The Journal of Antibiotics (2016) 69, 459–463

www.nature.com/ja

NOTE

# Amethysione and amethysamide, new metabolites from *Streptosporangium amethystogenes* BCC 27081

**Chollaratt Boonlarppradab, Chanwit Suriyachadkun, Sumalee Supothina and Pattiyaa Laksanacharoen**

*The Journal of Antibiotics* (2016) **69,** 459–463; doi:10.1038/ja.2015.128; published online 16 December 2015

Quinazolinone alkaloids belong to classes of fused heterocycles that are of considerable interest due to a broad spectrum of biological activities and diverse range of pharmacological properties, for example, anticancer, antimicrobial, antifungal, antiviral, antiprotozoan, antidepressant, anti-inflammatory and antimalarial activities.[1–5] Quinazolinone derivatives are considered an important chemical for the synthesis of various functional units or precursors with physiological significance and pharmacological utility. Their core heterocyclic frameworks are also present in many various drug molecules.[4,6] Among a diverse group of quinazolinone metabolites from both synthetic and natural origin, 2,4(1*H*,3*H*)quinazolinedione motifs are reported to exhibit anticonvulsant activity against electroshock and possess potential antihypertensive properties.[7–10] Majority of quinazoline-2,4-dione derivatives are known synthetically, whereas the natural occurrence of these molecules is quite uncommon.

During our screening program of rare actinomycetes from Thailand in search for novel and biologically active secondary metabolites, investigation of *Streptosporangium amethystogenes* BCC 27081 led to the isolation of two new compounds, amethysione (**1**) and amethysamide (**2**), together with their related derivatives including 2-(3-phenethyl-ureido)-benzoic acid (**3**), 2-(3-phenethyl-ureido)-benzoic acid methyl ester (**4**), *N*-phenethyl-2-(3-phenethyl-ureido)-benzamide (**5**), 1,3-diphenethyl-urea (**6**),[11] 3-(2-phenethyl)-2,4 (1*H*,3*H*)-quinazolinedione (**7**),[12] and 1-methyl-3-(2-phenylethyl)-2,4 (1*H*,3*H*)-quinazolinedione (**8**)[12]. In this paper, we report the isolation, structure elucidation and biological activities of these new metabolites and their derivatives (Figure 1).

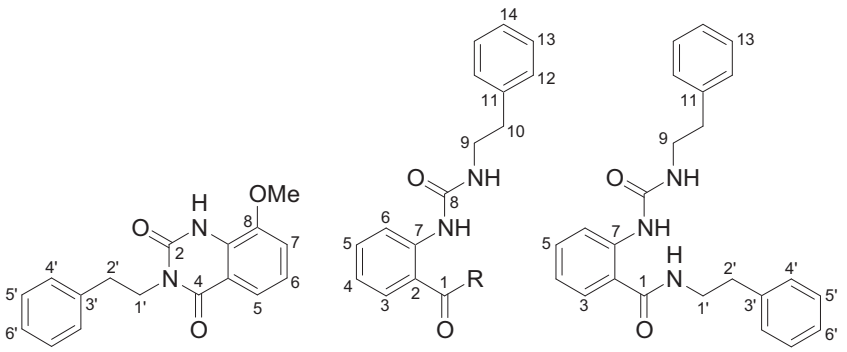
*S. amethystogenes* BCC 27081 culture was extracted with ethyl acetate and the obtained crude organic material was fractionated by partition on HP-20SS resin (Supelco, Bellefonte, PA, USA) and eluted with acetone/water mixtures. Further purification process by chromatography on a Sephadex LH-20 column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and reversed-phase $C_{18}$ HPLC (Waters Corporation, Milford, MA, USA) furnished compounds **1**–**8**.

Amethysione (**1**) was obtained as a white powder and its molecular formula was identified as $C_{17}H_{16}N_2O_3$ by HRESITOFMS (*m/z* 319.1059, $[M+Na]^+$) and from its NMR data. The LRESIMS spectra illustrated ion peaks corresponding to $[M+H]^+$ and $[M+Na]^+$ at *m/z* 297 and 319. Analysis of the IR spectroscopic data displayed very strong characteristic absorption bands at 1714 and 1649 $cm^{-1}$, which suggested the presence of carbonyl groups from amide functionality.[13]

The $^1H$ NMR spectrum of **1**, measured in acetone-$d_6$ (Table 1), illustrated a singlet oxygenated methyl signal at δ 3.98, two triplet methylene proton signals at δ 2.95 and δ 4.19 and eight olefinic proton signals (δ7.17–7.30). The $^1H$-$^1H$ COSY NMR analysis showed the first spin system as 1,2,3-trisubstituted aromatic ring system, which consisted of triplet and doublets at δ 7.17, 7.59 and δ 7.29. The second spin system involved the other five olefinic proton multiplets (δ7.21–7.30), which constructed a benzene ring system. The final spin system comprised just two correlated triplet methylene signals (δ 2.95 and δ 4.19).

The $^{13}C$ NMR/DEPT spectrum of **1** showed 17 carbon resonances including one oxygenated methyl group (δ 55.8), two methylene carbons (δ 33.7 and 41.7), two carbonyl carbons (δ 149.7 and δ 161.8), eight non-oxygenated olefinic/aromatic methine carbons and four olefinic/aromatic quaternary carbons with one bearing oxygen. The interpretation of COSY and HMBC correlations (Figure 2) enabled the establishment of quinazoline-2,4-dione backbone. The oxygenated methyl signal at δ 3.98 could be assigned to a methoxy group attached to the substituted aromatic ring at C-8 (δ 146.1) according to its HMBC correlation. The aromatic methine at H-5 (δ 7.59) correlated to carbonyl at C-4 (δ 161.8) indicated the carbonyl position adjacent to the trisubstituted aromatic ring. Two correlated methylene proton signals between H-1′ (δ 4.19) and H-2′ (δ 2.95) connected the benzene ring system to the core structure of quinazoline-2,4-dione. The methylene signal at H-2′ showed HMBC correlations to the aromatic quaternary carbon at C-3′ (δ 139.0) and methine carbon at C-4′ (δ 128.8), confirming the presence of phenylethyl functionality. The adjacent methylene proton at H-1′ correlated to both carbonyl groups at C-2 (δ 149.7) and C-4 (δ 161.8), allowing the assembly of quinazoline-2,4-dione structure. The interpretation of NOESY NMR spectroscopic data further supported this

National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand Science Park, Pathum Thani, Thailand
Correspondence: Dr C Boonlarppradab, Bioresources Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Thanon Phahonyothin, Tambon Khlong Neung, Amphoe Klong Luang, Pathum Thani 12120, Thailand.
E-mail: chollaratt.boo@biotec.or.th
Received 28 July 2015; revised 30 September 2015; accepted 12 November 2015; published online 16 December 2015

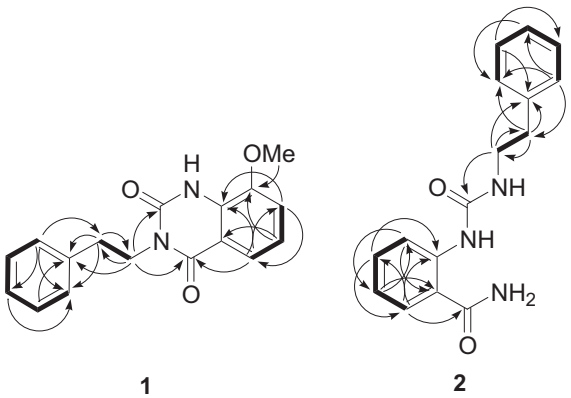


**1** **2** R = $NH_2$ **3** R = OH **4** R = OMe **5**

**Figure 1** The structures of amethysione (**1**), amethysamide (**2**), 2-(3-phenethyl-ureido)-benzoic acid (**3**), 2-(3-phenethyl-ureido)-benzoic acid methyl ester (**4**) and *N*-phenethyl-2-(3-phenethyl-ureido)-benzamide (**5**) from *Streptosporangium amethystogenes* BCC 27081.

**Table 1 $^{1}H$ and $^{13}C$ NMR spectroscopic data (acetone-$d_6$) for amethysione (1) and amethysamide (2)**

| *Amethysione (**1**)* | | | *Amethysamide (**2**)* | | |
|---|---|---|---|---|---|
| *Position* | *$\delta_C$, type* | *$\delta_H$ (J in Hz)* | *Position* | *$\delta_C$, type* | *$\delta_H$ (J in Hz)* |
| 1 | | | 1 | 171.6, C | |
| 2 | 149.7, C | | 2 | 118.1, C | |
| 3 | | | 3 | 128.1, CH | 7.72, d (7.9) |
| 4 | 161.8, C | | 4 | 120.1, CH | 6.92, t (7.6, 7.5) |
| 4a | 114.9, C | | 5 | 132.2, CH | 7.39, t (8.2, 7.5) |
| 5 | 118.6, CH | 7.59, d (8.0) | 6 | 119.9, CH | 8.55, d (8.5) |
| 6 | 122.4, CH | 7.17, t (8.0) | 7 | 142.6, C | |
| 7 | 114.7, CH | 7.29, m | 8 | 155.1, C | |
| 8 | 146.1, C | | 9 | 41.7, $CH_2$ | 3.45, t (7.7, 7.3) |
| 8-OMe | 55.8, $CH_3$ | 3.98, s | 10 | 36.5, $CH_2$ | 2.87, t (7.7, 7.3) |
| 8a | 129.7, C | | 11 | 140.1, C | |
| 1′ | 41.7, $CH_2$ | 4.19, t (8.0) | 12 | 129.0, CH | 7.28, m |
| 2′ | 33.7, $CH_2$ | 2.95, t (8.0) | 13 | 128.5, CH | 7.27–7.28, m |
| 3′ | 139.0, C | | 14 | 126.2, CH | 7.19, m |
| 4′ | 128.8, CH | 7.30, m | | | |
| 5′ | 128.4, CH | 7.29–7.30, m | | | |
| 6′ | 126.3, CH | 7.21, m | | | |



**1** **2**

**Figure 2** Selected COSY (bold lines) and HMBC (arrows) correlations observed for **1** and **2**.

elucidation. The methylene signal at H-2′ showed strong NOESY correlation to methine protons at H-4′ (δ 7.30), whereas the methoxy signal at δ 3.98 correlated to a methine proton at H-7 (δ 7.29). The structure of amethysione (**1**) is very similar to **7** and **8**, except that it contains a methoxy group at C-8 position and does not possess *N*-methyl functional group. Even though considerable amount of metabolites with this heterocyclic 2,4(1*H*,3*H*)quinazolinedione are widely known synthetically, there are not many reports regarding isolation from the natural sources. Quinazolines are quite well-known among plant alkaloids, but there are only few of them which were obtained as secondary metabolites from microorganisms.[14]

Amethysamide (**2**) was obtained as a white powder and its structure was also elucidated on the basis of the interpretation of 1D and 2D NMR data (Table 1). The molecular formula was established as $C_{16}H_{17}N_3O_2$ by HRESITOFMS (*m/z* 306.1214, $[M+Na]^+$). The $^{1}H$ NMR spectrum exhibited two triplet methylene proton signals at δ 2.87 and δ 3.45 and nine olefinic proton signals (δ 6.92–8.55). The major portion of this compound could be assembled through the interpretation of $^{1}H$-$^{1}H$ COSY and HMBC correlations (Figure 2). The analysis by COSY NMR showed correlation pattern almost identical to **1** as both metabolites were closely related structurally. For amethysamide, instead of a heterocyclic quinazoline-2,4-dione core structure, the bond between a carbonyl carbon at C-1 and nitrogen attached to C-9 was disrupted to break open the ring, and the substitution of aromatic methoxy functional group was absent. In accordance, the correlation pattern of HMBC NMR spectroscopic data was also revealed to be very similar to **1** (Figure 2), except for a lack of correlation from methylene signal at H-9 (δ 3.45) to a carbonyl carbon at C-1 (δ 171.6), confirming that quinazolinedione moiety no longer existed. The HMBC NMR interpretation demonstrated that nine olefinic methine protons belonged to two aromatic rings system; a benzamide and a benzene attached to two correlated methylene groups of H-9 and H-10. Correlation of HMBC from the low-field methylene signal at H-9 to the carbonyl carbon at C-8 (δ 155.1) suggested that the benzamide and phenylethyl group was connected through a urea motif.

2-(3-Phenethyl-ureido)-benzoic acid (**3**), 2-(3-phenethyl-ureido)-benzoic acid methyl ester (**4**) and *N*-phenethyl-2-(3-phenethyl-ureido)-benzamide (**5**) were obtained by synthesis previously but

**Table 2** $^{1}H$ and $^{13}C$ NMR spectroscopic data (acetone-$d_6$) of compound 3, 4 and 5

| | (3) | | (4) | | (5) | |
|---|---|---|---|---|---|---|
| *Position* | $\delta_C$, *type* | $\delta_H$ *(J in Hz)* | $\delta_C$, *type* | $\delta_H$ *(J in Hz)* | $\delta_C$, *type* | $\delta_H$ *(J in Hz)* |
| 1 | 169.7, C | | 168.4, C | | 169.1, C | |
| 1-OMe | | | 51.5, $CH_3$ | 3.88, s | | |
| 2 | 113.7, C | | 113.8, C | | 119.4, C | |
| 3 | 131.1, CH | 8.02, dd (8.0, 1.5) | 130.5, CH | 7.95, dd (8.0, 1.6) | 127.2, CH | 7.57, dd (7.9, 1.1) |
| 4 | 119.9, CH | 6.97, t (7.6) | 120.0, CH | 6.96, m (7.6, 1.1) | 120.0, CH | 6.89, t (7.6) |
| 5 | 134.0, CH | 7.50, m (7.9, 1.6) | 133.9, CH | 7.50, m (7.9, 1.6) | 131.5, CH | 7.36, m (7.9, 1.4) |
| 6 | 119.1, CH | 8.63, d (8.5) | 119.4, CH | 8.63, dd (8.6, 1.0) | 119.9, CH | 8.50, d (8.5) |
| 7 | 144.0, C | | 143.7, C | | 141.9, C | |
| 8 | 154.7, C | | 154.6, C | | 155.0, C | |
| 9 | 41.4, $CH_2$ | 3.46, t (7.7, 7.3) | 41.4, $CH_2$ | 3.48, t (7.7, 7.2) | 41.6, $CH_2$ | 3.47, m |
| 10 | 36.1, $CH_2$ | 2.86, t (7.7, 7.3) | 36.1, $CH_2$ | 2.88, t (7.7, 7.2) | 36.3, $CH_2$ | 2.88, t (7.7, 7.2) |
| 11 | 139.8, C | | 139.8, C | | 139.8, C | |
| 12 | 128.7, CH | 7.28, m | 128.7, CH | 7.29, m | 128.8, CH | 7.29, m |
| 13 | 128.3, CH | 7.27–7.28, m | 128.3, CH | 7.28–7.29, m | 128.4, CH | 7.28–7.29, m |
| 14 | 126.0, CH | 7.19, m | 126.0, CH | 7.20, m | 126.2, CH | 7.20, m |
| 1′ | | | | | 41.1, $CH_2$ | 3.61, m |
| 2′ | | | | | 35.4, $CH_2$ | 2.93, t (7.6, 7.3) |
| 3′ | | | | | 139.6, C | |
| 4′ | | | | | 128.7, CH | 7.29, m |
| 5′ | | | | | 128.3, CH | 7.28–7.29, m |
| 6′ | | | | | 126.0, CH | 7.20, m |

**Table 3 Cytotoxic activities against Vero cell and various cancer cell lines of 1–8**

| | *Vero cells* | *KB* | *MCF-7* | *NCI-H187* |
|---|---|---|---|---|
| *Compound* | $IC_{50}$ *(μg ml*$^{-1}$*)* | $IC_{50}$ *(μg ml*$^{-1}$*)* | $IC_{50}$ *(μg ml*$^{-1}$*)* | $IC_{50}$ *(μg ml*$^{-1}$*)* |
| **1** | >50 | 16.94 | >50 | 36.99 |
| **2** | >50 | >50 | >50 | >50 |
| **3** | >50 | >50 | >50 | >50 |
| **4** | 48.29 | >50 | 19.50 | 26.56 |
| **5** | >50 | 31.24 | >50 | >50 |
| **6** | >50 | >50 | >50 | >50 |
| **7** | >50 | >50 | >50 | >50 |
| **8** | 49.22 | 17.86 | 27.27 | 27.15 |

Vero cell was treated with metabolites for 4 days, while KB, MCF-7 and NCI-H187 were treated for 3 days.

not from natural sources, and their references with NMR data were not accessible. The isolation of these compounds as naturally derived secondary metabolites from microorganisms has been reported here for the first time. All three compounds possessed exactly the same core skeleton (C-1 to C-14) as amethysamide. Accordingly, both $^{1}H$ and $^{13}C$ NMR chemical shifts of **3**, **4** and **5** were quite comparable in comparison to **2** (Table 2), except the only major difference at carbonyl carbon C-1 attributed to various functional groups attached to it.

The molecular formula for **3** was established as $C_{16}H_{16}N_2O_3$ by HRESITOFMS (*m/z* 285.1235, $[M+H]^+$) and only differed from **2** by having a hydroxy group rather than amine at C-1 position. The HRESITOFMS spectrum of compound **4** suggested a molecular formula of $C_{17}H_{18}N_2O_3$ (*m/z* 299.1388, $[M+H]^+$) and was different from **3** by having one additional methoxy group instead of a hydroxy residue attached to C-1 carbonyl carbon. The $^{1}H$ and $^{13}C$ NMR chemical shifts of **3** and **4** for major part of the structure were mostly the same. Detailed analysis of COSY and HMBC NMR spectroscopic data showed similar correlations pattern between **2**, **3** and **4**. The HMBC correlation of **4** from an oxygenated methyl signal at δ 3.88 to the carbonyl carbon at C-1 (δ 168.4) confirmed the position of methoxy group at C-1 position.

The molecular formula of compound **5** was determined to be $C_{24}H_{25}N_3O_2$ by HRESITOFMS (*m/z* 410.1836, $[M+Na]^+$). The similar pattern of $^{1}H$-$^{1}H$ COSY and HMBC NMR correlations for the same core structure (C-1 to C-14) in comparison to **2**, **3** and **4** have been observed. The only difference is the alteration in functional group attached to C-1 carbonyl carbon as mentioned earlier. The $^{13}C$ NMR/DEPT spectrum of **5** showed 24 carbon residues including 4 methylene carbons, 2 carbonyl carbons (δ 155.0 and δ 169.1), 14 non-oxygenated olefinic/aromatic methine carbons and 4 olefinic/aromatic quaternary carbons. The analysis by 2D NMR data indicated that the amine group in **2** was replaced by the phenylethyl moiety. Two connected methylene signals between H-1′ (δ 3.61) and H-2′ (δ 2.93) were confirmed through COSY correlation. The methylene protons at H-2′ showed HMBC correlations to the quaternary carbon at C-3′ (δ 139.6) and aromatic carbons at C-4′ (δ 128.7), while the adjacent low-field methylene at H-1′ correlated to the carbonyl carbon at C-1 (δ 169.1). The structure of **5** resembles a tris-phenethyl urea, molleurea A, isolated from the ascidian *Didemnum molle*.[15]

All isolated metabolites **1–8** were evaluated for the following biological activities including anti-plant pathogens (*Magnaporthe grisea, Colletotrichum capsici* and *C. gloeosporioides*), antibacterial (*Mycobacterium tuberculosis*, *Enterococcus faecium*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*), antifungal (*Candida albicans*), antimalarial (*Plasmodium falciparum* K1) and cytotoxic activities (KB, MCF-7, NCI-H187 and Vero cells). None of them exhibited growth inhibition in any of these screening assays except for cytotoxic activities. The new quinazolinedione amethysione (**1**)

showed weak activities against two cancer cell lines, KB and NCI-H187, with respective $IC_{50}$ values of 16.94 μg ml$^{-1}$ and 36.99 μg ml$^{-1}$, whereas **2** was inactive in all cytotoxic screenings (Table 3).[16,17] Compounds **4** and **8** were also active in most cytotoxic assays while **5** only displayed weak cytotoxicity against KB cell line. On the other hand, compounds **3**, **6** and **7** were all inactive in every bioassay that was performed. MIC and $IC_{50}$ values $>50$ μg ml$^{-1}$ were reported as inactive.

## EXPERIMENTAL PROCEDURE

### General

UV spectra were taken on a SPEKOL 1200 spectrophotometer (Analytik Jena AG, Jena, Germany). FT-IR spectra were measured using a Bruker Alpha-E spectrometer (Bruker Optik GmbH, Ettlingen, Germany). $^{1}$H, $^{13}$C, DEPT, COSY, HMQC, HMBC and NOESY NMR spectra were recorded on a Bruker DRX400 and a Bruker AV500D spectrometer (Bruker BioSpin AG, Fällanden, Switzerland). ESI-TOF mass spectrometer measurements were obtained using Micromass LCT (Micromass UK Limited, Manchester, UK) and Bruker micrOTOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany).

### Biological material

The actinomycete *S. amethystogenes* (BCC 27081/A-T 1522) was isolated from the soil under a banana tree collected at evergreen forest, Thung Salaeng Luang National Park, Phitsanulok, Thailand. The collection and taxonomic identification were performed by Chanwit Suriyachadkun (BIOTEC, Pathum Thani, Thailand). This actinomycete strain was identified by 16S ribosomal DNA sequence analysis. A specimen has been deposited at BIOTEC Culture Collection (BCC) and registered as BCC 27081.

### Culture conditions

*S. amethystogenes* BCC 27081 was maintained on International Streptomyces project medium 2 (ISP-2) at 28 °C for 3 weeks. The agar was cut into small pieces (1 cm$^2$) and inoculated into 9 × 250 ml Erlenmeyer flasks containing 25 ml of Bio 19 medium (glucose 20 g, peptone 5.0 g, yeast extract 3.0 g, meat extract 5.0 g, NaCl 0.5 g and vitamin complex solution 2 ml, per 1 l of distilled water). Vitamin complex solution was prepared by dissolving three tablets of multivitamins+minerals supplement (Blackmores Ltd, NSW, Australia) in 100 ml of distilled water. Each primary seed culture was incubated at 28 °C on a rotary shaker (250 r.p.m.) for 7 days and was later transferred into a 1000 ml Erlenmeyer flask containing 250 ml of the same liquid medium as a secondary seed culture. After the incubation under 28 °C for 7 days on a rotary shaker (250 r.p.m.), 25 ml aliquots of these secondary cultures were transferred to 80 × 1000 ml Erlenmeyer flasks, each containing 250 ml of LS2 production medium (mannitol 20 g, soytone 20 g, trace element mix 1 ml and vitamin complex solution 2 ml, per 1 l of distilled water). The mixture of trace elements consisted of (w/v): $CaCl_2$, 0.4%; $ZnSO_4$, 0.2%; $Na_2B_4O_7$, 0.01%; $FeSO_4$, 0.5%; KI, 0.005%; $CoCl_2$, 0.05%; $CuSO_4$, 0.02%; $MnCl_2$, 0.2%; $Na_2MoO_4$, 0.005%; (v/v) $H_2SO_4$ (95–97% p.a.), 0.1%. Total of ~22 l (80 × 275 ml) of BCC 27081 cultures were incubated on rotary shakers (250 r.p.m.) for 7 days at 28 °C.

## EXTRACTION AND ISOLATION

The organic constituents from a 22 l culture of *S. amethystogenes* BCC 27081 were extracted with an excess amount of ethyl acetate three times. Afterward, the crude organic layer was concentrated *in vacuo* to yield a sticky dark brown substance (3.4 g), that was partitioned on Diaion HP-20SS (Supelco, Bellefonte, PA, USA) column chromatography (3.5 × 27 cm, acetone/water) to obtain nine fractions (20, 30, 40, 50, 60, 70, 80, 90 and 100% acetone mixtures). The fractions which eluted with 60% (138.1 mg) and 70% (176.6 mg) acetone/water were combined and further purified by gradient preparative HPLC using a reversed-phase column (Phenomenex Luna 10 u C18(2) 100 A (Phenomenex LUNA, Torrance, CA, USA), 21.2 × 250 mm, 10 μm; 10 ml min$^{-1}$, 50% MeCN/$H_2O$ over 7 min, 50–100% MeCN/$H_2O$ over 40 min, 100% MeCN over 20 min) to obtain **2** (54.3 mg) and **3** (15.2 mg). The combined 80% (1.4 g) and 90% (57.7 mg) acetone/water fractions were subjected to the purification by gradient HPLC (Phenomenex Luna $C_{18}$ preparative (Phenomenex LUNA)), 10 ml min$^{-1}$, 45% MeCN/$H_2O$ over 7 min, 45–100% MeCN/$H_2O$ over 40 min, 100% MeCN over 20 min) to afford **1** (16.5 mg), **2** (15.7 mg), **3** (18.2 mg), **4** (14.7 mg), **5** (51.1 mg), **8** (4.5 mg) and mixtures of **6** and **7**. The chromatography by Sephadex LH-20 column (2.5 × 50 cm) with methanol as eluent enabled the separation of **6** (38.7 mg) and **7** (24.4 mg) from the mixed fractions.

### Amethysione (1)

White amorphous powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 234 nm (4.20), 314 nm (3.70); IR $\nu_{max}$ (neat) 2931, 2854, 1714, 1649, 1516, 1454, 1434, 1270 cm$^{-1}$; NMR data, see Table 1; ESIMS $[M+Na]^+$ *m/z* 319; ESIMS $[M+H]^+$ *m/z* 297; HRESITOFMS $[M+Na]^+$ *m/z* 319.1059 (calcd for $C_{17}H_{16}N_2O_3Na$, 319.1059).

### Amethysamide (2)

White amorphous powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 222 nm (4.13), 248 nm (4.12), 306 nm (3.74); IR $\nu_{max}$ (neat) 3336, 2923, 2853, 1656, 1615, 1589, 1522, 1449, 1391, 1287, 1236 cm$^{-1}$; NMR data, see Table 1; ESIMS $[M+Na]^+$ *m/z* 306; ESIMS $[M+H]^+$ *m/z* 284; HRESITOFMS $[M+Na]^+$ *m/z* 306.1214 (calcd for $C_{16}H_{17}N_3O_2Na$, 306.1218).

### 2-(3-phenethyl-ureido)-benzoic acid (3)

White amorphous powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 224 nm (4.15), 246 nm (4.09), 310 nm (3.78); IR $\nu_{max}$ (neat) 3314, 2929, 2855, 1667, 1587, 1538, 1452, 1385, 1255 cm$^{-1}$; NMR data, see Table 2; ESIMS $[M+Na]^+$ *m/z* 307; ESIMS $[M+H]^+$ *m/z* 285; HRESITOFMS $[M+H]^+$ *m/z* 285.1235 (calcd for $C_{16}H_{17}N_2O_3$, 285.1239).

### 2-(3-phenethyl-ureido)-benzoic acid methyl ester (4)

White amorphous powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 225 nm (4.15), 248 nm (4.05), 314 nm (3.75); IR $\nu_{max}$ (neat) 3313, 2924, 2853, 1676, 1588, 1527, 1450, 1253 cm$^{-1}$; NMR data, see Table 2; ESIMS $[M+Na]^+$ *m/z* 321; ESIMS $[M+H]^+$ *m/z* 299; HRESITOFMS $[M+H]^+$ *m/z* 299.1388 (calcd for $C_{17}H_{19}N_2O_3$, 299.1396).

### *N*-phenethyl-2-(3-phenethyl-ureido)-benzamide (5)

White amorphous powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 220 nm (4.23), 247 nm (4.20), 302 nm (3.81); IR $\nu_{max}$ (neat) 3320, 2925, 2853, 1635, 1597, 1519, 1445, 1276 cm$^{-1}$; NMR data, see Table 2; ESIMS $[M+Na]^+$ *m/z* 410; ESIMS $[M+H]^+$ *m/z* 388; HRESITOFMS $[M+Na]^+$ *m/z* 410.1836 (calcd for $C_{24}H_{25}N_3O_2Na$, 410.1844).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

We are very grateful to Bioresources Research Network (BRN) of the National Center for Genetic Engineering and Biotechnology (BIOTEC) for financial support.

1 Connolly, D. J., Cusack, D., O'Sullivan, T. P. & Guiry, P. J. Synthesis of quinazolinones and quinazolines. *Tetrahedron* **61**, 10153–10202 (2005).
2 Mhaske, S. B. & Argade, N. P. The chemistry of recently isolated naturally occurring quinazolinone alkaloids. *Tetrahedron* **62**, 9787–9826 (2006).
3 Rajput, R. & Mishra, A. P. A review on biological activity of quinazolinones. *Int. J. Pharm. Pharm. Sci.* **4**, 66–70 (2012).

4 Asif, M. Chemical characteristics, synthetic methods, and biological potential of quinazoline and quinazolinone derivatives. *Int. J. Med. Chem.* **2014**, 395637 (2014).
5 Abdel Gawad, N. M., Georgey, H. H., Youssef, R. M. & El-Sayed, N. A. Synthesis and antitumor activity of some 2, 3-disubstituted quinazolin-4(3*H*)-ones and 4, 6-disubstituted- 1, 2, 3, 4-tetrahydroquinazolin-2*H*-ones. *Eur. J. Med. Chem.* **45**, 6058–6067 (2010).
6 Abida, Nayyar, P. & Arpanarana, M. An updated review: newer quinazoline derivatives under clinical trial. *Int. J. Pharm. Biol. Sci. Arch* **2**, 1651–1657 (2011).
7 Wenzel, D. G. Anticonvulsant activity of some uracils and related compounds. *J. Pharm. Sci.* **44**, 550–553 (1955).
8 Hayao, S., Havera, H. J., Strycker, W. G. & Hong, E. Hypotensive, antiadrenergic, and antihistaminic 3-substituted 2-methyl-(or 2-phenyl-) 4(3H)-quinazolones. *J. Med. Chem.* **12**, 936–938 (1969).
9 Hayao, S. *et al.* New Sedative and hypotensive 3-substituted 2,4(1H,3H)-Quinazolinediones. *J. Med. Chem.* **8**, 807–811 (1965).
10 Hayao, S. Quinazolinedione derivatives. US3274194 (1965).
11 Iwai, Y., Hirano, A., Awaya, J., Matsuo, S. & Ōmura, S. 1,3-diphenethylurea from *Streptomyces* sp. No. AM-2498. *J. Antibiot.* **31**, 375–376 (1978).
12 Dreyer, D. L. & Brenner, R. C. Alkaloids of some Mexican *Zanthoxylum* species. *Phytochemistry* **19**, 935–939 (1980).
13 Pretsch, E., Simon, W., Seibl, J. & Clerc, T. *Structure Determination of Organic Compounds: Tables of Spectral Data*, 2nd edn, I150 (Springer-Verlag, Berlin, Germany, 1989).
14 D'yakonov, A. L. & Telezhenetskaya, M. V. Quinazoline alkaloids in nature. *Chem. Nat. Compd.* **33**, 221–267 (1997).
15 Lu, Z. *et al.* Thiazoline peptides and a tris-phenethyl urea from *Didemnum molle* with anti-HIV activity. *J. Nat. Prod.* **75**, 1436–1440 (2012).
16 Changsen, C., Franzblau, S. G. & Palittapongarnpim, P. Improved green fluorescent protein reporter gene-based microplate screening for antituberculosis compounds by utilizing an acetamidase promoter. *Antimicrob. Agents Chemother.* **47**, 3682–3687 (2003).
17 O'Brien, J., Wilson, I., Orton, T. & Pognan, F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur. J. Biochem.* **267**, 5421–5426 (2000).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)