in benzene (1.0 mL) was added to a stirred solution of alcohol **7** (0.12 g, 0.67 mmol) in anhydrous benzene (2.0 mL) at 0 °C. The mixture was heated to 60 °C for 45 min, then cooled to rt, and saturated aqueous $NaHCO_3$ (100 mL) was added until a neutral pH was achieved. The mixture was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were washed with water, dried (Na_2SO_4), and concentrated to give the bromide as a yellow oil, 0.12 mg, 76% yield: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.90 (1H, s), 8.13 (1H, d, *J* = 8.4 Hz), 8.08 (1H, d, *J* = 8.4 Hz), 7.66 (1H, s), 7.58 (1H, d, *J* = 8.4 Hz), 7.39 (1H, m), 3.65 (2H, t, *J* = 7.2 Hz), 3.34 (1H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 400 MHz) 150.2, 147.4, 137.2, 135.7 × 2, 130.5, 129.8, 127.1, 121.3, 39.1, 32.5.

6-(1′-Ethyl-2′-azido)quinoline (9). To a stirred solution of bromide **8** (0.12 g, 0.5 mmol) in anhydrous DMF (3 mL) was added NaN_3 (0.12g, 1.84 mmol, 3.7 equiv). The reaction mixture was heated to 90 °C for 30 min, cooled, diluted with ethyl acetate (30 mL), washed with water (30 mL) and brine (30 mL), dried ($MgSO_4$), and concentrated to give the azide **9** as a brown oil, 91 mg, 91% yield: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.91 (1H, s), 8.13 (1H, d, *J* = 8 Hz), 8.07 (1H, d, *J* = 8 Hz), 7.67 (1H, s), 7.59 (1H, d, *J* = 8.8 Hz), 7.41 (1H, dd, *J* = 8, 4 Hz), 3.63 (2H, t, *J* = 7 Hz), 3.09 (1H, t, *J* = 7 Hz); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 150.2, 147.4, 137.8, 136.4, 135.7, 130.6, 129.8, 127.1, 121.3, 52.2, 35.3.

6-(1′-Ethyl-2′-amino)quinoline (10). To a stirred solution of azide **9** (0.089 g, 0.45 mmol) in anhydrous THF (3 mL), cooled to 0 °C under N_2, was added dropwise $LiAlH_4$ (1 M solution in THF, 0.75 mL, 0.75 mmol, 1.65 equiv). Stirring at 0 °C was continued for 45 min. The reaction was completed by the dropwise addition of H_2O (30 μL), 15% NaOH (30 μL), and H_2O (90 μL) successively, with stirring continued for 20 min. The mixture was diluted with ethyl acetate (10 mL) and dried (Na_2SO_4), and the solution filtered. The solid phase was washed with ethyl acetate (5 mL), and the organic layers were combined, concentrated, and dried under reduced pressure to give amine **8** as a yellow oil (quantitative yield): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.88 (1H, m), 8.11 (1H, d, *J* = 8.3 Hz), 8.06 (1H, d, *J* = 8.7 Hz), 7.63 (1H, s), 7.59 (1H, d, *J* = 8.5 Hz), 7.39 (1H, dd, *J* = 8, 4 Hz), 3.09 (2H, t, *J* = 7 Hz), 2.95 (2H, t, *J* = 7 Hz), 1.81 (2H, br s); ^{13}C NMR ($CDCl_3$, 120 Hz) δ 149.9, 147.3, 137.6, 135.6, 131.0, 129.6, 126.9, 121.2, 43.2, 39.9.

6-(1′-Ethyl-2′-amido-Boc-Dap)quinoline (11). A solution of Boc-Dap[6] (0.153 g, 0.5 mmol) in anhydrous CH_2Cl_2 (1 mL) was added to a solution of the amine **10** (0.083 g, 0.5 mmol) in anhydrous CH_2Cl_2 (3 mL), and the resulting mixture cooled to 0 °C. Triethylamine (0.3 mL, 2.1 mmol) and DEPC (0.2 mL, 1.2 mmol) were added, and the solution was stirred at 0 °C for 2 h. Concentration under reduced pressure led to a dark orange oil, which was separated on a silica gel column. Elution with a gradient 3:2 → 1:1 → 2:3 of hexanes−acetone gave the desired amide as a colorless oil, 111 mg, 54% yield: TLC R_f 0.5 (hexanes−acetone, 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.88 (1H, s), 8.10−8.04 (2H, m), 7.65−7.58 (2H, m), 7.39 (1H, m), 6.42, 5.73 (1H, br s, NH), 3.85−3.42 (4H, m), 3.35 (3H, s), 3.37−3.27 (1H, m), 3.19−3.10 (1H, m), 3.09−3.01 (2H, m), 2.40−2.21 (1H, m), 1.84−1.71 (3H, m), 1.53−1.42 (10H, m), 1.19 (3H, nm); (+)-HRAPCIMS *m*/*z* 442.2695 $(M + H)^+$ (calcd for $C_{25}H_{36}N_3O_4$, 442.2706).

6-(1′-Ethyl-2′-amido-Dap)quinoline trifluoroacetate salt (12). To a stirred solution of amine **11** (0.1 g, 0.23 mmol) in anhydrous CH_2Cl_2 at 0 °C under N_2 was added TFA (1 mL). The solution was stirred for 2 h at 0 °C and then concentrated under reduced pressure to give a residue, which was dissolved in toluene and reconcentrated (×2). The oily TFA salt was dried under reduced pressure and used immediately for the next reaction.

6-(1′-Ethyl-2′-amido-Dap-Dil-Val-Dov)quinoline [quinstatin 6 (4)]. To a stirred solution of TFA salt **12** (0.96 mg) and Dov-Val-Dil TFA[7] (0.114 g, 0.21 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C under N_2 was added TEA (0.12 mL, 0.86 mmol) followed by DEPC (0.035 mL, 0.21 mmol), and the solution stirred at 0 °C for 2 h. The solvent was removed at room temperature under reduced pressure to give a yellow oil, which was separated by chromatography on a short silica gel column (7 × 3 cm), by eluting slowly with hexanes−acetone (1:1), to give quinstatin 6 (**4**) as a yellow froth, 78 mg (47%): TLC R_f 0.23 (CH_2Cl_2−MeOH 6%); $[\alpha]^{22}_D$ −6.5 (*c* 0.34, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.88 (1H, dd, *J* = 4.4, 1.6 Hz), 8.10 (1H, d, *J* = 8 Hz), 8.03 (1H, d, *J* = 8.8 Hz), 7.66 (1H, s), 7.61 (1H, dd, *J* = 8, 2 Hz), 7.39 (1H, dd, *J* = 8.0, 4.0 Hz), 6.88 (1H, d, *J* = 10 Hz), 6.72 (1H, t, *J* = 4.4 Hz), 4.79 (1H, dd, *J* = 10, 6.8 Hz), 4.09−4.03 (1H, m), 3.98 (1H, m), 3.82 (1H, dd, *J* = 8.8 Hz, 1.6 Hz), 3.71 (1H, m), 3.59 (1H, m), 3.43−3.35 (2H, m), 3.33 (3H, s, OCH_3), 3.28 (3H, s, OCH_3), 3.07 (2H, t, *J* = 8 Hz), 3.03 (3H, s), 2.49−2.43 (1H, m), 2.40−2.31(2H, m), 2.28−2.24 (7H, m), 2.14−1.84 (5H, m), 1.66 (2H, m), 1.39−1.26 (2H, m), 1.24−1.14 (4H, m), 1.06−0.92 (16H, m), 0.85−0.82 (3H, m); ^{13}C NMR ($CDC1_3$, 100 MHz) δ 174.4, 174.1, 173.5, 171.8, 170.5, 170.2, 150.l, 147.4, 137.9, 135.7, 131.3, 130.7, 129.9, 129.5, 128.4, 127.l, 121.6, 121.3, 86.0, 81.8, 78.6, 76.6, 61.7, 60.7, 59.6, 59.2, 58.3, 58.0, 59.9, 47.8, 45.0, 44.7, 43.0, 40.6, 37.5, 35.4, 33.1, 31.2, 27.8, 25.8, 25.0, 23.5, 20.3, 20.0,

DOI: 10.1021/acs.jnatprod.6b01006
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19.6, 18.4, 17.9, 16.0, 15.7, 15.2, 10.9, 10.6; (+)-HRAPCIMS m/z 753.5279 $[M + H]^+$ (calcd for $C_{42}H_{69}N_6O_6$, 753.5279).

Quinstatin 7. The general experimental procedure summarized above for the synthesis of the 2′-ethylamine intermediate from the corresponding 3- and 6-quinoline acetic acids was employed for preparation of the amine intermediate required for the synthesis of quinstatin 7.

7-(1′-Ethyl-2′-hydroxy)quinoline: yellow oil that solidified on standing to a waxy solid, yield 86%; TLC R_f 0.25 (CH_2Cl_2−CH_3OH 3%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.81 (1H, dd, J = 4.6, 1.7 Hz), 8.09 (1H, d, J = 8.5 Hz), 7.93 (1H, s), 7.73 (1H, d, J = 8.8 Hz), 7.42 (1H, dd, J = 8.8, 0.16 Hz), 7.32 (1H, dd, J = 8.5, 4.6 Hz), 3.99 (2H, t, J = 6.0 Hz), 3.07 (2H, t, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.3, 140.7, 135.9 (×2C), 128.4, 127.8, 126.9, 120.6, 63.2, 39.4.

7-(1′-Ethyl-2′-bromo)quinoline: colorless oil (66% yield); TLC R_f = 0.6 CH_2Cl_2−MeOH 3%; ^{1}H NMR ($CDCl_3$ 400 MHz) δ 8.89 (1H, dd, J = 4.0, 1.2 Hz), 8.81 (1H, d, J = 8.7 Hz), 7.98 (1H, s), 7.79 (1H, d, J = 8.7 Hz), 7.41 (2H, m), 3.65 (2H, t, J = 7.9 Hz), 3.36 (2H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 149.9, 147.3, 141.1, 136.8, 128.1, 128.06, 127.8, 127.2, 120,.9, 39.2, 32.2.

7-(1′-Ethyl-2′-azido)quinoline: brown oil, quantitative yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.88 (1H, dd, J = 4.0, 1.6 Hz), 8.11 (1H, d, J = 8 Hz), 7.93 (1H, s), 7.76 (1H, d, J = 8 Hz), 7.40 (1H, dd, J = 8.8, 2 Hz), 7.35 (1H, dd, J = 8, 4 Hz), 3.61 (2H, t, J = 7.4 Hz), 3.09 (2H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.9, 148.5, 139.9, 136.0, 128.9, 128.3, 128.0, 127.3, 121.1, 52.3, 35.7.

7-(1′-Ethyl-2′-amino)quinoline: yellow residue (91% yield); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.86 (1H, dd, J = 4.4, 1.8 Hz), 8.11 (1H, dd, J = 8, 1.2 Hz), 7.9 (1H, s), 7.74 (1H, d, J = 8 Hz), 7.39 (1H, dd, J = 8, 1.5 Hz), 7.34 (1H, dd, J = 8, 4.4 Hz), 3.08 (2H, t, J = 6.8 Hz), 2.96 (2H, t, J = 6.8 Hz), 1.63 (br s, NH_2).

7-(1′-Ethyl-2′-amido-Boc-Dap)quinoline. To a solution of the preceding amine (0.06 g, 0.35 mmol) in anhydrous CH_2Cl_2 was added a solution of Boc-Dap[6] (0.1 g, 0.35 mmol) in anhydrous CH_2Cl_2 (1 mL), and the resulting mixture cooled to 0 °C. TEA (0.2 mL, 1.43 mmol, 4 equiv) and DEPC (0.185 mL, 0.199 g, 1.22 mmol, 3.5 equiv) were added. The mixture was stirred at 0 °C for 8 h, then overnight at rt for 24 h. The reaction mixture was next concentrated to a dark brown solid, extracted with water, dried (Na_2SO_4), and concentrated. Purification on silica gel flash chromatography using CH_2Cl_2−MeOH 5% gave the product as a yellow oil (65 mg, 43% yield based on Boc-Dap): TLC R_f 0.4 (CH_2Cl_2−MeOH 5%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.82 (1H, br s), 8.08 (1H, br d, J = 9 Hz), 7.87 (1H, s), 7.71 (1H, d, J = 8 Hz), 7.39 (1H, m), 7.31 (1H, m), 6.51, 5.99 (1H, br s, NH), 3.77 (1H, m), 3.70 (1H, m), 3.66−3.52 (3H, m), 3.43−3.35 (1H, m), 3.29 (3H, s) 3.16−2.96 (2H, m), 2.33−2.15 (1H, m), 1.84−1.64 (2H, m), 1.63−1.51 (2H, m), 1.43, 1.39 (9H, s), 1.16−1.08 (3H, m); (+)-HRAPCIMS m/z 442.2696 $[M + H]^+$ (calcd for $C_{25}H_{36}N_3O_4$, 442.2706).

7-(1′-Ethyl-2′-amido-Dap)quinoline trifluoroacetate salt. A solution of the preceding ethylamide (0.06 g, 0.136 mmol) in dry CH_2Cl_2 was stirred and cooled to 0 °C under N_2. TFA (0.15 mL) was added, and the solution stirred at 0 °C for 3 h. The solvent was removed under reduced pressure using toluene as an azeotrope, to yield a residue, which was further dried under high vacuum for 16 h. The salt was used as-is in the next step.

7-(1′-Ethyl-2′-amido-Dap-Dil-Val-Dov)quinoline (quinstatin 7). The preceding TFA salt (0.06 g) and Dov-Val-Dil-TFA[7] (0.078 g, 0.143 mmol, 1 equiv) in anhydrous CH_2Cl_2 were stirred at 0 °C. TEA (0.1 mL, 0.72 mmol, 5 equiv) and DEPC (0.022 mL, 1.43 mmol) were added in succession, and the solution was stirred under argon for 4 h at 0 °C. The solvent was removed under reduced pressure, and the residue separated by chromatography using flash silica gel, eluting with CH_2Cl_2−MeOH 5%; column size 20 cm × 2 cm. The product was obtained as a light yellow powder, 65 mg (61% yield based on the TFA salt precursor): TLC R_f 0.27 (CH_2Cl_2−MeOH 5%); $[\alpha]^{23}_D$ −4.5 (c 0.2, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.82 (1H, br s), 8.07 (1H, d, J = 8 Hz), 7.85 (1H, s), 7.71 (1H, d, J = 8.8 Hz), 7.41(1H, d, J = 8.3 Hz), 7.31(1H, dd, J = 8, 4.7 Hz), 6.88 (1H, d, J = 8.6 Hz), 6.71 (1H, m), 4.73 (1H, dd, J = 9.6, 6.8 Hz), 4.02 (1H, m), 3.95 (1H, m), 3.78 (1H, dd, J = 8.7, 1.6 Hz), 3.71−3.48 (3H, m), 3.33−3.28 (2H, m), 3.27 (3H, s), 3.22 (3H, s), 3.02 (2H, t, J = 7.6 Hz), 2.97 (3H, br s), 2.40 (1H, d, J = 7.1 Hz), 2.34−2.26 (2H, m), 2.20 (7H, s), 2.09−1.71 (5H, m), 1.62 (2H, m), 1.30 (1H, m), 1.16 (3H, d, J = 7.2 Hz), 0.99−0.84 (16H, m), 0.76 (3H, t, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4, 173.6, 171.9, 171.6, 170.6, 170.2, 150.6, 148.5, 141.3, 140.6, 139.2, 135.9, 129.0, 128.7, 128.3, 128.0, 127.1,120.8, 81.9, 78.5, 76.6, 61.7, 60.7, 59.6, 58.1, 54.0, 47.8, 46.7, 44.6, 43.0, 42.9, 40.5, 37.6, 35.9, 33.2, 31.1, 27.8, 25.9, 25.0, 20.3, 19.9, 19.7, 18.4, 18.1, 17.9, 16.0, 15.7, 15.1, 10.9; (+)-HRAPCIMS m/z 753.5292 $[M + H]^+$ (calcd for $C_{42}H_{69}N_6O_6$, 753.5279).

Quinstatin 8. *8-(1′-Ethyl-2′-amido-Boc-Dap)quinoline.* 8-(1′-Ethyl-2′-amino)quinoline (0.1 mL, 0.7 mmol) was added to a solution of Boc-Dap[6] (0.2 g, 0.7 mmol) in anhydrous CH_2Cl_2 (3 mL), and the reaction mixture was stirred at 0 °C. Next, TEA (0.3 mL, 2.15 mmol, 3 equiv) and DEPC (0.15 mL, 0.16 g, 0.98 mmol, 1.4 equiv) were added. The reaction mixture was stirred at 0 °C for 7 h and concentrated to an orange-colored oil, which was separated by silica gel flash chromatography using CH_2Cl_2−MeOH 3.5%; column size 33 cm × 2 cm. Fractions were combined according to TLC data. The product was obtained as a colorless oil (139 mg, 0.32 mmol, 45%): TLC R_f 0.26 (CH_2Cl_2−CH_3OH 3%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (l H, dd, J = 4.0, 1.6 Hz), 8.06 (1H, d, J = 7.2 Hz), 7.61 (1H, d, J = 7.4 Hz), 7.49 (1H, d, J = 7.0 Hz), 7.37 (1H, t, J = 8.0 Hz), 7.31 (1H, m), 6.91 (1H, m), 3.70 (1H, m), 3.57 (3H, m), 3.44−3.30 (3H, m), 3.23 (3H, s, OCH_3), 3.05 (1H, m), 2.22−1.96 (1H, m), 1.64 (2H, m), 1.54−1.30 (11H, m), 1.01 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) (two conformers observed) δ 174.2, 173.6, 154.6, 154.3, 149.4, 147.1, 138.4, 136.7, 136.6, 130.2, 128.5, 126.9, 126.8, 126.6, 121.0, 83.9, 82.3, 79.6, 79.0, 60.9, 60.8, 58.8, 53.5, 46.9, 46.5, 44.5, 44.0, 41.5, 41.3, 30.8,28.6, 25.6, 25.3, 24.4, 24.0, 14.3, 14.0; (+)-HRAPCIMS m/z 442.2703 $[M + H]^+$ (calcd. for $C_{25}H_{36}N_3O_4$, 442.2706).

8-(1′-Ethyl-2′-amido-Dap)quinoline trifluoroacetate salt. A solution of the preceding amide (0.3 g, 0.68 mmol) in dry CH_2Cl_2 (10 mL) was stirred at 0 °C under N_2. TFA (1.0 mL, 1.49 g, 13 mmol, 19 equiv) was added, and the reaction mixture stirred at 0 °C for 3 h and monitored by TLC using CH_2Cl_2−MeOH 5% as solvent. The solvent was removed under reduced pressure and with toluene as an azeotrope, then dried using high vacuum for 16 h to yield a brown oil, which was used without further purification for the next step.

8-(1′-Ethyl-2′-amido-Dap-Dil-Val-Dov)quinoline (quinstatin 8). The preceding TFA salt (0.3 g, 0.66 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and stirred at 0 °C. Dov-Val-Dil-TFA[7] (0.37 g, 0.66 mmol, 1 equiv) was added followed by TEA (0.5 mL, 3.6 mmol, 5 equiv) and DEPC (0.11 mL, 7.15 mmol, 11 equiv). The reaction mixture was stirred under N_2 for 4 h at 0 °C. Solvent was removed under reduced pressure, and the residue was dried under high vacuum. Chromatographic separation was achieved using flash silica gel, eluting with CH_2Cl_2−MeOH 5%; column size 20 cm × 4 cm. This gave the desired product as a light yellow powder (0.32 g, 64% yield): TLC R_f 0.4 (CH_2Cl_2−MeOH 5%); $[\alpha]^{23}_D$ −12.2 (c 0.5, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz), doubling of signals in the proton and carbon spectra indicating the presence of two isomers, a pattern observed in dolastatin 10 and discovered to be due to conformational isomers arising from *cis*−*trans* isomerism at the Dil-Dap bond,[3b] δ 8.89 (1H, s), 8.16, 8.12 (1H, d, J = 8 Hz), 7.70, 7.66 (1H, d, J = 8 Hz), 7.57 (1H, d, J = 6.8 Hz), 7.48, 7.44 (1H, d, J = 8 Hz), 7.46−7.36 (1H, m), 6.96 (1H, t, J = 4.4 Hz), 6.86 (1H, d, J = 8.8 Hz), 4.85, 4.74 (1H, dd, J = 6.4, 9.6 Hz), 4.0 (1H, m), 3.80 (1H, dd, J = 7.6, 2.9 Hz), 3.72−3.56 (2H, m), 3.53−3.19 (11H, m), 3.30 (3H, s, OCH_3), 3.27 (3H, s, OCH_3), 2.96 (3H, br s), 2.50−2.32 (2H, m), 2.23−2.20 (7H, br s), 2.08−1.77 (5H, m), 1.69−1.51 (2H, m), 1.31 (1H, m), 1.10 (2H, d, J = 7 Hz), 1.0−0.83 (17H, m), 0.77 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) 174.1, 173.7, 173.4, 171.9, 170.2, 149.6, 149.59, 147.3, 147.2, 138.6, 138.5, 137.1, 136.8., 130.6, 130.3, 128.6, 127.2, 127.0, 126.9, 126.7, 121.3, 121.2, 86.3, 82.3, 78.2,76.6, 61.8, 60.5, 59.2, 59.1, 58.2, 58.1, 53.9, 47.7, 46.6, 44.3, 43.0, 41.9, 41.3, 37.7, 33.3, 31.1, 31.0, 30.9, 27.8, 25.9, 25.1, 24.8, 23.6, 20.3, 20.0, 19.7, 18.0, 17.9, 15.4, 14.2, 10.9, 10.4; (+)-HRAPCIMS m/z 753.5285 $(M + H)^+$ (calcd for $C_{42}H_{69}N_6O_6$, 753.5279).

Cancer Cell Line Procedures. Inhibition of human cancer cell growth was assessed using the standard sulforhodamine B assay of the U.S. National Cancer Institute, as previously described.[10] In summary,

DOI: 10.1021/acs.jnatprod.6b01006
J. Nat. Prod. XXXX, XXX, XXX−XXX

cells in a 5% fetal bovine serum/RPMI1640 medium were inoculated in 96-well plates and incubated for 24 h. Next, serial dilutions of the compounds were added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI_{50} or the drug concentration causing a 50% reduction in the net protein increase) was calculated from optical density data with Immunosoft software.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b01006.

^{1}H and ^{13}C NMR spectra of quinstatins 2 (**3**), 3−5, 6 (**4**), 7, and 8 along with the HRMS of 6-(1′-ethyl-2′-amido-Boc-Dap)quinoline (**11**) and quinstatin 8 (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

With appreciation, we acknowledge and thank for financial support the Arizona Biomedical Research Commission, the J.W. Kiecknefer Foundation, and the Margaret T. Morris Foundation.

■ DEDICATION

In memory of Dr. Carl Djerassi (1923−2015), a most extraordinarily productive professor of chemistry, humanitarian, and mentor as well as discoverer of the first successful oral and dedicated to Professor Phil Crews, of the University of California, Santa Cruz, for his pioneering work on bioactive natural products.

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DOI: 10.1021/acs.jnatprod.6b01006
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