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A Straightforward Total Synthesis of (−)-Chaetominine

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Dedicated to the Centenary of the Italian Chemical Society

Abstract: A total synthesis of the tripeptide alkaloid (−)-chaetominine (**1**) was achieved in 9.3% overall yield starting from commercially available D-tryptophan methyl ester, based on a short and straightforward (nine steps) sequence. The early stage introduction (first step) of the quinazolinone moiety and the late stage introduction (penultimate step) of the hydroxy group allowed a synthetic strategy devoid of protective groups. The key step of the process is the **a**–**c** tricyclic ring construction via an unprecedented NCS-mediated *N*-acyl cyclization on an indole ring to give tetrahydro-1*H*-pyrido[2,3-*b*]indole **11**. In the penultimate step, oxidation of the tetracyclic intermediate **14** with oxaziridine **15** gave only one of the four possible diastereoisomers, the *cis*-diastereoisomer **16** resulting from the attack of the oxaziridine to the double bond face opposite to the **c**–**d** ring substituents. In the last step, the complete stereocontrol of the Et_3SiH/TFA reduction of compound **16**, probably involving a *N*-acyliminium ion, can be attributed to ring constrain, which forces the **b**–**c** ring junction in the more stable *cis*-orientation. (−)-Chaetominine (**1**) showed a negligible inhibitory activity on several cancer cell lines.

Keywords: alkaloids · amino acids · lactams · natural products · total synthesis

Introduction

Endophytic fungi[1] represent one of the largest (conservatively 1.5×10^6 species) and relatively unexplored resource of secondary metabolites.[2] As a single strain is supposed to produce multiple bioactive principles,[3] the overall chemical diversity is virtually uncountable, thus rendering these microorganisms an excellent source of new potential drug leads.[4] Among the endophytic fungi, the *Chaetomium* species produces a vast array of structurally different natural products, such as alkaloids (e.g., chetomin,[5] chaetocin,[6] and chaetoglobosins[7]), terpenes (e.g., heptelidic acid[8] and FR207944[9]) and phenols (e.g., globosumones[2] and orsellides[10]), that show a broad spectrum of biological activities. The strain IFB-E015, pertaining to this species and colonizing the leaves of *Adenophora axilliflora* (family of Campanulaceae), was reported in 2006 by Tan and co-workers[11] to produce a novel tripeptide alkaloidal metabolite, (−)-chaetominine (**1**, Figure 1). This secondary metabolite was nicely demonstrated to possess an unprecedented framework, partially shared by the non-*Chaetomium* species-derived tetrapeptide alkaloids tryptoquivalines,[12] [for example, tryptoquivalin G (**2**), Figure 1] (rings **a**–**b**, **d** and **e**–**f**), the fumiquinazolines[13] [for example, fumiquinazoline A (**3**), Figure 1] and the cyclic peptides kapakahines[14] [for example, kapakahine B (**4**), Figure 1] (rings **a**–**d**). The intriguing topology of **1**, associated with the reported[11] cytotoxic activity in the double-digit nanomolar range on a couple of human cancer cell lines, left (−)-chaetominine (**1**) undefeated by total synthesis for a very short period of time. By capitalizing on an advanced intermediate along the preparation of fumiquinazolines,[15] the Snider group published in 2007 a synthesis of **1**,[16] less than one year after its isolation.

More recently, a second total synthesis of **1** was reported by Evano and co-workers.[17] In the isolation and structure determination manuscript,[11] Tan proposed a plausible bio-

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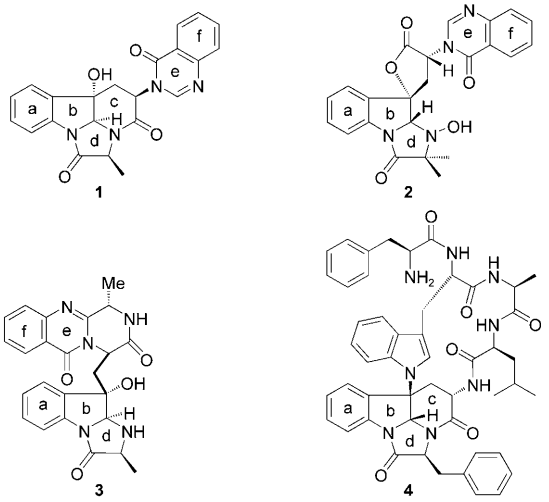
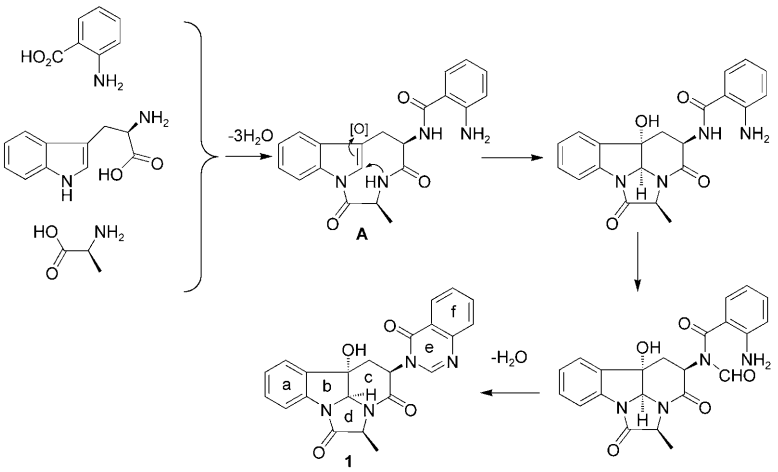


Figure 1. Chaetominine (**1**), tryptoquivalin G (**2**), fumiquinazoline A (**3**), and kapakahine B (**4**).

synthetic pathway for (−)-chaetominine (**1**) involving L-alanine, anthranilic acid and D-tryptophan, the last two being closely linked through the shikimate pathway.[18]

According to the biosynthetic proposal, rings **c** and **d** of the tetracyclic core of **1** should simultaneously form through oxidation[14a] of a putative nine-membered ring cyclic tripeptide intermediate (**A**, Scheme 1). The final assembly of the quinazolinone moiety (rings **e**–**f**) would require a C1 unit in the formic acid oxidation state.



Scheme 1. Proposed biosynthetic pathway for (−)-chaetominine (**1**).

Although a biomimetic route towards **1** seemed attractive, a closer inspection of the postulated biosynthetic intermediate **A**, including two amides and one *trans* C−C double bond in a nine-membered ring, revealed the potential problems associated with its preparation and/or stability. Therefore we envisioned a different approach, where the sequence of synthetic events was carefully planned in order to minimize the use of protective groups.[19] The key step of this new route is the **a**–**c** tricyclic ring construction via an unprecedented NCS-mediated tetrahydro-1*H*-pyrido[2,3-*b*]indole formation (Scheme 2).

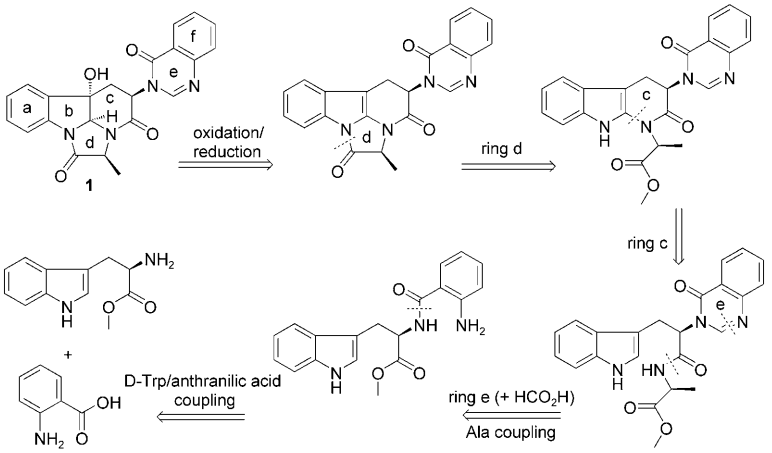
Results and Discussion

Starting from commercially available D-tryptophan methyl ester (**5**), we planned to introduce the quinazolinone moiety (ring **e**–**f** of chaetominine) from the very beginning of the synthesis (Scheme 3).

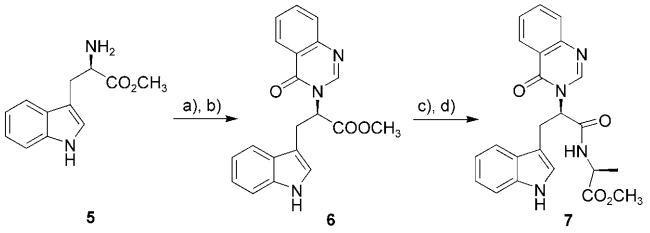
Thus reaction of compound **5** with anthranilic acid using TBTU as activating agent, followed by treatment with $(EtO)_3CH$ in the presence of a catalytic amount of TsOH in EtOAc at 50 °C,[20] delivered the tryptophan−quinazolinone intermediate **6** in excellent overall yield (90 %).[21] Subsequent hydrolysis of methyl ester **6** afforded the corresponding acid which, upon coupling with L-alanine methyl ester,[22] was transformed into compound **7** again in excellent yield (90 %). At this stage, the presence of a minor diastereoisomer (≤10 %), originated by partial racemization of the quinazolinone-bearing stereocenter during the LiOH-mediated hydrolysis of methyl ester **6**, was detected by ^{1}H NMR analysis.[23] The minor diastereoisomer could not be separated, and the 9:1 mixture was processed further. Intermediate **7** possesses all the nitrogen and carbon atoms present in chaetominine, and sets the stage for a stepwise ring **c** and ring **d** construction, according to our retrosynthetic analysis (see Scheme 2).

N-Acylated and *N*-carbamoylated derivatives of tryptophan are known to give tricyclic dihydropyrrolo[2,3-*b*]indoles by reaction with NBS, in phosphate buffer at pH 9 or with *tert*-butyl hypochlorite in CH_2Cl_2 and Et_3N.[24] These cyclizations presumably occur via 3-haloindolenines, which ring close to 3-haloindolines. On standing, the dihydropyrrolo-[2,3-*b*]indoles arise by spontaneous or base-catalyzed dehydrohalogenation of the 3-haloindolines.[24a] However, to the best of our knowledge, no examples were ever reported where a six-membered lactam ring is formed following the aforementioned reaction protocol.

After extensive experimental efforts (selected conditions are reported in Table 1), tricyclic tetrahydro-1*H*-pyrido[2,3-*b*]indole (**8**) was finally secured in 40–63 % yield using a slight excess of NCS in CH_2Cl_2 in the presence of Et_3N at −78 °C (the minor isomer at the quinazolinone-bearing stereocenter was separated at this stage). A variable amount (15–20 %) of the corresponding 3-chloroindolenine (**9**) was

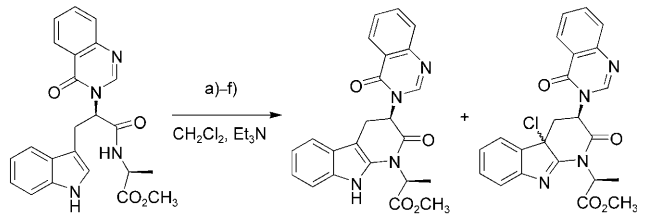


Scheme 2. Retrosynthetic analysis of (−)-chaetominine (**1**).



Scheme 3. Synthesis of intermediate **7**. a) Anthranilic acid, TBTU, DIPEA, DMF, RT; b) $(EtO)_3CH$, TsOH (cat.), AcOEt, 50 °C (90% overall yield); c) 0.2 N LiOH, THF, RT (100%); d) H-L-Ala-OMe·HCl, EDC·HCl, DhBtOH, DIPEA, DMF, RT (90%).

Table 1. Optimization of the cyclization conditions to tricyclic tetrahydro-1*H*-pyrido[2,3-*b*]indole (**8**).



Entry	Conditions	*T* [°C]	**8** [%][a]	Isolated yield **8** [%]	Isolated yield **9** [%]
a	*t*BuOCl	RT	53	50	27
b	1.2 equiv NCS	RT	40	[d]	[d]
c	1.5 equiv NCS	RT	[c]	[d]	[d]
d	1.2 equiv NCS	−78	70	51	16
e	1.3 equiv NCS	−78	55–75	40–63	20–15
f	XeF_2[b]	RT	30	[d]	[d]

[a] Measured by integration of the HPLC trace at 254 nm. [b] See ref. [19b]. [c] Complex mixture. [d] Not determined.

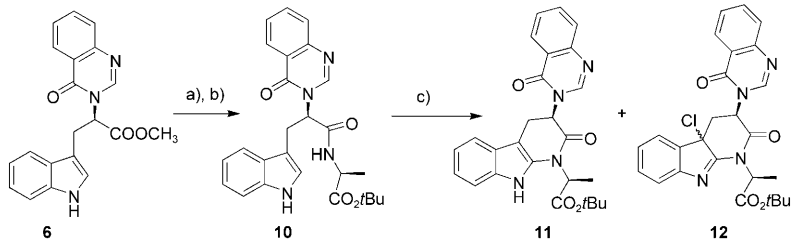
also produced, probably due to a competing electrophilic halogenation of the more electron-rich 2-carbonylaminoindole **8**. However, despite repeated attempts, this reaction proved capricious and not reproducible on a larger scale. For this reason, we turned our attention to the corresponding *tert*-butyl ester **10**, rapidly available from methyl ester **6** (Scheme 4). Compound **10**, again processed as a 9:1 mixture of diastereoisomers,[23] cyclized on a gram scale to give the tricyclic tetrahydro-1*H*-pyrido[2,3-*b*]indole (**11**) in an acceptable 52% isolated yield as a single diastereoisomer (the minor isomer at the quinazolinone-bearing stereocenter was separated at this stage). HPLC analysis of the reaction mixture revealed the presence of the intermediates stereoisomeric 3-chloroindolines,[24a] whose slower dehydrohalogenation (slower in the *tert*-butyl ester series compared to the methyl ester series) seems to protect 2-carbonylamino-indole **11** from further halogenation by excess NCS, thus reducing the amount of 3-chloroindolenine (**12**) formed (13%).

TFA-mediated cleavage of *tert*-butyl ester **11** afforded acid **13** in quantitative yield, which was subjected to intramolecular cyclization via the corresponding acid chloride in the presence of diisopropylethylamine (DIPEA) (Scheme 5). Tetracyclic intermediate **14** was thus obtained in 75% yield as a single diastereoisomer. Careful HPLC-MS and NMR analysis of the crude reaction mixture revealed no presence of epimerized material.

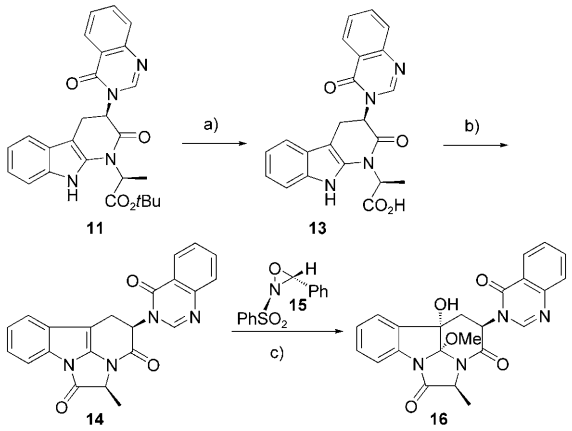
At this point, completion of the synthesis required the stereoselective manipulation of the indole double bond via an oxidation–reduction sequence. Hydroxylation at C-3 of the indole ring in compound **14** was performed using the classical Davis (±)-oxaziridine **15**.[25] As desired and expected, the reaction turned out to be highly stereoselective, under substrate control governed by the two stereogenic centers of the rigid tetracycle **14**. Also the addition of methanol to the postulated acyliminium intermediate occurred with complete stereocontrol in favor of the *cis*-diastereoisomer (**16**). The excellent stereoselectivity of this entire process marks the difference with the results reported by Snider on less rigid substrates, where mixtures of diastereoisomers were obtained in various ratios.[15,16]

Hydroxyl-directed reduction of **16** with sodium borohydride in acetic acid[26] did not afford any desired product. Also attempts to reduce **16** with either $NaBH(OAc)_3$ or $NaBH_3CN$/HCl failed. Pleasingly, chaetominine (**1**) was secured in a reasonable yield (65%) using Et_3SiH as reducing agent,[15,27] in CH_2Cl_2/TFA at room temperature (Scheme 6).

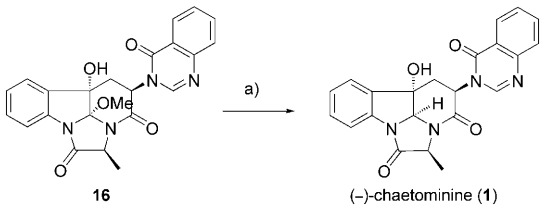
The observed complete stereocontrol of the reduction, probably involving a *N*-acyliminium ion, can be attributed to ring constrain, which forces the ring **b–c** junction in the more favorable *cis*-orientation. Synthetic (−)-chaetominine (**1**) was fully characterized by spectroscopic and analytical methods and proved to be identical to the natural product.[11] In gloomy contrast to the results reported by Tan,[11] (−)-chaetominine (**1**) proved to possess, in our hands, a neg-



Scheme 4. Synthesis of tricyclic tetrahydro-1*H*-pyrido[2,3-*b*]indole **11**. a) 0.2 N LiOH, THF, RT (100%); b) H-L-Ala-O*t*Bu·HCl, EDC·HCl, DhBtOH, DIPEA, DMF, RT (80%); c) NCS, Et_3N, CH_2Cl_2, −78°C (52% **11**, 13% **12**).



Scheme 5. Synthesis of tetracyclic intermediate **16**. a) 20% TFA in CH_2Cl_2, RT (100%); b) $(COCl)_2$, DIPEA, DMF cat., CH_2Cl_2, RT (75%); c) CH_2Cl_2/MeOH 2:1, RT (50%).



Scheme 6. Synthesis of (−)-chaetominine (**1**). a) Et_3SiH, CH_2Cl_2, TFA, RT (65%).

ligible (2%) inhibitory activity at 72 h on human leukaemia cell line K562 at 10 μM. The growth of other cancer cell lines (A2780 and MCF7) was similarly unaffected by **1** (see Experimental Section, Table 2).

Table 2. Inhibitory activity of (−)-chaetominine (**1**) at 10 μM concentration on selected cell lines.

Cancer cell lines	A2780	K562	MCF7
% inhibition in the presence of 10 μm **1**	2.89	1.70	9.61

Conclusions

In conclusion, a new total synthesis of (−)-chaetominine (**1**) was achieved. The natural product was obtained in 9.3% overall yield, by capitalizing on a concise (nine steps) and straightforward synthetic sequence, starting from commercially available D-tryptophan methyl ester. Compared to the previously published total syntheses,[16,17] the early stage introduction (first step) of the quinazolinone moiety and the late stage introduction (penultimate step) of the hydroxy group allow a synthetic strategy characterized by a minimal use of protective groups.

In this paper, we also document the first example of tricyclic tetrahydro-1*H*-pyrido[2,3-*b*]indole synthesis via NCS mediated *N*-acyl cyclization on an indole ring. In the penultimate step, oxidation of the tetracyclic intermediate **14** with oxaziridine **15** gave only one of the four possible diastereoisomers, the *cis*-diastereoisomer **16** resulting from the attack of the oxaziridine to the double bond face opposite to the **c–d** ring substituents. In the last step, the complete stereocontrol of the Et_3SiH/TFA reduction of compound **16**, probably involving a *N*-acyliminium ion, can be attributed to ring constrain, which forces the **b–c** ring junction in the more stable *cis*-orientation.

Experimental Section

All solvents were reagent grade and all reagents were used as supplied. Flash chromatography was performed with silica gel 60 Å (particle size 230–400 mesh) supplied by Aldrich. Melting points were recorded on a Buchi 535 instrument. NMR spectra were performed at 25, 50 and 80°C in [D_6]DMSO on a Varian Inova 500 spectrometer equipped with 5 mm $^1H\{^{13}C,^{15}N\}$ *z*-axis-PFG indirect detection cold probe and on a Varian Inova 400 spectrometer equipped with 5 mm $^1H\{^{15}N,^{31}P\}$ *z*-axis-PFG indirect detection probe. Residual solvent signal was used as reference (δ = 2.50 ppm for ^1H and δ = 39.5 for ^{13}C). Standard two-dimensional sequences provided by Varian (gradient-enhanced HSQC, HMBC and T-roesy) were used to assign carbons and stereochemistry. Electrospray (ESI) mass spectra were obtained on a LCQ Deca XP (Thermo) ion trap mass spectrometer. HPLC-UV-MS analyses, used to assess compound purity, were carried out combining the ion trap MS instrument with a Surveyor HPLC system (Thermo) equipped with an autosampler and UV6000 diode array detector (UV detection 215–400 nm). Instrument control, data acquisition and processing were performed by using Xcalibur 1.4 software (Thermo). HPLC chromatography was run at room temperature, and 1 mL min^{-1} flow rate, using a Phenomenex Gemini C18 column (4.6×50 mm; 3.0 μm). Mobile layer A was ammonium acetate 5 mM buffer (pH 5.5 with acetic acid): acetonitrile 95:5, and mobile layer B was ammonium acetate 5 mM buffer (pH 5.5 with acetic acid): acetonitrile 5:95; the gradient was from 0 to 100% B in 7 min then hold 100% B for 2 min before re-equilibration. Exact mass data ESI(+) were obtained on a Waters Q-Tof Ultima directly connected with micro HPLC 1100 Agilent as previously described.[28] Optical rotation measurements were performed on a Perkin–Elmer 241 polarimeter.

(*R*)-3-(1*H*-Indol-3-yl)-2-(4-oxo-4*H*-quinazolin-3-yl)-propionic acid methyl ester (6): To a solution of D-triphtophane methyl ester hydrochloride (2 g, 7.89 mmol) in dry DMF (100 mL) kept at 0°C, TBTU (3.8 g, 11 mmol) and DIPEA (5.40 mL, 32 mmol) were subsequently added under argon atmosphere. Anthranilic acid (1.62 g, 11 mmol) was then added portionwise in 1 h. After 5 h, the reaction mixture was poured into

water and extracted with AcOEt (2×100 mL). The combined organic layers were washed with brine (100 mL) and saturated $NaHCO_3$ (100 mL) then dried over Na_2SO_4, filtered and eventually concentrated in vacuo. The crude light yellow solid (2.4 g, 7.1 mmol) was dissolved under an inert atmosphere in AcOEt (65 mL) at room temperature and *p*-TsOH (120 mg, 0.63 mmol) and triethyl orthoformate (1.3 mL, 7.8 mmol) were added. The solution was warmed at 50 °C for 5 h. The reaction mixture was then washed with water (2×100 mL), dried over Na_2SO_4, filtered and eventually concentrated in vacuo. The crude light yellow solid was purified by flash chromatography (EtOAc/CH_2Cl_2 1:9–2:8) affording **6** (2.46 g, 7.1 mmol, 90% over two steps) (**6**). M.p. 98–101 °C; $[\alpha]_D^{20}$ = (c = 0.99, MeOH) = +375°; ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 3.61 (dd, ^2J(H,H) = 15.1, ^3J(H,H) = 5.4 Hz, 1H; C*H*H), 3.66 (dd, ^2J(H,H) = 15.1, ^3J(H,H) = 10.4 Hz, 1H; CH*H*), 3.73 (s, 3H, OCH_3), 5.53 (dd, ^3J(H,H) = 10.4, 5.4 Hz, 1H; C*H*CH_2), 6.84–6.90 (m, 1H; ArH of ring a), 6.97–7.03 (m, 2H; ArH of ring b and ArH of ring a), 7.26 (d, ^3J(H,H) = 8.1 Hz, 1H; ArH of ring a), 7.45 (d, ^3J(H,H) = 7.7 Hz, 1H; ArH of ring a), 7.51–7.55 (m, 1H; ArH of ring f), 7.55–7.58 (m, 1H; ArH of ring f), 7.80 (ddd, ^3J(H,H) = 8.3, 7.0, ^4J(H,H) = 1.6 Hz, 1H; ArH of ring f), 7.99 (s, 1H; ArH of ring e), 8.11 (ddd, ^3J(H,H) = 8.0, 1.6, ^4J(H,H) = 0.5 Hz, 1H; ArH of ring f), 10.78 ppm (brs, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 24.1 (CH_2), 52.5 ($COOCH_3$), 59.6 ($CHCH_2$), 108.5 (ArC of ring b), 111.7 (ArCH of ring a), 118.0 (ArCH of ring a), 118.6 (ArCH of ring a), 121.3 (ArC of ring f), 121.4 (ArCH of ring a), 124.0 (ArCH of ring b), 126.4 (ArCH of ring f), 126.8 (ArC of ring a), 127.3 (2×ArCH of ring f), 134.8 (ArCH of ring f), 136.2 (ArC of ring a), 147.2 (ArCH of ring e), 147.4 (ArC of ring f), 160.0 (NCO of ring e), 169.7 ppm ($COOCH_3$); HRMS (ESI+): m/z: calcd for $C_{20}H_{17}N_3O_3$ + H 348.1343, found 348.1328.

(*S*)-2-[(*R*)-3-(1*H*-Indol-3-yl)-2-(4-oxo-4*H*-quinazolin-3-yl)-proprionylamino]propionic acid methyl ester (7): To a solution of **6** (2 g, 5.7 mmol) in THF (77 mL), aqueous 0.2 N LiOH (28.5 mL, 5.7 mmol) was added. The reaction was stirred for 3 h at room temperature, then poured into water, neutralized with 1 N HCl (5.7 mL) and extracted with AcOEt (2× 100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo affording the product as a white solid (1.89 g, 5.7 mmol). The product thus obtained was dissolved in dry DMF (78 mL) at 0 °C. DhBtOH (1.022 g, 6.3 mmol), DIPEA (3.9 mL, 23 mmol) and L-alanine methyl ester (0.8 g, 5.7 mmol) were subsequently added under argon atmosphere. EDC·HCl (1.22 g, 6.3 mmol) was then added portionwise in 30 min. After 12 h, the reaction mixture was poured into water and extracted with CH_2Cl_2 (2×100 mL), saturated $NaHCO_3$ (2×100 mL), 0.3 N $KHSO_4$ (2×100 mL) and eventually with brine. The combined organic layers were filtered and concentrated in vacuo to give a light yellow solid which was purified through flash chromatography (AcOEt/CH_2Cl_2 5:95–20:80) affording **7** (2.14 g, 5 mmol, 90%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 1.33 (d, ^3J(H,H) = 7.3 Hz, 3H; C*H*$_3$CH), 3.51–3.58 (m, 2H; CH_2), 3.61 (s, 3H; OCH_3), 4.35 (dq, ^3J(H,H) = 7.3, 7.0 Hz, 1H; C*H*CH_3), 5.98- 6.00 (m, 1H; C*H*CH_2), 6.93–6.97 (m, 1H; ArH of ring a), 7.00–7.04 (m, 1H; ArH of ring a), 7.09 (d, ^3J(H,H) = 2.3 Hz, 1H; ArH of ring b), 7.24 (d, ^3J(H,H) = 7.9 Hz, 1H; ArH of ring a), 7.45–7.49 (m, 1H; ArH of ring f), 7.60 (d, ^3J(H,H) = 7.7 Hz, 1H; ArH of ring f), 7.74–7.80 (m, 2H; ArH of ring f and ArH of ring a), 8.01 (dd, ^3J(H,H) = 8.2, ^4J(H,H) = 1.3 Hz, 1H; ArH of ring f), 8.46 (s, 1H; ArH of ring e), 9.09 (d, ^3J(H,H) = 7.0 Hz, 1H; CONH), 10.73 ppm (brs, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 16.5 (CH_3CH), 26.4 (CH_2), 47.7 ($CHCH_3$), 51.9 (OCH_3), 55.0 ($CHCH_2$), 108.7 (ArC of ring b), 111.3 (ArCH of ring a), 118.5 (ArCH of ring a), 118.7 (ArCH of ring a), 121.1 (ArCH of ring a), 121.2 (ArC of ring f), 124.0 (ArCH of ring b), 126.1 (ArCH of ring f), 126.8 (2×ArCH of ring f), 127.1 (ArC of ring a), 134.5 (ArCH of rinf f), 136.1 (ArC of ring a), 146.7 (ArCH of ring e), 147.5 (ArC of ring f), 160.3 (NCO of ring e), 169.5 (CH*C*ONH), 173.2 ppm ($COOCH_3$); HRMS (ESI+): m/z: calcd for $C_{23}H_{22}N_4O_4$ + H 419.1714, found 419.1719.

(*S*)-2-[(*R*)-2-Oxo-3-(4-oxo-4*H*-quinazolin-3-yl)-2,3,4,9-tetrahydropyrido-[2,3-*b*]indol-1-yl]-propionic acid methyl ester (8): To a solution of **7** (0.820 g, 1.96 mmol) in dry CH_2Cl_2 (45 mL), kept at −78 °C and under argon atmosphere, Et_3N (1.09 mL, 7.84 mmol) and NCS (0.348 g, 2.61 mmol) were added. The reaction mixture was kept at −78 °C for 3 h then poured into water at 0 °C, extracted with CH_2Cl_2 (2×100 mL), and then washed with brine (3×50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo, delivering a light brown solid which was purified by flash chromatography (EtOAc/CH_2Cl_2 1:9–3:7). Product **8** (0.513 g, 1.235 mmol, 63%) was obtained along with side-product **9** (0.185 g, 0.412 mmol, 21%). **8**: m.p. 235–237 °C (decomp); $[\alpha]_D^{20}$ (c = 0.7, $CHCl_3$) = +24°; ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 1.59 (d, ^3J(H,H) = 6.9 Hz, 3H; C*H*$_3$CH), 3.34 (dd partially obscured by DMSO, ^2J(H,H) = 14.0, ^3J(H,H) = 8.2 Hz, 1H; C*H*H), 3.56 (t, ^2J(H,H) = 14.0, ^3J(H,H) = 14.0 Hz, 1H; CH*H*), 3.68 (s, 3H; OCH_3), 5.06 (q, ^3J-(H,H) = 6.9 Hz, 1H; C*H*CH_3), 5.70–5.75 (m, 1H; C*H*CH_2), 6.99–7.08 (m, 2H; ArH of ring a), 7.31–7.40 (m, 2H; ArH of ring a), 7.60 (t, ^3J(H,H) = 7.5 Hz, 1H; ArH of ring f), 7.74 (d, ^3J(H,H) = 8.2 Hz, 1H; ArH of ring f), 7.83–7.92 (m, 1H; ArH of ring f), 8.18 (d, ^3J(H,H) = 7.7 Hz, 1H; ArH of ring f), 8.35 (s, 1H; ArH of ring e), 11.42 ppm (brs, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 14.6 (CH_3CH), 21.6 (CH_2), 52.1 (OCH_3), 52.3 ($CHCH_3$), 56.1 ($CHCH_2$), 90.6 (ArC of ring b), 111.0 (ArCH of ring a), 116.5 (ArCH of ring a), 119.6 (2×ArCH of ring a), 121.7 (ArC of ring f), 125.9 (ArC of ring a), 126.0 (ArCH of ring f), 127.2 (2×ArCH of ring f), 133.6 (ArC of ring a), 134.6 (ArCH of ring f), 135.0 (ArC of ring b), 147.1 (ArCH of ring e), 147.8 (ArC of ring f), 160.3 (NCO of ring e), 166.2 (CH*C*ON of ring c), 170.6 ppm ($COOCH_3$); HRMS (ESI+): m/z: calcd for $C_{23}H_{20}N_4O_4$ + H 417.1558, found 417.1556.

Compound 9: ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 1.61 (d, ^3J-(H,H) = 6.9 Hz, 3H; C*H*$_3$CH), 2.67 (dd, ^2J(H,H) = 14.1, ^3J(H,H) = 10.7 Hz, 1H; C*H*H), 3.39 (dd, ^2J(H,H) = 14.1, ^3J(H,H) = 6.3 Hz, 1H; CH*H*), 3.61 (s, 3H; OCH_3), 5.57 (q, ^3J(H,H) = 6.9 Hz, 1H; C*H*CH_3), 5.67 (dd, ^3J(H,H) = 10.7, 6.3 Hz, 1H; C*H*CH_2), 7.25–7.30 (m, 1H; ArH of ring a), 7.43–7.50 (m, 2H; ArH of ring a), 7.55–7.58 (m, 1H; ArH of ring f), 7.62 (d, ^3J(H,H) = 7.4 Hz, 1H; ArH of ring a), 7.74 (d, ^3J(H,H) = 8.0 Hz, 1H; ArH of ring f), 7.87–7.90 (m, 1H; ArH of ring f), 8.06 (d, ^3J(H,H) = 7.8 Hz, 1H; ArH of ring f), 8.73 ppm (s, 1H; ArH of ring e); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 13.7 (CH_3CH), 31.5 (CH_2), 51.8 ($CHCH_3$), 52.2 (OCH_3), 56.0 ($CHCH_2$), 65.4 (CCl), 120.2 (ArCH of ring a), 121.4 (ArC of ring f), 123.2 (ArCH of ring a), 125.8 (ArCH of ring a), 126.3 (ArCH of ring f), 127.7 (2×ArCH of ring f), 131.4 (ArCH of ring a), 135.3 (ArCH of ring f), 136.0 (ArC of ring a), 147.8 (ArC of ring f), 148.6 (ArCH of ring e), 151.8 (ArC of ring a), 160.1 (NCO of ring e), 166.2 (CH*C*ON of ring c), 167.6 (ArC of ring b), 169.6 ppm ($COOCH_3$); HRMS (ESI+): m/z: calcd for $C_{23}H_{19}ClN_4O_4$ + H 451.1168, found 451.1157.

(*S*)-2-[(*R*)-3-(1*H*-Indol-3-yl)-2-(4-oxo-4*H*-quinazolin-3-yl)-propionylamino]propionic acid *tert*-butyl ester (10): The same procedure as for compound **7** has been followed, starting from **6** (1 g, 3 mmol). To a solution of **6** in dry DMF (40 mL), DhBtOH (717 mg, 4.4 mmol), DIPEA (2.24 mL, 13 mmol) and L-alanine *tert*-butyl ester hydrochloride (543 mg, 3 mmol) were added at 0 °C. EDC·HCl (843 mg, 4.4 mmol) was added portionwise to this solution in 40 min. The mixture was left under stirring at room temperature overnight. After dilution with AcOEt, the organic layer was washed with water, saturated solution of $NaHCO_3$, 0.3 N solution of $KHSO_4$ and brine. It was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude was purified by flash chromatography (Hexane/AcOEt 7:3) affording **10** as white solid (1.084 g, 2.35 mmol, 80%). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 1.30 (d, ^3J-(H,H) = 7.3 Hz, 3H; C*H*$_3$CH), 1.34 (s, 9H; *t*Bu), 3.50–3.62 (m, 2H; CH_2), 4.19 (dq, ^3J(H,H) = 7.3, 6.8 Hz, 1H; C*H*CH_3), 5.99 (dd, ^3J(H,H) = 10.2, 6.3 Hz, 1H; C*H*CH_2), 6.94–6.98 (m, 1H; ArH of ring a), 7.01–7.04 (m, 1H; ArH of ring a), 7.10 (d, ^3J(H,H) = 2.2 Hz, 1H; ArH of ring b), 7.24 (d, ^3J(H,H) = 8.1 Hz, 1H; ArH of ring a), 7.45–7.49 (m, 1H; ArH of ring f), 7.61 (d, ^3J(H,H) = 7.8 Hz, 1H; ArH of ring f), 7.77–7.81 (m, 2H; ArH of ring a and ArH of ring f), 8.00–8.03 (m, 1H; ArH of ring f), 8.49 (s, 1H; ArH of ring e), 9.02 (d, ^3J(H,H) = 6.8 Hz, 1H; CONHCH), 10.76 ppm (s, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 16.7 (CH_3CH), 26.3 (CH_2), 27.1 (*t*Bu), 48.6 ($CHCH_3$), 54.8 ($CHCH_2$), 80.7 (C-$(CH_3)_3$), 108.7 (ArC of ring b), 111.1 (ArCH of ring a), 118.4 (ArCH of ring a), 118.8 (ArCH of ring a), 121.1 (ArCH of ring a), 121.2 (ArC of ring f), 124.0 (ArCH of ring b), 126.1 (ArCH of ring f), 126.7 (2×ArCH of ring f), 127.0 (ArC of ring e), 134.4 (ArCH of ring f), 136.2 (ArC of ring e), 146.5 (ArCH of ring e), 147.5 (ArC of ring f), 160.3 (NCO), 169.2

(CHCONH), 171.7 ppm (COO*t*Bu); HRMS (ESI+): *m/z*: calcd for $C_{26}H_{28}N_4O_4$ +H 461.2184, found 461.219.

(*S*)-2-[(*R*)-2-Oxo-3-(4-oxo-4*H*-quinazolin-3-yl)-2,3,4,9-tetrahydropyrido-[2,3-*b*]indol-1-yl]-propionic acid *tert*-butyl ester (11): To a solution of **10** (350 mg, 0.76 mmol) and Et_3N (0.42 mL, 3.04 mmol) in dry CH_2Cl_2 (35 mL) at −78 °C under argon, NCS (135 mg, 1.01 mmol) was added. The mixture was kept at −78 °C and the reaction was monitored by HPLC. After 4.5 h further NCS was added (90 mg, 0.674 mmol) and the reaction was stirred at the same temperature for additional 2 h. The reaction was then quenched with water and let rise to room temperature overnight. The mixture was diluted with CH_2Cl_2 and washed twice with water, the organic layer was dried over Na_2SO_4 and the solvent was removed under vacuum. The crude was purified by flash chromatography (hexane/AcOEt 70:30–65:35) affording **11** (180 mg, 0.395 mmol, 52%) and **12** (50 mg, 0.101 mmol, 13%) as side-product. **11**: m.p. 176–178 °C; $[\alpha]_D^{20}$ (c = 0.9, MeOH) = +49.5°; ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 1.40 (s, 9H; *t*Bu), 1.51 (d, ^3J(H,H) = 7.0 Hz, 3H, CH_3CH), 3.31–3.35 (m obscured by water, 1H; CH*H*-α), 3.59 (t, ^2J(H,H) = 14.1, ^3J(H,H) = 14.1 Hz, 1H; C*H*H-β), 4.71 (q, ^3J(H,H) = 7.0 Hz, 1H; C*H*CH$_3$), 5.66 (brs, 1H; C*H*CH$_2$), 7.01–7.06 (m, 2H; ArH of ring a), 7.34–7.38 (m, 2H; ArH of ring a), 7.58–7.62 (m, 1H; ArH of ring f), 7.74 (d, ^3J(H,H) = 8.1 Hz, 1H; ArH of ring f), 7.87–7.90 (m, 1H; ArH of ring f), 8.16–8.20 (m, 1H; ArH of ring f), 8.30 (s, 1H; ArH of ring e), 11.44 ppm (s, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 14.4 (*C*H$_3$CH), 21.8 (CH$_2$), 27.1 (*t*Bu), 53.7 (*C*HCH$_3$), 55.3 (*C*HCH$_2$), 81.2 (*C*(CH$_3$)$_3$), 89.9 (ArC of ring b), 111.2 (ArCH of ring a), 116.7 (ArCH of ring a), 120.0 (2×ArCH of ring a), 121.9 (ArC of ring f), 126.1 (ArCH of ring f), 126.4 (ArC of ring a), 127.4 (2×ArCH of ring f), 133.4 (ArC of ring a), 134.8 (ArCH of ring f), 135.4 (ArC of ring b), 147.4 (ArCH of ring e), 147.7 (ArC of ring f), 160.0 (NCO), 165.6 (CH*C*ON), 168.9 ppm (*C*OO*t*Bu); HRMS (ESI+): *m/z*: calcd for $C_{26}H_{26}N_4O_4$ +H 459.2027, found 459.2018.

Compound 12: ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 1.34 (s, 9H, *t*Bu), 1.55 (d, ^3J(H,H) = 7.1 Hz, 3H; CH_3CH), 2.67 (dd, ^2J(H,H) = 14.1, ^3J(H,H) = 10.9 Hz, 1H; C*H*H-β), 3.39 (dd, ^2J(H,H) = 14.1, ^3J(H,H) = 6.5 Hz, 1H; CH*H*-α), 5.39 (q, ^3J(H,H) = 7.1 Hz, 1H; C*H*CH$_3$), 5.68 (dd, ^3J(H,H) = 10.9, 6.5 Hz, 1H; C*H*CH$_2$), 7.27–7.30 (m, 1H; ArH of ring a), 7.45–7.50 (m, 2H; ArH of ring a), 7.57 (t, ^3J(H,H) = 7.3 Hz, 1H; ArH of ring a), 7.63 (d, ^3J(H,H) = 7.3 Hz, 1H; ArH of ring a), 7.75 (d, ^3J(H,H) = 8.0 Hz; 1H; ArH of ring f), 7.90 (t, ^3J(H,H) = 7.3 Hz, 1H; ArH of ring f), 8.06 (d, ^3J(H,H) = 8.05 Hz, 1H; ArH of ring f), 8.74 ppm (s, 1H; ArH of ring e); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 13.9 (*C*H$_3$CH), 27.4 (*t*Bu), 31.8 (CH$_2$), 52.3 (*C*HCH$_3$), 56.2 (*C*HCH$_2$), 65.6 (CCl), 81.3 (*C*(CH$_3$)$_3$), 120.0 (ArCH of ring a), 121.6 (ArC of ring f), 123.2 (ArCH of ring a), 126.0 (ArCH of ring a), 126.2 (ArCH of ring f), 127.7 (ArCH of ring f), 128.0 (ArCH of ring f), 131.6 (ArCH of ring a), 135.5 (ArCH of ring f), 136.2 (ArC of ring a), 147.9 (ArC of ring f), 148.7 (ArCH of ring e), 151.9 (ArC of ring a), 160.3 (NCO of ring e), 166.6 (CH*C*ON of ring c), 167.0 (ArC of ring b), 168.1 ppm (*C*OO*t*Bu); HRMS (ESI+): *m/z*: calcd for $C_{26}H_{25}ClN_4O_4$ +H 493.1637, found 493.1635.

(*S*)-2-[(*R*)-2-Oxo-3-(4-oxo-4*H*-quinazolin-3-yl)-2,3,4,9-tetrahydropyrido-[2,3-*b*]indol-1-yl]-propionic acid (13): A solution of **11** (104 mg, 0.227 mmol) in TFA/CH_2Cl_2 (20%; 3 mL) was stirred at room temperature for 5 h. The solvent was then removed under reduced pressure and the residue was triturated with Et_2O affording **13** as a light yellow solid that was used in the next reaction, without any further purification (90 mg, 0.224 mmol, quantitative yield). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 1.58 (d, ^3J(H,H) = 7.1 Hz, 3H; CH_3CH), 3.32 (dd, ^2J(H,H) = 14.4, ^3J(H,H) = 7.8 Hz, 1H; CH*H*-α), 3.55 (dd, ^2J(H,H) = 14.4, ^3J(H,H) = 14.1 Hz, 1H; C*H*H-β), 5.02 (brs, 1H; C*H*CH$_3$), 5.74 (brs, 1H; C*H*CH$_2$), 7.00–7.06 (m, 2H; ArH of ring a), 7.33–7.39 (m, 2H; ArH of ring a), 7.58–7.61 (m, 1H; ArH of ring f), 7.74 (d, ^3J(H,H) = 8.0 Hz, 1H; ArH of ring f), 7.86–7.92 (m, 1H; ArH of ring f), 8.16–8.21 (m, 1H; ArH of ring f), 8.35 (s, 1H; ArH of ring e), 11.33 (brs, 1H; NH), 12.95 ppm (brs, 1H; COOH); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C and 100 MHz, [D$_6$]DMSO, 80 °C): δ = 14.2 (*C*H$_3$CH), 21.9 (CH$_2$), 52.3 (*C*HCH$_3$), 54.9 (*C*HCH$_2$), 90.3 (ArC of ring b), 110.9 (ArCH of ring a), 116.5 (ArCH of ring a), 119.6 (2×ArCH of ring a), 121.8 (ArC of ring f), 126.1 (ArCH of ring f), 126.2 (ArC of ring a), 127.1 (ArCH of ring f), 127.3 (ArCH of ring f), 133.7 (ArC of ring a), 134.6 (ArCH of ring f), 135.2 (ArC of ring b), 147.3 (ArCH of ring e), 147.9 (ArC of ring f), 160.6 (NCO of ring e), 166.2 (CH*C*ON), 171.7 ppm (COOH); HRMS (ESI+): *m/z*: calcd for $C_{22}H_{18}N_4O_4$+H 403.1401, found 403.1396.

(2*S*,4*R*)-2-Methyl-4-(4-oxo-4*H*-quinazolin-3-yl)-4,5-dihydro-2a,9b-diaza-cyclopenta[*jk*]fluorene-1,3-dione (14): To a solution of **13** (31 mg, 0.077 mmol) in dry CH_2Cl_2 (3 mL) under argon, DIPEA (0.052 mL, 0.309 mmol), oxalyl chloride (0.010 mL, 0.116 mmol) and catalytic DMF were added at room temperature. The mixture was stirred for 1 h, than it was diluted with CH_2Cl_2 and washed with water (3×10 mL). The organic layer was dried over Na_2SO_4 and taken to dryness under vacuum. The crude was purified by flash chromatography (CH_2Cl_2/acetone 9:1) yielding **14** as white solid (22 mg, 0.057 mmol, 75%). M.p. 256 °C; $[\alpha]_D^{20}$ (c = 0.63, CHCl$_3$) = + 27.6°; ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 1.69 (d, ^3J(H,H) = 7.2 Hz, 3H; CH_3CH), 3.29 (dd, ^2J(H,H) = 15.0, ^3J(H,H) = 11.2 Hz, 1H; C*H*H-β), 3.47 (dd, ^2J(H,H) = 15.0, ^3J(H,H) = 9.0 Hz, 1H; CH*H*-α), 5.06 (q, ^3J(H,H) = 7.2 Hz, 1H; C*H*CH$_3$), 5.61 (brs, 1H; C*H*CH$_2$), 7.20–7.23 (m, 1H; ArH of ring a), 7.30–7.33 (m, 1H; ArH of ring a), 7.43 (d, ^3J(H,H) = 7.6 Hz, 1H; ArH of ring a), 7.59–7.62 (m, 1H; ArH of ring f), 7.73–7.77 (m, 2H; ArH of ring a and ArH of ring f), 7.89–7.92 (m, 1H; ArH of ring f), 8.18 (d, ^3J(H,H) = 7.9 Hz, 1H; ArH of ring f), 8.57 ppm (s, 1H; ArH of ring e); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C and 100 MHz, [D$_6$]DMSO, 80 °C): δ = 15.7 (*C*H$_3$CH), 24.7 (CH$_2$), 57.9 (*C*HCH$_2$), 62.7 (*C*HCH$_3$), 85.6 (ArC of ring b), 113.6 (ArCH of ring a), 118.9 (ArCH of ring a), 121.8 (ArCH of ring a and ArC of ring f), 125.1 (ArCH of ring a), 126.5 (ArCH of ring f), 127.5 (2× ArCH of ring f), 128.8 (ArC of ring a), 134.1 (ArC of ring a), 135.0 (ArCH of ring f), 143.0 (NCN), 148.1 (ArCH of ring e and ArC of ring f), 160.1 (NCO of ring e), 163.5 (CH*C*ON), 166.7 ppm (NCO of ring d); HRMS (ESI+): *m/z*: calcd for $C_{22}H_{16}N_4O_3$ +H 385.1295, found 385.1296.

(2*S*,4*R*,5a*S*,9c*R*)-5a-Hydroxy-9c-methoxy-2-methyl-4-(4-oxo-4*H*-quinazolin-3-yl)-4,5,5a,9c-tetrahydro-2a,9b-diaza-cyclopenta[*jk*]fluorene-1,3-dione (16): To a solution of **14** (50 mg, 0.13 mmol) in CH_2Cl_2/MeOH 2:1 (0.5:0.25 mL) and (±)-trans-2-(phenylsulfonyl)-3-phenyloxaziridine **15** (0.373 g, 0.143 mmol) was added. The resulting mixture was stirred at room temperature for 2 h. After removal of the solvent under reduced pressure, the mixture was purified by flash chromatography on silica gel (CH_2Cl_2/acetone 8:2) giving **16** (28.1 mg, 0.065 mmol, 50%) as a white solid. M.p. 163–164 °C; $[\alpha]_D^{20}$ (c = 0.77, CHCl$_3$) = −30.1°; ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 1.61 (brs, 3H; CH_3CH), 2.49–2.57 (m obscured by DMSO, 1H; CH*H*-α), 2.97–3.09 (brs, 1H; C*H*H-β), 3.49 (s, 3H; OCH$_3$), 4.87 (q, ^3J(H,H) = 6.9 Hz, 1H; C*H*CH$_3$), 6.07 (brs, 1H; C*H*CH$_2$), 6.66 (s, 1H; OH), 7.24–7.30 (m, 1H; ArH of ring a), 7.41–7.45 (m, 1H; ArH of ring a), 7.45–7.48 (m, 1H; ArH of ring a), 7.50–7.53 (m, 1H; ArH of ring a), 7.55–7.63 (brs, 1H; ArH of ring f), 7.69 (d, ^3J(H,H) = 7.9 Hz, 1H; ArH of ring f), 7.84–7.90 (m, 1H; ArH of ring f), 8.19 ppm (brs, 2H; ArH of ring f and ArH of ring e); ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80 °C): δ = 1.63 (d, ^3J(H,H) = 6.8 Hz, 3H; CH_3CH), 2.51–2.55 (dd partially obscured by DMSO, ^2J(H,H) = 12.7, ^3J(H,H) = 3.2 Hz, 1H; CH*H*-α), 3.01 (t partially obscured by water, ^2J(H,H) = 12.7, ^3J(H,H) = 12.7, 1H; C*H*H-β), 3.52 (s, 3H; OCH$_3$), 4.81 (q, ^3J(H,H) = 6.8 Hz, 1H; C*H*CH$_3$), 5.84 (brs, 1H; C*H*CH$_2$), 6.37 (brs, 1H; OH), 7.24–7.30 (m, 1H; ArH of ring a), 7.41–7.45 (m, 1H; ArH of ring a), 7.45–7.48 (m, 1H; ArH of ring a), 7.50–7.53 (m, 1H; ArH of ring a), 7.55–7.63 (m, 1H; ArH of ring f), 7.69 (d, ^3J(H,H) = 8.2 Hz, 1H; ArH of ring f), 7.84–7.90 (m, 1H; ArH of ring f), 8.17 (d, ^3J(H,H) = 7.7 Hz, 1H; ArH of ring f), 8.25 ppm (s, 1H; ArH of ring e); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 13.9 (*C*H$_3$CH), 39.5 (CH$_2$), 51.5 (OCH$_3$), 59.7 (*C*HCH$_3$), 77.7 (C-OH), 105.2 (NCN), 114.2 (ArCH of ring a), 120.9 (ArC of ring f), 124.7 (ArCH of ring a), 125.6 (ArCH of ring a), 126.5 (ArCH of ring f), 127.4 (2×ArCH of ring f), 130.1 (ArCH of ring a), 134.8 (ArCH of ring f), 136.1 (2×ArC of ring a), 146.2 (ArCH of ring e), 147.3 (ArC of ring f), 160.0 (NCO of ring e), 167.1 (CH*C*ON), 171.6 ppm (NCO of ring d); *C*HCH$_2$ not seen because the ^{1}H signal is too broad to detect the ^{1}H-^{13}C correlations in gHSQC and gHMBC spectra; HRMS (ESI+): *m/z*: calcd for $C_{23}H_{20}N_4O_5$+H 433.1507, found 433.1509.

(−)-Chaetominine (1): To a solution of **16** (20 mg, 0.0463 mmol) in CH_2Cl_2/TFA 2:1 (0.4:0.2 mL) Et_3SiH (0.011 mL, 0.069 mmol) was added. The resulting mixture was stirred at room temperature for 2 h. After removal of the solvent under reduced pressure, the crude material was treated with HF (49% wt. in H_2O)/CH_3CN at room temperature overnight to deprotect TES-derived chaetominine[16,17] formed in a variable amount under reaction conditions. The reaction mixture was then diluted with AcOEt, washed with saturated $NaHCO_3$ solution and brine, the organic layer dried over Na_2SO_4 and concentrated. The crude residue was purified through flash chromatography on silica gel (CH_2Cl_2/acetone 8:2). **1** was recovered as a white solid (12 mg, 0.03 mmol, 65%). M.p. 160–162°C; $[\alpha]_D^{20}$ (c=0.25, MeOH) = −47.9° in agreement with literature data;[16,17] ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25°C): δ = 1.60 (d, ^3J(H,H) = 6.4 Hz, 3H; CH_3CH), 2.49–2.55 (m obscured by DMSO, 1H; CHH-α), 2.93 (t, ^2J(H,H) = 12.8, ^3J(H,H) = 12.8 Hz, 1H; CHH-β), 4.61 (q, ^3J(H,H) = 6.9 Hz, 1H; CHCH$_3$), 5.60 (s, 1H; NCHN), 5.92 (brs, 1H; CHCH$_2$), 6.69 (brs, 1H; OH), 7.22–7.28 (m, 1H; ArH of ring a), 7.41–7.44 (m, 1H; ArH of ring a), 7.49 (d, ^3J(H,H) = 7.8 Hz, 1H; ArH of ring a), 7.50 (d, ^3J(H,H) = 8.0 Hz, 1H; ArH of ring a), 7.58 (t, ^3J(H,H) = 7.5 Hz, 1H; ArH of ring f), 7.69 (d, ^3J(H,H) = 8.1 Hz, 1H; ArH of ring f), 7.83–7.89 (m, 1H; ArH of ring f), 8.19 (brs, 1H; ArH of ring f), 8.28 ppm (brs, 1H; ArH of ring e); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 50°C): δ = 1.60 (d, ^3J(H,H) = 7.0 Hz, 3H; CH_3CH), 2.49–2.55 (m obscured by DMSO, 1H; CHH-α), 2.93 (t, ^2J(H,H) = 12.8, ^3J(H,H) = 12.8 Hz, 1H; CHH-β), 4.61 (q, ^3J(H,H) = 7.0 Hz, 1H; CHCH$_3$), 5.60 (s, 1H; NCHN), 5.78 (brs, 1H; CHCH$_2$), 6.59 (brs, 1H; OH), 7.22–7.28 (m, 1H; ArH of ring a), 7.41–7.44 (m, 1H; ArH of ring a), 7.49 (d, ^3J(H,H) = 7.1 Hz, 1H; ArH of ring a), 7.50 (d, ^3J(H,H) = 8.0 Hz, 1H; ArH of ring a), 7.56–7.59 (m, 1H; ArH of ring f), 7.69 (d, ^3J(H,H) = 8.1 Hz, 1H; ArH of ring f), 7.83–7.89 (m, 1H; ArH of ring f), 8.19 (d, ^3J(H,H) = 7.6 Hz, 1H; ArH of ring f), 8.26 ppm (brs, 1H; ArH of ring e); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 50°C): δ = 13.4 (*C*H$_3$CH), 37.8 (CH$_2$), 59.4 (*C*HCH$_3$), 76.0 (C-OH), 82.3 (NCHN), 114.2 (ArCH of ring a), 121.2 (ArC of ring f), 124.7 (ArCH of ring a), 125.3 (ArCH of ring a), 126.3 (ArCH of ring f), 126.9 (2×ArCH of ring f), 129.5 (ArCH of ring a), 134.5 (ArCH of ring f), 136.5 (ArC of ring a), 138.6 (ArC of ring a), 147.0 (ArC of ring f), 160.0 (NCO of ring e), 165.2 (CH*C*ON), 172.0 ppm (NCO of ring d); *C*HCH$_2$ and ArCH of ring e not seen because the ^{1}H signal is too broad to detect the ^{1}H-^{13}C correlations in gHSQC and gHMBC spectra; HRMS (ESI+): m/z: calcd for $C_{22}H_{18}N_4O_4$+H 403.1401, found 403.1402.

Antiproliferative assay: K-562 human myelogenous leukemia, A2780 human ovarian and MCF7 human breast cancer cells (1250 cells/well) were seeded in white 384well-plates in complete medium (RPMI1640 or EMEM plus 10% fetal bovine serum) and treated with **1** dissolved in 0.1% DMSO, 24 h after seeding. The cells were then incubated at 37°C in the presence of 5% CO_2. At the end of treatment time the plates were processed using CellTiter-Glo assay (Promega) following the manufacturer's instruction. CellTiter-Glo is a homogenous method based on the quantification of the ATP present, an indicator of metabolitically active cells. ATP is quantified using a system based on luciferase and D-luciferin resulting into light generation. The luminescent signal is proportional to the number of cells present in culture.

Briefly, 25 μL per well reagent solution are added to each well and, after 5 min shacking, microplates are read by a luminometer. The luminescent signal is proportional to the number of cells present in culture. Inhibitory activity was evaluated comparing treated versus control data, using Assay Explorer (MDL) program. IC_{50} was calculated using sigmoidal interpolation curve.

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