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Total synthesis of (±)-auranthine confirmed its refined structure†

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Auranthine, isolated in 1986 from *Penicillium aurantiogriseum*, is a fungal benzodiazepine. Through the successful total synthesis of (±)-auranthine, we confirmed the refined structure of natural (−)-auranthine. We established that natural (−)-auranthine is a fused quinazolino benzodiazepine dione **1** featuring an acyclic aliphatic nitrile moiety, thereby disproving the proposed structure **2**.

Introduction

Auranthine (**1**) is a fungal benzodiazepine first isolated in 1986 from a plant pathogen *Penicillium aurantiogriseum*.[1] The analysis of the ^{1}H-NMR, ^{13}C-NMR, and mass spectra led to the proposal of the initial structure of auranthine as structure **2** (Fig. 1).[1] No absolute configuration of auranthine **2** has been reported at this point. It was suggested that auranthine **2** is naturally produced by the fungus in a condensation reaction between two molecules of anthranilic acid and a molecule of glutamine.[2] Since benzodiazepines are known to have a diverse spectrum of biological activities and are often used as therapeutic agents,[3] auranthine, a newly isolated benzodiazepine with a rather unusual structure **2**, has attracted the attention of synthetic chemists.

In 2002, the research group of Bergman attempted the synthesis of auranthine **2**.[4] Despite not achieving the desired auranthine **2**, the team successfully synthesized compound **3**, a structurally relevant *C*-acetyl derivative of auranthine **2**.[4] Later, in 2010, a group led by Argade, starting from Cbz-protected glutamic anhydride and Boc-protected *o*-aminobenzyl amine, successfully accomplished the first total synthesis of proposed racemic auranthine **2** over seven synthetic steps.[5] The authors achieved explicit confirmation of the structure of the synthesized racemic auranthine **2** through a combination of NMR analysis and X-ray diffraction. Nevertheless, the ^{1}H-NMR spectrum of the synthesized compound failed to match the spectral profile of the natural auranthine isolated back in 1986.[5] This observation raised significant doubts regarding the accuracy of the initially proposed structure of auranthine (**2**).

To verify this, in 2018, we chemically modified natural auranthine to obtain its reduced derivative **4** (Fig. 1).[6] The chemical modification of auranthine was required to obtain a derivative capable of producing X-ray-diffractable crystals as natural auranthine failed to give suitable crystals. The X-ray crystallography data of semi-synthetic derivative **4** revealed that its structure significantly differs from the originally proposed structure of auranthine **2**. Based on the confirmed structure of **4** and subsequently performed detailed analysis of natural auranthine using NMR, mass, and CD spectroscopy as well as DFT calculations, we refined the original structure of auranthine **2** and suggested that auranthine is a fused quinazolino benzodiazepinedione **1** bearing an acyclic aliphatic nitrile moiety (Fig. 1).[6] The refined auranthine (**1**) shares structural similarity with other known fungal secondary metabolites such as auranomide C (**5**),[7] asperlicin (**6**),[8,9] sclerotigenin (**7**),[10] and benzomalvin A (**8**)[11] (Fig. 1), total synthesis of which has also been reported.[12–15]

Although we have presented substantial evidence proving the proposed structure of auranthine (**1**), it is important to acknowledge that these findings, despite their strength, do not provide absolute certainty. Of all the spectroscopic data at our disposal, the X-ray crystal structure of derivative **4** emerged as

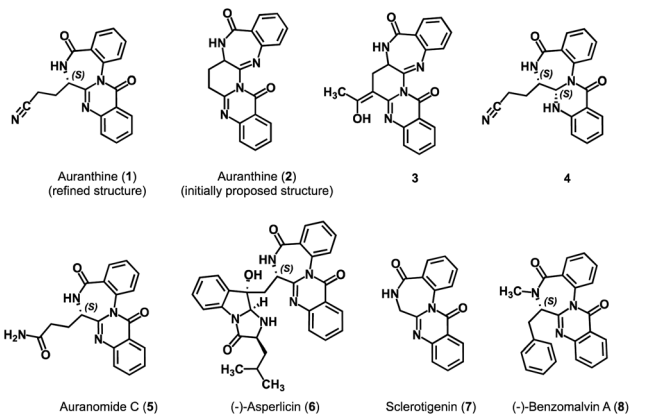

Fig. 1 Refined structure of auranthine (**1**), initially proposed structure of auranthine (**2**), (semi)synthetic derivatives of auranthine **3** and **4** as well as structurally related natural products **5**–**8**.

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the most convincing and definitive piece of evidence supporting the refined structure of auranthine (**1**). Nevertheless, compound **4** was obtained by the treatment of natural auranthine with a reducing agent $NaBH_3CN$ comprising a nitrile moiety. Thus, there is a small possibility that the nitrile group in derivative **4** may have been derived from the reducing agent rather than intrinsic to the original structure of auranthine (**1**). To unambiguously establish the accuracy of the refined structure of auranthine (**1**), it was necessary to perform its total synthesis. We herein report the total synthesis of racemic auranthine (**1**).

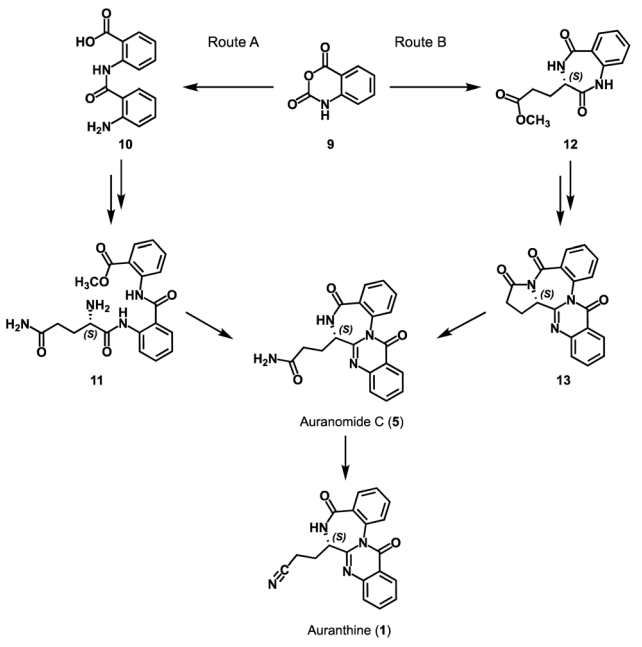
Results and discussion

To confirm the refined structure of auranthine (**1**), its total synthesis should be performed. Since auranthine (**1**) and another fungal metabolite auranomide C (**5**) differ only in the structure of the aliphatic side chain (amide moiety *vs.* nitrile moiety, Fig. 1), we decided that auranthine (**1**) could be synthetically accessed *via* a dehydration of the amide moiety of auranomide C (**5**). For this purpose, at first, auranomide C (**5**) is to be synthesized following one of the suggested routes A or B, both starting from a common precursor – isatoic anhydride (**9**) (Scheme 1).

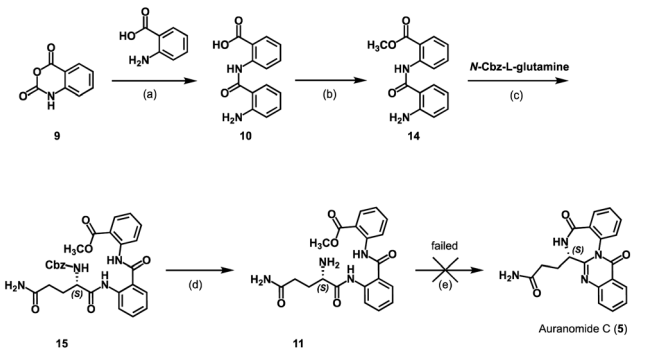
Rote A is inspired by the reported synthesis of structurally related quinazolino[3,2-*a*]benzodiazepinediones including natural products sclerotigenin (**7**) and asperlicin C but not auranomide C (**5**).[13] In this synthetic route, isatoic anhydride (**9**) is converted into amide **10** consisting of two anthranilic acid moieties, which then is transformed into the anthranilate-containing tripeptide **11**, subsequent double dehydrocyclization of which should yield auranomide C (**5**) (Scheme 1).[13] Alternatively, auranomide C (**5**) can be accessed following synthetic route B. Here, key intermediate benzodiazepine **12** over several steps is converted into quinazolinobenzodiazepine **13**, followed by its lactam ring opening to afford auranomide C (**5**) as previously reported.[14]

At first, we attempted to obtain auranthine (**1**) *via* auranomide C (**5**) following synthetic rout A (Scheme 2). For this, isatoic anhydride (**9**) was refluxed with anthranilic acid in water to afford bis(anthranilic acid) **10**, which was then esterified yielding corresponding methyl ester **14**.[13] Subsequently performed amide coupling between **14** and *N*-Cbz-L-glutamine afforded anthranilate-containing tripeptide **15** in 62% yield. The hydrogenolytic Cbz-group removal from **15** allowed for almost quantitative formation of the key intermediate **11** possessing the primary amino group (Scheme 2). It was anticipated that by applying the literature-described procedure,[13] intermediate **11** should undergo two intramolecular cyclocondensation reactions forming the 4-quinazolinone scaffold fused with the 1,4-benzodiazepine moiety to afford auranomide C (**5**). This microwave-assisted reaction was shown to be promoted by metal triflates, which being Lewis acids presumably increase the electrophilicity of the carbonyl C-atoms involved in the corresponding cyclization steps.[13] Nevertheless, despite our numerous efforts, under conditions identical to those previously described (microwave irrad. at 140 °C in DMF with $Sn(OTf)_2$),[13] compound **11** failed to produce auranomide C (**5**). Additional efforts to optimize the reaction, such as changing the reaction time, temperature, solvent and Lewis acid, also proved to be fruitless. Presumably, the reaction was incompatible with substrate **11** comprising an additional amide moiety, which in the presence of Lewis acids underwent side intramolecular reactions.

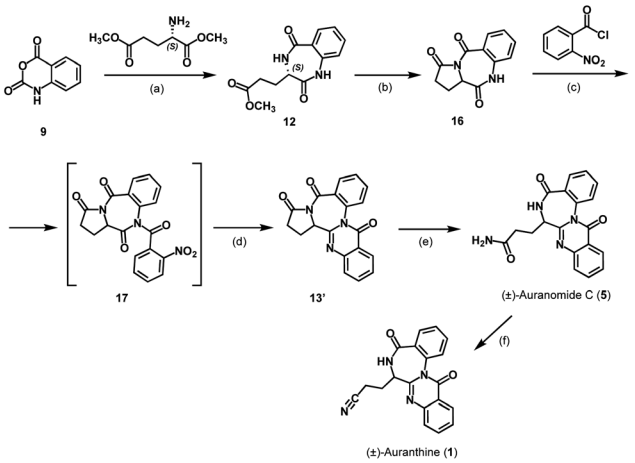
Next, we attempted the synthesis of auranomide C (**5**) following previously reported route B (Scheme 3).[14] For this, isatoic anhydride (**9**) was reacted with dimethyl L-glutamate to undergo two sequential addition–elimination reactions affording the cyclization product **12** exhibiting a 1,4-benzodiazepine-2,5-dione scaffold. It has been reported that derivatives of compound **12** exhibiting a flexible side chain are prone to racemization under reaction conditions used for the 1,4-benzodiazepine-2,5-dione scaffold annulation, making them



Scheme 1 Planned synthetic routes (A and B) towards auranthine (**1**).



Scheme 2 Attempted synthesis of auranthine (**1**): (a) H_2O, 2 h, reflux, 76%; (b) CH_3OH, H_2SO_4, 96 h, reflux, 69%; (c) DCM, EDCI, DMAP, r.t., 18 h, 62%; (d) THF/CH_3OH (4 : 1), $Pd(OH)_2/C$, H_2, r.t., 3 h, 95%; (e) DMF, $Sn(OTf)_2$, μ-wave (30 W), 140 °C, 15 min.



Scheme 3 Synthesis of racemic auranthine (**1**): (a) pyridine, 120 °C, 16 h, 17%; (b) DMA, 180 °C, 20 h, 61%; (c) DCM, DMAP, Et_3N, 0 °C, 0.5 h; (d) DCM, Zn, AcOH, −20 °C to r.t., 3 h, 25% over two steps; (e) THF-NH_3 (0.5 M), 50 °C, 18 h, 76%; (f) DMF, pyridine, TsCl, r.t., 24 h, 34%.

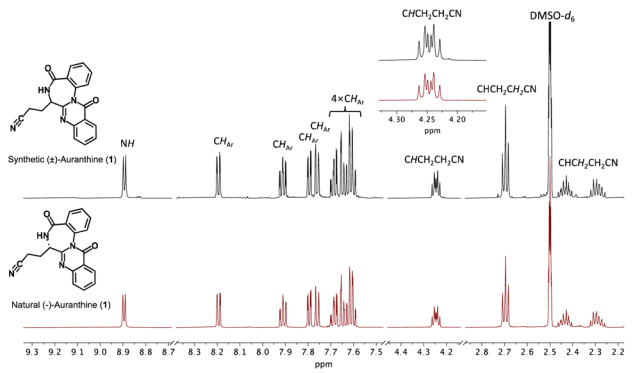


Fig. 2 Comparison of ^{1}H-NMR spectra in DMSO-d_6 of synthetic (upper spectrum, black) and natural auranthine (lower spectrum, red). The fragment of ^{1}H-NMR spectra with assigned proton signals is given.

problematic substrates for enantioselective synthesis. In contrast, under otherwise similar reaction conditions, tricyclic benzodiazepines like compound **16** are reported to retain their chiral information.[14,16] Therefore, the synthesis was continued in the direction of the tricyclic intermediate **16** reported earlier,[14] which in our hands, however, was obtained as a racemic mixture.

This defined the subsequent intermediates and the final product as racemates and the synthesis as non-stereospecific (Scheme 3). While this was somewhat disappointing, it wasn't a critical setback because the primary aim of this synthesis was to verify the structure of the refined auranthine (**1**), rather than to establish a new synthetic pathway. The physicochemical properties of synthetic (±)-auranthine, such as ^{1}H-NMR, ^{13}C-NMR, and IR spectra, are expected to be indistinguishable from those of natural (−)-auranthine. The alignment of these spectral characteristics will ultimately allow to confirm the structure of natural (−)-auranthine. We, therefore, proceeded with the racemate of **16**. In the next synthetic steps, tricyclic

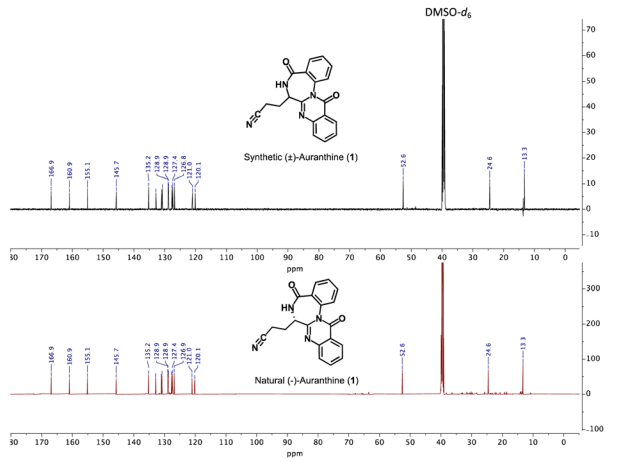


Fig. 3 Comparison of ^{13}C-NMR spectra in DMSO-d_6 of synthetic (upper spectrum, black) and natural auranthine (lower spectrum, red).

intermediate **16** was acylated with 2-nitrobenzoyl chloride to afford **17**, the nitro group of which was then reduced to facilitate further intramolecular cyclization reaction yielding quinazolinobenzodiazepine **13′**. The lactam ring opening of **13′** with ammonia yielded (±)-auranomide C (**5**) as reported previously.[14] Finally, (±)-auranomide C (**5**) was treated with *p*-toluenesulfonyl chloride in a DMF/pyridine mixture to dehydrate its primary amide group and produce desired (±)-auranthine (**1**) in 34% yield with 95% purity. To our knowledge, this reaction has not been documented in the literature for auranomide C (**5**).

Now, having synthetic (±)-auranthine in hands, we recorded its spectral characteristics to compare them with a sample of natural (−)-auranthine isolated from *Penicillium aurantiogriseum* as we reported previously.[6] Recorded ^{1}H-NMR spectra (DMSO-d_6) of synthetic (upper spectrum) and natural auranthine (lower spectrum) appeared to be nearly identical (Fig. 2). Both ^{1}H-NMR spectra feature characteristic proton signals of structure **1**, which include one amide proton at 8.89 ppm, eight aromatic protons between 7.58 and 8.20 ppm, one methine proton at the asymmetric C-atom (4.25 ppm), and three signals of two methylene groups of propanenitrile moiety at 2.70 ppm, 2.44 ppm, and 2.29 ppm (Fig. 2).

Similarly, the ^{13}C-NMR spectra (DMSO-d_6) of synthetic and natural auranthine appeared to be identical featuring characteristic signals, among which the C-atom of the –CN moiety appeared in both spectra at 120.1 ppm (Fig. 3). Also, the IR spectra of synthetic and natural auranthine matched exhibiting, for instance, the absorption band of the –CN moiety at 2249.00 cm^{-1} in both spectra (ESI†). Considering the identical spectral characteristics of synthetic and natural auranthine, we concluded that the synthesis of racemic auranthine was successful, and, more importantly, that the structure of natural auranthine (**1**) was correctly refined by us previously.[6]

Conclusions

In summary, we successfully accomplished the total synthesis of (±)-auranthine **1**, thus enabling us to unambiguously

confirm the refined structure of natural (−)-auranthine isolated from *Penicillium aurantiogriseum*. It is now established that natural (−)-auranthine is indeed a fused quinazolino benzodiazepinedione **1** with an acyclic aliphatic nitrile moiety, dispelling the previously proposed structure (compound **2**) from 1986. This finding allows for further investigations into the biosynthetic pathway of auranthine. Additionally, the reported synthesis will facilitate the preparation of synthetic analogs of auranthine for the purpose of studying their biological activity.

Experimental

General experimental procedures

THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (TLC): silica gel 60 F_{254} plates (Merck). Flash chromatography (FC): silica gel 60, 40–63 μm (Macherey-Nagel). Automatic flash column chromatography: Isolera One (Biotage); brackets include eluent, cartridge-type. Melting point (m.p.): melting point apparatus SMP 3 (Stuart Scientific), uncorrected. ^{1}H NMR (400 MHz), ^{1}H NMR (600 MHz), and ^{13}C NMR (151 MHz): Agilent DD2 400 and 600 MHz spectrometers; chemical shifts (δ) are reported in ppm against the reference substance TMS and calculated using the solvent residual peak of the undeuterated solvent. IR: IR Prestige-21 (Shimadzu). HRMS: MicrOTOF-QII (Bruker). The microwave irradiation experiments: single-mode cavity system CEM Discover LabMate (CEM Corporation, NC). HPLC method to determine the purity of compounds: equipment 1: pump: L-7100, degasser: L-7614, autosampler: L-7200, UV detector: L-7400, interface: D-7000, data transfer: D-line, data acquisition: HSMS software (all from LaChrom, Merck Hitachi); equipment 2: pump: LPG-3400SD, degasser: DG-1210, autosampler: ACC-3000T, UV detector: VWD-3400RS, interface: Dionex UltiMate 3000, data acquisition: Chromeleon 7 (Thermo Fisher Scientific); column: LiChrospher 60 RP-select B (5 μm), LiChroCART 250–4 mm cartridge; flow rate: 1.0 mL min^{-1}; injection volume: 5.0 μL; detection at λ = 210 nm; solvents: (A) demineralized water with 0.05% (v/v) trifluoroacetic acid, (B) acetonitrile with 0.05% (v/v) trifluoroacetic acid; gradient elution (% A): 0–4 min: 90%; 4–29 min: gradient from 90 to 0%; 29–31 min: 0%; 31–31.5 min: gradient from 0 to 90%; 31.5–40 min: 90%.

2-(2-Aminobenzamido)benzoic acid (10)

Synthesis was performed as previously reported.[13] Isatoic anhydride (2.51 g, 15.4 mmol) and anthranilic acid (2.30 g, 16.8 mmol) were dissolved in H_2O (50 mL) and stirred under reflux for 2 h. After cooling to r.t., the precipitate was filtered off, washed with H_2O und dried under reduced pressure. Product **10** was obtained as an off white solid (2.98 g, 11.6 mmol, 76%). M.p.: 203 °C. TLC: R_f = 0.66 (EtOAc). ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.92 (s, 1H), 8.65 (dd, J = 8.5/1.2 Hz, 1H), 8.04 (dd, J = 7.9/1.7 Hz, 1H), 7.63 (ddd, J = 8.8/7.3/1.7 Hz, 1H), 7.60 (dd, J = 8.1/1.5 Hz, 1H), 7.24 (ddd, J = 8.4/7.0/1.5 Hz, 1H), 7.17 (ddd, J = 8.2/7.4/1.2 Hz, 1H), 6.80 (dd, J = 8.4/1.2 Hz, 1H), 6.62 (ddd, J = 8.1/7.0/1.2 Hz, 1H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ (ppm) = 170.0, 167.4, 150.6, 141.4, 134.2, 132.8, 131.2, 127.3, 122.5, 119.9, 117.1, 116.3, 115.2, 114.0. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1600, 1649, 2980, 3356, 3468. HRMS (APCI): m/z = 257.0906, calcd 257.0921 for $C_{14}H_{13}N_2O_3$ $[M + H]^+$. HPLC: t_R = 16.55 min; purity: 95.3%.

Methyl 2-(2-aminobenzoylamino)benzoate (14)

Synthesis was performed as previously reported.[13] Compound **10** (2.82 g, 11.00 mmol) was dissolved in MeOH (50 mL), conc. sulfuric acid (3.20 mL) was added, and the reaction mixture was stirred at reflux for 96 h. The solvent was removed, the residue was dissolved in H_2O (20 mL), the pH was adjusted to 8 using 3 M NaOH upon cooling. The resulting mixture was extracted with DCM, the combined organic layers were dried over Na_2SO_4, and the solvent was removed *in vacuo*. After purification with flash column chromatography (cyclohexane/EtOAc = 100/0 → 50/50), product **14** was obtained as an off-white solid (2.04 g, 7.55 mmol, 69%). M.p.: 114 °C. TLC: R_f = 0.33 (cyclohexane/EtOAc = 9/1). ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 11.39 (s, 1H), 8.52 (dd, J = 8.5/1.2 Hz, 1H), 8.00 (dd, J = 8.0/1.6 Hz, 1H), 7.68–7.64 (m, 1H) 7.64–7.60 (m, 1H), 7.25 (ddd, J = 8.1/6.9/1.3 Hz, 1H), 7.23–7.18 (m, 1H), 6.80 (dd, J = 8.3/1.2 Hz, 1H), 6.65 (ddd, J = 8.1/7.0/1.2 Hz, 1H), 6.56 (s, 2H), 3.88 (s, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6): δ (ppm) = 168.1, 167.4, 150.5, 140.6, 134.2, 132.8, 130.7, 127.4, 122.9, 120.7, 117.1, 116.8, 115.2, 113.9, 52.6. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1655, 1682, 3337, 3474. HRMS (APCI): m/z = 271.1077, calcd 271.1064 for $C_{15}H_{15}N_2O_3$ $[M + H]^+$. HPLC: t_R = 19.58 min; purity: 98.2%.

Methyl (*S*)-2-(2-(5-amino-2-(((benzyloxy)carbonyl)amino)-5-oxopentanamido)benzamido)benzoate (15)

To a solution of **14** (3.51 g, 13.0 mmol) in DCM (50 mL), DMAP (3.17 g, 26 mmol), EDCI (4.98 g, 26 mmol), and *N*-carbobenzoxy-L-glutamine (5.46 g, 19.5 mmol) was added. The reaction mixture was stirred at r.t. under N_2-atmosphere for 18 h. The resulting precipitate was filtrated off and washed with 1 M HCl and H_2O and dried under reduced pressure. The filtrate was extracted (3×) with DCM and 1 M HCl, the combined organic layers were dried over Na_2SO_4, and the solvent evaporated *in vacuo*. The residue was purified twice by flash chromatography (cyclohexane/EtOAc = 50/50 → 0/100 and DCM/MeOH = 70/30) and combined with the solids. The product **15** was obtained as a white solid (4.28 g, 8.0 mmol, 62%). M.p.: 207 °C. TLC: R_f = 0.56 (EtOAc). ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.41 (s, 1H), 11.25 (s, 1H), 8.41 (dd, J = 8.4/1.2 Hz, 1H), 8.35 (dd, J = 8.4/1.2 Hz, 1H), 8.00 (dd, J = 7.9/1.6 Hz, 1H), 7.96 (d, J = 7.0 Hz, 1H), 7.90 (dd, J = 8.0/1.6 Hz, 1H), 7.61 (ddd, J = 8.7/7.3/1.5 Hz, 1H), 7.54 (ddd, J = 8.6/7.4/1.7 Hz, 1H), 7.38–7.28 (m, 6H), 7.28–7.24 (m, 2H), 6.76 (s, 1H), 5.01 (d, J = 12.5 Hz, 1H), 4.93 (d, J = 12.5 Hz, 1H), 4.02 (ddd, J = 9.7/7.0/4.7 Hz, 1H), 3.86 (s, 3H), 2.23–2.14 (m, 2H), 2.12–2.02 (m, 1H), 1.86–1.71 (m, 1H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ (ppm) = 173.5, 171.0, 167.7, 166.7, 156.2, 139.3, 138.4, 136.7, 133.9, 132.7, 130.7, 128.3 (2C), 127.82, 127.77, 127.7 (2C), 124.0, 123.6, 122.3, 121.9, 121.2, 118.6, 65.8, 56.0, 52.6, 31.4, 26.7. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1657,

1695, 3289, 3412. HRMS (APCI): m/z = 533.2042, calcd 533.2031 for $C_{28}H_{29}N_4O_7$ $[M + H]^+$. HPLC: t_R = 19.88 min; purity: 95.3%.

Methyl (*S*)-2-(2-(2,5-diamino-5-oxopentanamido)benzamido) benzoate (11)

Compound **15** (1.00 g, 1.88 mmol) and $Pd(OH)_2/C$ (0.15 g, 15 wt%) were dissolved in THF/MeOH (4/1, 50 mL) under N_2-atmosphere. H_2-atmosphere was applied, and the reaction mixture stirred for 3 h at r.t. The solution was then filtered through Celite washing with MeOH. The filtrate was evaporated under reduced pressure to obtain product **11** as a white solid (0.71 g, 1.79 mmol, 95%). M.p.: 98–99 °C. TLC: R_f = 0.36 (DCM/MeOH = 9.5/0.5). ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.35 (s, 1H), 8.52 (dd, J = 8.4/1.2 Hz, 1H), 8.39 (dd, J = 8.3/1.2 Hz, 1H), 7.99 (dd, J = 7.9/1.6 Hz, 1H), 7.85 (dd, J = 7.8/1.6 Hz, 1H), 7.70 (ddd, J = 8.2/7.3/1.7 Hz, 1H), 7.58 (ddd, J = 8.6/7.3/1.5 Hz, 1H), 7.31–7.23 (m, 3H), 6.72 (s, 1H), 3.86 (s, 3H), 3.33–3.28 (m, 1H), 2.23–2.10 (m, 2H), 2.00–1.93 (m, 1H), 1.67 (dtd, J = 13.6/8.4/6.3 Hz, 1H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ (ppm) = 174.4, 174.1, 167.7, 166.4, 139.5, 138.3, 134.1, 132.3, 130.6, 127.7, 123.9, 123.1, 122.9, 121.7, 120.9, 118.4, 55.4, 52.6, 31.6, 30.4. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1649, 1682, 3333, 3385. HRMS (APCI): m/z = 399.1656, calcd 399.1663 for $C_{20}H_{23}N_4O_5$ $[M + H]^+$. HPLC: t_R = 14.75 min; purity: 96.3%.

Methyl (*S*)-3-(2,5-dioxo-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepin-3-yl) propanate (12)

Synthesis was performed as previously reported.[14] L-Glutamic acid dimethyl ester hydrochloride (6.02 g, 28.4 mmol) and isatoic anhydride (**9**) (4.64 g, 28.4 mmol) were dissolved in pyridine (60 mL) and refluxed at 120 °C for 16 h. Pyridine was evaporated under reduced pressure and the residue was purified by column chromatography (cyclohexane/EtOAc = 35/65 → 30/70). Product **12** was obtained as a white solid (1.28 g, 4.88 mmol, 17%). M.p.: 180 °C. TLC: R_f = 0.69 (MeOH/DCM = 0.5/9.5). ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.40 (s, 1H), 8.46 (d, J = 5.6 Hz, 1H), 7.74 (dd, J = 7.8/1.6 Hz, 1H), 7.51 (ddd, J = 8.1/7.3/1.7 Hz, 1H), 7.26–7.18 (m, 1H), 7.10 (dd, J = 8.1/1.1 Hz, 1H), 3.70 (dt, J = 8.0/6.1 Hz, 1H), 3.56 (s, 3H), 2.46–2.35 (m, 2H), 2.18–1.96 (m, 1H), 1.91–1.77 (m, 1H). ^{13}C-NMR (101 MHz, DMSO-d_6): δ (ppm) = 172.9, 171.3, 167.8, 136.6, 130.4, 126.2, 124.0, 120.9, 51.3, 50.9, 29.6, 23.1. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1447, 1485, 1659, 1678, 1728, 3059. HRMS (APCI): m/z = 263.1022, calcd 263.1026 for $C_{13}H_{15}N_2O_4$ $[M + H]^+$. HPLC: t_R = 11.13 min; purity: 96.2%.

(±)-1,11a-Dihydro-3*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-3,5,11(2*H*,10*H*)-trione (16)

Synthesis was performed as previously reported.[14] Compound **12** (0.47 g, 1.79 mmol) was dissolved in DMA (2.40 mL) and stirred under reflux at 180 °C for 20 h. The solvent was removed *in vacuo*, the residue was dissolved in EtOAc and H_2O, and layers were separated. The organic layer was dried with Na_2SO_4, and the solvent was removed under *vacuum*. After purification with flash column chromatography (DCM/MeOH = 98/2 → 95/5), product **16** was obtained as a beige solid (0.25 g, 1.10 mmol, 61%). M.p.: 133–134 °C. TLC: R_f = 0.65 (DCM/MeOH = 9.8/0.2). ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) = 10.71 (s, 1H), 7.81 (dd, J = 7.9/1.6 Hz, 1H), 7.61 (ddd, J = 8.1/7.3/1.6 Hz, 1H), 7.31–7.27 (m, 1H), 7.17 (dd, J = 8.2/1.1 Hz, 1H), 4.66 (dd, J = 8.4/1.0 Hz, 1H), 2.60–2.51 (m, 2H), 2.49–2.43 (m, 1H), 2.14–2.04 (m, 1H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ (ppm) = 173.3, 169.6, 164.1, 136.6, 133.7, 131.3, 125.8, 124.4, 121.6, 56.0, 31.0, 17.8. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1477, 1667, 1690, 1759, 2978. HRMS (APCI): m/z = 231.0751, calcd 231.0764 for $C_{12}H_{11}N_2O_3$ $[M + H]^+$.

(±)-6,7-Dihydro-8*H*,10*H*-benzo[6,7]pyrrolo[2′,1′:3,4][1,4]diazepino[2,1-*b*]quinazoline-8,10,16(5b*H*)-trione (13′)

Synthesis was performed as previously reported.[14] Compound **16** (0.559 g, 2.43 mmol) was dissolved in DCM (5.6 mL) and cooled down to 0 °C. Triethylamine (0.68 mL), DMAP (0.119 g, 0.97 mmol) and 2-nitrobenzoyl chloride (0.39 mL, 2.91 mmol) were added, and the mixture was stirred for 0.5 h at room temperature. The reaction mixture was cooled down to −20 °C and Zn dust (1.57 g, 24.0 mmol, 9.9 eq) and acetic acid (5.60 mL) was added. The reaction mixture was stirred for 1 h before slowly warming up to −5 °C and stirring for another 2 h at this temperature before letting it warm up to room temperature. The mixture was filtered through Celite and the residue was washed with DCM. The filtrate was washed with saturated $NaHCO_3$, the combined organic layers dried with Na_2SO_4 and the solvent evaporated. Product **13′** was obtained after flash chromatography (DCM/MeOH = 99/1 → 95/5) as a white solid (0.198 g, 0.60 mmol, 25%). M.p.: 259–260 °C. TLC: R_f = 0.58 (DCM/MeOH = 9.8/0.2). ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) = 8.19 (dd, J = 7.9/1.5 Hz, 1H), 7.90 (ddd, J = 8.4/7.1/1.6 Hz, 1H), 7.85 (dd, J = 7.8/1.6 Hz, 1H), 7.78–7.72 (m, 2H), 7.66–7.60 (m, 3H), 5.10–5.07 (m, 1H), 2.96–2.89 (m, 1H), 2.79 (ddd, J = 17.5/11.7/9.4 Hz, 1H), 2.56–2.51 (m, 1H), 2.32–2.22 (m, 1H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ (ppm) = 173.2, 163.6, 161.1, 152.7, 145.7, 135.0, 132.8, 131.9, 131.3, 130.3, 129.2, 128.8, 127.7, 127.6, 126.8, 121.5, 58.5, 31.7, 19.0. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1593, 1759, 1690, 2978. HRMS (APCI): m/z = 332.1014, calcd 332.1030 for $C_{19}H_{14}N_3O_3$ $[M + H]^+$.

(±)-Auranomide C (5)

Synthesis was performed as previously reported with minor variations.[14] To compound **13′** (0.118 g, 0.36 mmol) THF-NH_3 (0.5 M, 3.50 mL) was added. The reaction mixture was heated to 50 °C for 18 h. The solvent was evaporated, and auranomide C (**5**) was obtained after purification with flash column chromatography (DCM/MeOH = 97/3 → 95/5) as a white solid (0.094 g, 0.27 mmol, 76%). M.p.: 220–221 °C. TLC: R_f = 0.44 (DCM/MeOH = 9.5/0.5). ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) = 8.81 (d, J = 6.2 Hz, 1H), 8.19 (dd, J = 7.9/1.5 Hz, 1H), 7.91 (ddd, J = 8.4/7.2/1.5 Hz, 1H), 7.80–7.74 (m, 2H), 7.69–7.63 (m, 2H), 7.63–7.57 (m, 2H), 7.24 (s, 1H), 6.74 (s, 1H), 4.19–4.13 (m, 1H), 2.38–2.22 (m, 3H), 2.20–2.10 (m, 1H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ (ppm) = 173.7, 166.9, 161.0, 155.8, 145.9, 135.1, 133.0, 131.2, 130.7, 128.85, 128.81, 128.6, 127.6, 127.4, 126.8, 121.0, 53.2, 30.8, 24.1. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1647, 1678, 2920, 3071, 3318, 3418. HRMS (APCI): m/z = 349.1321, calcd 349.1222 for $C_{19}H_{17}N_4O_3$ $[M + H]^+$.

HPLC: t_R = 12.54 min; purity: 94.3%. Spectra are in agreement with previously published data.[14]

(±)-Auranthine (1)

(±)-Auranomide C (**5**) (0.094 g, 0.27 mmol) was dissolved in DMF (1.20 mL), then pyridine (0.11 mL) and 4-toluenesulfonyl chloride (0.113 g, 0.59 mmol) was added, and the mixture stirred at room temperature. After 24 h, the solvent was evaporated, the residue was dissolved in EtOAc and washed with aqueous 10% citric acid. The organic layer was dried with Na_2SO_4 and the solvent evaporated. After purification with flash column chromatography (DCM/MeOH = 97/3 → 90/10 and then cyclohexane/EtOAc = 50/50 → 0/100), (±)-auranthine (**1**) was obtained as a white solid (0.03 g, 0.09 mmol, 34%). M.p.: 157–159 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) = 8.89 (d, J = 6.3 Hz, 1H), 8.19 (ddd, J = 7.9/1.6/0.5 Hz, 1H), 7.91 (ddd, J = 8.2/7.2/1.6 Hz, 1H), 7.79 (dd, J = 7.6/1.6 Hz, 1H), 7.76 (dd, J = 8.3/1.1 Hz, 1H), 7.69 (ddd, J = 8.2/7.1/1.6 Hz, 1H), 7.65 (dd, J = 8.1/1.4 Hz, 1H), 7.63–7.58 (m, 2H), 4.25 (dt, J = 8.7/5.9 Hz, 1H), 2.70 (t, J = 7.6, 2H), 2.47–2.38 (m, 1H), 2.33–2.25 (m, 1H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ (ppm) = 166.9, 160.9, 155.1, 145.7, 135.2, 132.9, 131.0, 130.8, 128.94, 128.91, 128.7, 127.7, 127.4, 126.8, 121.0, 120.1, 52.6, 24.6, 13.3. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3174, 3067, 2924, 2249, 1686, 1655, 1613, 1597, 1454, 1393, 1250, 775, 694. HRMS (APCI): m/z = 331.1190, calcd 331.1117 for $C_{19}H_{15}N_4O_2$ [M + H]$^+$. HPLC: t_R = 15.00 min; purity: 94.6%. Spectra are in agreement with those of natural auranthine.[6]

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval.

Conflicts of interest

There are no conflicts to declare.

Abbreviations

APCI	Atmospheric pressure chemical ionization
CD	(Circular dichroism) spectroscopy
DCM	Dichloromethane
DFT	Density functional theory
DMA	Dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide,
HRMS	High-resolution mass spectrometry
THF	Tetrahydrofuran
TLC	Thin layer chromatography

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References

1 S. E. Yeulet, P. G. Mantle, J. N. Bilton, H. S. Rzepa and R. N. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1891–1894, DOI: 10.1039/p19860001891.
2 S. E. Yeulet and P. G. Mantle, *FEMS Microbiol. Lett.*, 1987, **41**, 207–210.
3 N. Arora, P. Dhiman, S. Kumar, G. Singh and V. Monga, *Bioorg. Chem.*, 2020, **97**, 103668.
4 A. Witt, A. Gustavsson and J. Bergman, *J. Heterocycl. Chem.*, 2003, **40**, 29–35.
5 U. A. Kshirsagar, V. G. Puranik and N. P. Argade, *J. Org. Chem.*, 2010, **75**, 2702–2705.
6 S. A. Kalinina, D. V. Kalinin, Y. Hovelmann, C. G. Daniliuc, C. Muck-Lichtenfeld, B. Cramer and H. U. Humpf, *J. Nat. Prod.*, 2018, **81**, 2177–2186.
7 F. H. Song, B. A. Ren, K. Yu, C. X. Chen, H. Guo, N. Yang, H. Gao, X. T. Liu, M. Liu, Y. J. Tong, H. Q. Dai, H. Bai, J. D. Wang and L. X. Zhang, *Mar. Drugs*, 2012, **10**, 1297–1306.
8 M. A. Goetz, M. Lopez, R. L. Monaghan, R. S. L. Chang, V. J. Lotti and T. B. Chen, *J. Antibiot.*, 1985, **38**, 1633–1637.
9 R. S. L. Chang, V. J. Lotti, R. L. Monaghan, J. Birnbaum, E. O. Stapley, M. A. Goetz, G. Albersschonberg, A. A. Patchett, J. M. Liesch, O. D. Hensens and J. P. Springer, *Science*, 1985, **230**, 177–179.
10 B. K. Joshi, J. B. Gloer, D. T. Wicklow and P. F. Dowd, *J. Nat. Prod.*, 1999, **62**, 650–652.
11 H. H. Sun, C. J. Barrow, D. M. Sedlock, A. M. Gillum and R. Cooper, *J. Antibiot.*, 1994, **47**, 515–522.
12 F. He, B. M. Foxman and B. B. Snider, *J. Am. Chem. Soc.*, 1998, **120**, 6417–6418.
13 M. C. Tseng, C. Y. Lai, Y. W. Chu and Y. H. Chu, *Chem. Commun.*, 2009, **4**, 445.
14 K. Sorra, K. Mukkanti and S. Pusuluri, *Synthesis*, 2014, **46**, 189–194.
15 T. Sugimori, T. Okawa, S. Eguchi, A. Kakehi, E. Yashima and Y. Okamoto, *Tetrahedron*, 1998, **54**, 7997–8008.
16 P. E. Zhichkin, X. M. Jin, H. L. Zhang, L. H. Peterson, C. Ramirez, T. M. Snyder and H. S. Burton, *Org. Biomol. Chem.*, 2010, **8**, 1287–1289.