Pyramidamycins A-D and 3-hydroxyquinoline-2-carboxamide; cytotoxic benzamides from *Streptomyces* sp. DGC1

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Four new benzamides, pyramidamycins A-D (2–5) along with the new natural 3-hydroxyquinoline-2-carboxamide (6) were isolated from the crude extract of *Streptomyces* sp. DGC1. Additionally, five other known compounds, namely 2-aminobenzamide (anthranilamide) (1), 4',7-dihydroxyisoflavanone (7), 2'-deoxy-thymidine, 2'-deoxy-uridine and adenosine were also isolated and identified. The structures of the new compounds 2–6 were elucidated by 1D and 2D NMR studies along with HR MS analyses. The isolated compounds 1–6 contained the same amide side chain. The isolated compounds 1–7 were biologically evaluated in comparison with landomycin A against a prostate cancer cell line (PC3) and non-small cell lung cancer cell line (H460) for 48 h and against several bacterial strains. Pyramidamycin C (4) was the most active compound against both PC3 and H460 cell lines (GI₅₀ = 2.473 and 7.339 μM, respectively). Benzamides (1–3) demonstrated inhibitory activity against *Kocuria rosea* B-1106 (a diameter halo of 13 ± 2 mm for 1; 10 ± 2 mm for 2 and 3). Compound 6 was slightly active against both *Escherichia coli* DH5α and *Micrococcus luteus* NRRL B-2618 (diameter halos 8 ± 2 and 9 ± 2 mm, respectively). Taxonomically, the amplified 500-bp 16S rRNA fragment of the *Streptomyces* sp. DGC1 had 99% identity (BLAST search) to the 16S rRNA gene of *Streptomyces atrovirens* strain NRRL B-16357.

**Introduction**

Most currently marketed antibiotics are natural products of microbial origin, and > 120 of the most important medicines in use today are obtained from terrestrial microorganisms.¹,² Often because of drug-resistance phenomena, 17 million lives every year are lost to infectious diseases,³ leading to the global concern that we may soon be facing a post-antibiotic era with reduced capabilities to combat microbes. As a consequence, a concerted worldwide search for new antibiotics from microbial origin is on-going, with focus on the potential of marine and terrestrial bacteria as source for novel metabolites with interesting biological and pharmaceutical properties.⁴,⁵ Diverse habitats, for example, tropical forests, deep sea sediments, sites of extreme temperature, salinity or pH, were explored and were successful to yield new microorganisms, which in turn provide the potential for novel metabolic pathways and new bioactive natural compounds.⁸ *Streptomyces* spp. are widespread in nature and continue to have a significant role in the production of bioactive metabolites. *Streptomyces* spp. produce many classes of secondary metabolites with great biofunctional diversity (antibiotics, antifungal, antiviral, anticancer, immunosuppressants, insecticides, herbicides etc.) and diverse chemical structures, which makes them useful as pharmaceuticals and agricultural agents.¹⁰

During our continued search for bioactive constituents from bacteria, strain DGC1 was isolated from a soil sample collected from the Devil's Golf Course salt pan (Death Valley National Park, CA, USA). Phylogenetic studies of DGC1 strain were conducted as described earlier,¹¹ and the amplified 500-bp 16S rRNA fragment was found to have 99% identity (BLAST) to the 16S rRNA gene of *Streptomyces atrovirens* strain NRRL B-16357. The extract obtained from the small-scale fermentation of *Streptomyces* sp. DGC1 on SG medium¹²,¹³ exhibited several unusual green fluorescent bands under long UV (365 nm), which stained to yellow with anisaldehyde/sulfuric acid in the pre-screening. A large-scale fermentation of the strain in SG medium afforded a crude extract from which different chromatographic techniques led to the isolation of five new benzamides: pyramidamycins A-D (2–5) and 3-hydroxyquinoline-2-carboxamide (6), whose structures were determined by NMR (1D and 2D) spectroscopy and MS (ESI and HR-ESI) studies (Figure 1).

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Benzamides are of increased interest, because Ning et al. have demonstrated recently that the synthetic benzamide chidamide is a potent histone deacetylase inhibitor in T-cell lymphoma cell lines. The new compounds were examined for antimicrobial and cytotoxic activities.

RESULTS AND DISCUSSION

In our search for new bioactive compounds from streptomycetes, Streptomyces sp. DGC1 (Supplementary Figure S2) was cultivated on ISP4 agar plates at 28°C for 3 days. After grown over, small agar pieces (circa 1 cm³) of the strain were used to inoculate 12 2-l Erlenmeyer flasks, each containing 670 ml of SG medium. The cultures were kept on a rotary shaker for 4 days at 28°C. The reddish-brown broth was harvested, mixed with celite, filtered off and extracted with ethyl acetate, and the mycelium was extracted with ethyl acetate followed by acetone. The combined organic extracts from supernatant and cells were concentrated in vacuo to afford 2.30 g of yellow-solid crude extract.

An TLC analysis of the strain extract exhibited several UV yellowish-green fluorescent bands at 366 nm, which turned yellow by staining with anisaldehyde/sulfuric-acid spraying reagent. The HPLC-MS analysis of the crude extract displayed several components with UV spectrum (Supporting Information, Supplementary Figures S3 and S4). Work-up and purification of the 2.30-g crude extract using various chromatographic techniques (Figure 2) led to the isolation of five new compounds, including pyramidamycins A-D (2-5) and 3-hydroxyquinoline-2-carboxamide (6), all five possessing an amide group (-CONH₂). In addition, the five known compounds 2-aminobenzamide (anthranilamide, 1, Supplementary Figures S5–S8), 4',7-dihydroxyisoflavanone (daidzein, 7, Supplementary Figures S48–S56), 2'-deoxy-thymidine, 2'-deoxy-uridine and adenosine, were also isolated and characterized.

Figure 1 Chemical structures of compounds 1–7.

Figure 2 Work-up procedure of extracts from Streptomyces sp. DGC1.
Table 1 Physicochemical properties of pyramidamycins A-C (2–4)

| Appearance | Pyramidamycin A (2) | Pyramidamycin B (3) | Pyramidamycin C (4) |
|------------|---------------------|---------------------|---------------------|
| Anisaldehyde/H2SO4b | Pale yellow | Pale yellow | Yellow |
| Rf | 0.32 (CH3Cl2/5% MeOH) | 0.22 (CH3Cl2/5% MeOH) | 0.57 (CH3Cl2/2% MeOH), 0.30 (CH3Cl2) |
| Molecular formula | C10H12N2O4 | C10H12N2O4 | C10H12N2O3 |
| (−)-ESI MS: m/z | 165 [M-H]− | 182 [M-H]− | 181 [M-H]− |
| (−)-ESI MS: m/z | 168 [M + H]+, 190 [M + Na]+ | 184 [M + H]+, 206 [M + Na]+ | 183 [M + H]+, 205 [M + Na]+ |
| (+)-HR-ESI MS (m/z) | 168.0654 (M + H)+ and 190.0489 (M + Na)+ | 184.0609 (M + H)+, 206.0435 (M + Na)+ | 183.0774 (M + H)+, 205.0600 (M + Na)+ |
| Calculated | 168.0655 for C10H12N2O4 and 190.0475 for C10H12N2O4Na | 184.0604 for C10H12N2O4, 206.0424 for C10H12N2O4Na | 183.0770 for C10H12N2O4, 205.0589 for C10H12N2O4Na |
| UV/VIS (MeOH): λmax (log ε) | 212 (4.45), 255 (4.17), 295 (3.90) nm | 214 (4.20), 260 (3.94), 295 (3.62) nm | 235 (4.42), 271 (4.02), 314 (3.63) nm |

aSee also Supplementary Figures S4, S9–S11, S23 and S28–S30 (for comparison).

bColoration with anisaldehyde/sulfuric-acid spraying reagent and heating.

| Appearance | Pyramidamycin D (5) | 3-Hydroxyquinoline-2-carboxamide (6) |
|------------|---------------------|------------------|
| Anisaldehyde/sulfuric acidb | White powder, UV absorbing | Pale-yellow solid, UV absorbing, green fluorescence under long UV (365 nm) |
| Rf | 0.31 (CH3Cl2/7% MeOH) | — |
| Molecular formula | C10H12N2O4 | C10H12N2O2 |
| (−)-ESI MS: m/z | 233 [M-H]− | 187 [M-H]− |
| (−)-ESI MS: m/z | 225 [M + H]+ | 189 [M + H]+ |
| (+)-HR-ESI MS (m/z) | 225.0875 (M + H)+, 247.0704 (M + Na)+ and 263.0438 (M + K)+ | 189.0653 (M + H)+ and 211.0473 (M + Na)+ |
| Calculated | 225.0870 for C10H12N2O4, 247.0689 for C10H12N2O4Na and 263.0429 for C10H12N2O4NaK for C10H12N2O4NaK | 189.0658 for C10H12N2O4 and 211.0478 |
| UV/VIS (MeOH): λmax (log ε) | 235 (4.13), 271 (3.84), 297 (3.63) nm | 218 (4.18), 231 (4.24), 296 (4.59), 358 (3.58) nm |

aSee also Supplementary Figures S4, S35 and S40–S42 (for comparison).

coloration with anisaldehyde/sulfuric-acid spraying reagent and heating.

NMR and mass data, by comparison with literature data, Structures 1 and 7 were determined by 1D and 2D NMR studies (Supplementary Figures S5–S8, and S48–S53), and by comparison with literature data. Compound 2 was obtained as a white solid. It is UV absorbing, exhibits a blue fluorescence under long UV (365 nm) and gave a pale-yellow color discoloration on spraying with anisaldehyde/sulfuric acid. The molecular formula of 2 was determined by HR-ESI MS as C10H12N2O4 (Table 1, Supplementary Figures S9–S22). The proton NMR spectrum of 2 in DMSO-d6 (Table 3) displayed one chelated broad signal for a hydroxyl group at δ 13.40 along with two doublet singlets at δ 8.21 and 7.68, typical for an amide group (−CONH2), which converted to a broad signal (as known from anthramide 1) of 2H at δ 5.75 when measured in CDCl3 solvent. In addition, the 1H NMR spectrum displayed ortho-coupled protons at δ 7.76 (d, J = 9.0 Hz) and 6.43 (dd, J = 9.0, 2.5 Hz), a meta-coupled proton at δ 6.39 (d, J = 2.5 Hz) as well as a methoxy singlet at δ 3.75 (s), representing a trisubstituted benzene. The 13C NMR/HSQC spectra (Table 4) confirmed compound 2 to be 2-hydroxy-4-methoxybenzamide and showed the OH group at C-2 (δ 163.6) chelated with the amide carbonyl (δ 172.4) and the methoxy group (δ 55.5) located at C-4 (δ 164.0). The HMBC correlations (Figure 3) of compound 2 finalized the structure, showing correlations from the doublet proton H-6 (δ 7.76) to the amide carbonyl (δ 172.4), C-2 (δ 163.6) and C-4 (δ 164.0). The methoxy group (δ 3.75) could be determined as being attached at C-4 due to its significant HMBC correlation with C-4 (δ 164.0). Furthermore, NOESY correlations between this methoxy group and H-3 as well as H-5 were observed, all of which confirmed structure 2 to be 2-hydroxy-4-methoxybenzamide (Figure 3, Tables 3 and 4). A database search (chemical abstracts) confirmed the novelty of structure 2, which was subsequently named pyramidamycin A.

Compound 3 was obtained as a colorless solid, with a molecular weight of 183 Da corresponding to a molecular formula of C10H12N2O3.
Table 3 ¹H NMR (500 MHz) data of the benzamides 1–5 in DMSO-d₆, δ in p.p.m. relative to TMS, multiplicities (J Hz⁻¹)

| Position | Anthranilamide (1)a | Pyramidamycin A (2)a,b | Pyramidamycin B (3)a | Pyramidamycin C (4)a | Pyramidamycin D (5)a |
|----------|---------------------|------------------------|----------------------|----------------------|----------------------|
| 1-CONH₂  | 7.70 (1H, br s)     | 8.21 (1H, br s)        | 8.29 (1H, br s)      | 8.16 (1H, br s)      | 8.30 (1H, br s)      |
|          | 7.03 (1H, br s)     | 7.68 (1H, br s)        | 7.63 (1H, br s)      | 7.59 (1H, br s)      | 7.78 (1H, br s)      |
| 2-OH     | —                   | 13.40 (1H, br s)       | 13.49 (1H, br s)     | 13.0–11.0 (1H, br s) | 13.52 (1H, br s)     |
| 2-NH₂    | 6.54 (2H, br s)     | —                      | —                    | —                    | —                    |
| 3        | 6.66 (1H, dd, 8.5, 1.0) | 6.39 (1H, d, 2.5) | —                    | —                    |
| 3-NH     | —                   | —                      | —                    | —                    |
| 3-NHOCH₃ | —                   | —                      | 3.68 (3H, s)         | —                    |
| 3-NH₂    | —                   | —                      | —                    | 13.0–11.0 (2H, br s) | —                    |
| 3-OCH₃   | —                   | —                      | —                    | —                    |
| 4        | 7.12 (1H, td, 7.0, 1.5) | —                      | —                    | —                    |
| 4-OH     | —                   | —                      | 9.86 (1H, br s)      | —                    |
| 4-OCH₃   | —                   | —                      | 3.75 (3H, s)         | 3.80 (3H, s)         |
| 5        | 6.47 (1H, td, 8.0, 1.0) | 6.43 (1H, dd, 9.0, 2.5) | 6.33 (1H, d, 8.5) | 6.47 (d, 9.0) | 6.58 (d, 8.5) |
| 6        | 7.51 (1H, dd, 8.0, 1.5) | 7.76 (1H, d, 9.0) | 7.43 (1H, d, 9.0) | 7.16 (d, 9.0) | 7.77 (d, 9.0) |

Abbreviation: TMS, tetramethylsilane.
aSee also Supplementary Figures S5–S8, S12–S22, S24–S27, S31–S34, S36–S39 and S43–S47 for comparison.
bFor ¹H NMR data in CDCl₃, see the experimental part.
Underlined entries refer to the protons (or carbons) of the listed position.

Table 4 ¹³C NMR (125 MHz) data of benzamides 1–5 in DMSO-d₆, (δC, mult.)

| Position | Anthranilamide (1)a | Pyramidamycin A (2)a,b | Pyramidamycin B (3)a | Pyramidamycin C (4)a | Pyramidamycin D (5)a |
|----------|---------------------|------------------------|----------------------|----------------------|----------------------|
| 1        | 113.7 s             | 107.3 s                | 106.9 s              | 107.5 s              | 107.8 s              |
| 1-CO     | 171.3 s             | 172.4 s                | 172.9 s              | 173.4 s              | 172.5 s              |
| 2        | 150.2 s             | 163.6 s                | 156.7 s              | 149.6 s              | 158.8 s              |
| 3        | 116.4 d             | 101.2 d                | 135.1 s              | 124.9 s              | 113.9 s              |
| 3-NHCO   | —                   | —                      | —                    | —                    |
| 3-NH₂    | —                   | —                      | —                    | 13.0–11.0 (2H, br s) | —                    |
| 3-OCH₃   | —                   | —                      | —                    | —                    |
| 4        | 131.9 d             | 164.0 s                | 154.9 s              | 149.7 s              | 159.4 s              |
| 4-OCH₃   | 55.5 q              | 55.9 q                 | 55.9 q               | 55.9 q               |
| 5        | 114.5 d             | 106.1 d                | 107.0 d              | 102.3 d              | 102.0 d              |
| 6        | 128.5 d             | 129.5 d                | 123.5 d              | 123.5 d              | 127.1 d              |

aSee also Supplementary Figures S6, S13, S25, S32 and S37 for comparison.
bFor ¹³C NMR data in CDCl₃, see the experimental part.
Underlined entries refer to the protons (or carbons) of the listed position.

Figure 3 ¹H-¹H-COSY (bold lines) and selected HMBC (→) correlations in compounds 1–7.
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Figure 4 Chemical structures of compounds 8–10.

Table 5 1H (500 MHz) and 13C NMR (125 MHz) data of 3-hydroxyquinoline-2-carboxamide (6) in DMSO-d6, δ in p.p.m. relative to TMS

| Position | δC (mult.) | δH (J Hz) |
|----------|------------|-----------|
| 2        | 135.8 s    | —         |
| 2-CO     | 171.8 s    | —         |
| 2-CONH2  | —          | 8.78 (1H, br s), 8.23 (1H, br s) |
| 3        | 153.9 s    | —         |
| 3-OH     | —          | 12.32 (1H, br s) |
| 4        | 120.5 d    | 7.75 (1H, s) |
| 4a       | 132.2 s    | —         |
| 5        | 127.2 d    | 7.82 (1H, br d, 8.0) |
| 6        | 129.6 d    | 7.57 (1H, br, 6.5) |
| 7        | 128.3 d    | 7.59 (1H, br, 7.0) |
| 8        | 129.9 d    | 7.99 (1H, br d, 7.5) |
| 8a       | 141.6 s    | —         |

Abbreviation: TMS, tetramethylsilane.
*See also Supplementary Figures S43–S47 for comparison.
Underlined entries refer to the delta value of the carbon atom.

methoxybenzamide, and named pyramidamycin D (for spectra, see Supplementary Figures S35–S39).

Compound 6 was isolated from fraction FII as a pale-yellow solid. It shows green fluorescence under long UV (365 nm), and has a molecular weight of m/z 188, corresponding to the molecular formula C6H10N2O2 determined by HR-ESI MS. The 1H NMR spectrum revealed signals for a disubstituted benzene ring, a chelated OH group (δ 12.32), one singlet aromatic proton (δ 7.75) along with the typical broad signals of the amide protons (-CONH2) as in the above discussed compounds (1–5, Figure 3). The 13C NMR/HSQC spectra revealed ten carbons, five sp2 methine (δ 129.9, 129.6, 128.3, 127.2 and 120.5, Table 5) and four quaternary sp2 carbon atoms (δ 171.8, 153.9, 141.6, 135.8 and 133.2), of which the first one is the carbonyl amide. In the HMBC spectrum (Figure 3), the disubstituted benzene ring was confirmed and the chemical shift of one of its quaternary carbons at δ 141.6 should be linked to a heterocyclic nitrogen atom, such as in indole or quinoline moieties. The remaining singlet methine proton was in peri-position to H-5 (δ 7.82) based on the observed HMBC correlations between H-4 (δ 7.75) and C-5 (δ 127.2) and from H-5 (δ 7.82) to C-3 (δ 120.5). In addition, 31C-H HMBC correlations (Figure 3) were observed from the amide protons (-CONH2) and the singlet methine H-4 (δ 7.75) to C-2 (δ 135.8), along with the 31C-H HMBC coupling of H-4 (δ 7.75) to C-3 (δ 153.9), confirming the structure of 6 as 3-hydroxyquinoline-2-carboxamide. Based on literature search, compound 6 is a new
natural product. The compound was previously mentioned by Kaneko and Yameda\(^2\) as one of the intermediates of their synthesis of 3-hydroxyquinoline derivatives, however, no NMR and MS data were reported (for spectra, see Supplementary Figures S40–S47).

**Biological activity**

**Cytotoxicity assays.** The cytotoxic activity of antranilamide (1), pyramidamycins A-D (2–5), 3-hydroxy-quinoline-2-carboxamide (6) and isoflavanone (7) was determined in comparison with the known strong cytotoxic angucyclin landomyacin A, using PC3 (Prostate cancer) and H460 (non-small cell lung cancer) cell lines (Figures 5a and b, Table 6). Cell-viability assays showed that pyramidamycin C (4) was the most active compound against both PC3 and H460 cell lines (GI\(_{50}\) = 2.473 and 7.339 \(\mu\)M, respectively). It exhibited cytostatic activity at all tested concentrations, but was less active than landomyacin A (GI\(_{50}\) = 0.5505 and 4.109 \(\mu\)M, respectively). Pyramidamycin C (4) has a free amino group (-NH\(_2\)) connected at C-3, which may be responsible for its cytostatic activity. Acetylation of this amino group, as found in the congener pyramidamycin D (5), led to the complete loss of cytostatic activity. All other compounds showed low or no activity, except 3-hydroxy-quinoline-2-carboxamide (6), which revealed some moderate cytostatic activity in the PC3 cell line (Figure 5a, Table 6).

**Antibacterial activity.** The antibacterial activity of compounds 1–7 were also determined against the Gram-negative bacterium Escherichia coli DH5\(_{\alpha}\) (Invitrogen, Grand Island, NY, USA) and the Gram-positive bacteria Micrococcus luteus NRRL B-2618 and Kocuria rosea B-1106 (Table 7). K. rosea B-1106 was included in the testing as a second representative Gram-positive bacterium of the family Micrococcaceae. Of these compounds, benzamides 1–3 were the most active against the Gram-positive bacterium K. rosea B-1106, with 1 producing a diameter halo of 13 ± 2 mm. Compounds 5 and 6 were slightly active against Gram-negative E. coli DH5\(_{\alpha}\) (5 = 10 ± 2 mm, 6 = 8 ± 2 mm). Furthermore, compound 6 demonstrated the widest range of activities of those compounds tested, inhibiting also the Gram-positive M. luteus NRRL B-2618 (9 ± 2 mm).

**EXPERIMENTAL PROCEDURE**

**General experimental procedures**

UV spectra were recorded on a Shimadzu UV-1800 (Model TCC-240A) UV spectrometer (Shimadzu, Kyoto, Japan). NMR spectra were measured on a Varian Vnmr 500 (\(^1\)H, 500 MHz; \(^1\)C, 125.7 MHz) spectrometer; the \(\delta\) values were referenced to the solvent signals (62.5 and 40.66 p.p.m., respectively, for DMSO-d\(_6\), and 87.21 and 77.06 p.p.m., respectively, for CDCl\(_3\)). ESI mass spectra were recorded on a Finnigan LCQ ion trap mass spectrometer (Thermo Finnigan, Waltham, MA, USA). HR-ESI mass spectra were recorded on an Agilent LC/MSD TOF (resolution: 10 000; 3 p.p.m. mass accuracy; inlet systems: Agilent Technologies 1200 Series LC pumps) mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Samples were introduced by means of a syringe pump. Prep C\(_18\) 7-μm column (7.8 × 300 mm) on a binary LC system (solvent A: H\(_2\)O/0.2% formic acid; solvent B: acetonitrile; flow rate: 2.0 ml min\(^{-1}\); 0–15 min, 0–75% A (linear gradient); 15–20 min, 0% A and 100% B; 20–22 min, 0–75% A (linear gradient); 22–27 min, 75% A). HPLC-MS analyses were carried out using a Symmetry Anal C\(_18\) 5-μm column (4.6 × 250 mm\(^2\)) on a binary LC system. Flash chromatography was carried out on silica gel MN 60 (140–270 mesh ASTM). \(R_t\) values were measured on Polygram SIL G/UV254 (Macherey–Nagel & Co., Düren, Germany). Size-exclusion chromatography was performed on Sephadex LH-20 (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

**Taxonomy**

A soil sample containing DGC1 was collected from the Devil’s Golf Course salt pan (Death Valley National Park). Approximately 1 g of soil sample was
suspended in 25 ml sterile H2O and was subsequently heated to 80 °C for 30 min. After heating, the sample was briefly vortexed and the large sediment was allowed to settle before plating 100 μl serial dilutions of the supernatant (10-1, 10-2, 10-4 and 10-6) onto oatmeal-agar plates supplemented with naldixic acid (1 mg) and cycloheximide (10 mg). After 5 days of incubation at 30 °C, several bacterial colonies were observed. After a total incubation time of 7 days, over 50 individual colonies were streaked on oatmeal, M2, ISP2 and ISP4 agar plates. Colonies with general morphological similarities to streptomycetes were taken and streaked onto agar plates, in which they were found to have optimal growth (oatmeal, M2, ISP2 or ISP4). Finally, strains were grown in trypsic soy broth liquid media and stored as glycerol stocks for further study.

Phylogenetic studies of DGC1 were conducted as previously described.11

The amplified 500-bp 16S rRNA fragment was found to have 99% identity (BLAST search) to the 16S rRNA gene of S. atrovirens strain NRRL B-16357. The sequenced 16S rRNA gene fragment from DGC1 has been deposited in the NCBI nucleotide database with an accession number JN836739.

Cell-viability assay
Prostate cancer cell line PC3 and non-small cell lung cancer cell line H460 were used to determine the cytotoxicity of amides 1-7 in comparison with laminodycin A. Experiments were performed in four replicates and conducted as previously described.25

Antibacterial activity test
The Gram-negative bacterium E. coli DH5α (Invitrogen) and the Gram-positive bacteria M. luteus NRRL B-2618 and K. rosea B-1106 were maintained in lysogenic broth liquid media and Mueller-Hinton agar (beef infusion 2 g/l-1, casein peptone 17.5 g/l, starch 1.5 g/l-1, Difco granulated agar 17 g/l-1, 11 of double distilled H2O, autoclaved). A sterile loopful of culture material was allowed to settle before plating 100 μl amounts per each 6-mm sterile filter disc and were allowed to dry in vacuo.

Fermentation, extraction and isolation. Streptomyces sp. DGC1 was cultivated on ISP4 agar plates at 28 °C for 3 days. Pieces of well-grown agar subculture of the strain were used to inoculate 12-21 flask, each containing 670 ml of 5% medium, which was grown at 28 °C and harvested after 4 days. The obtained reddish brown culture broth was mixed with celite and filtered off. The water phase was extracted with EtOAc (4 x 21) and the biomass was extracted with EtOAc (4 x 500 ml), then with acetone (1 x 300 ml). The organic extracts were evaporated in vacuo at 38 °C, and then combined after evaporation based on the TLC profile affording 2.30 g of yellow-solid crude extract.

Separation of the obtained crude extract on silica gel column (column 2.5 x 50 cm2, 120 g), using a stepwise MeOH/CH2Cl2 gradient (0.41

0% MeOH→fraction FI, then 0.21% MeOH and 0.21% MeOH combined→fraction FII, then 0.21% MeOH combined→fraction FII, then 0.31% MeOH and 0.21% MeOH combined→fraction FIV), yielded four fractions, FII (350 mg, yellow oil), FIII (40.8 mg, yellow solid), FIII (700 mg, yellow solid) and FIV (400 mg, yellow solid). Fraction FII was identified as fats based on TLC, HPLC-MS and anisaldehyde/sulfuric-acid spraying reagent. Fraction FII was further purified using Sephadex LH-20 (2 x 50 cm2, 50% MeOH/CH2Cl2 to give 3-hydroxyquinoline-2-carboxamide (6; pale-yellow solid, 4.2 mg). Purification of fraction FIII was carried out by Sephadex LH-20 (2 x 50 cm2, 50% MeOH/CH2Cl2) followed by HPLC to yield anthranilamide (1; pale-yellow solid, 3.8 mg), pyridamycin A (2; white solid, 5.6 mg), B (3; white solid, 7.2 mg) and C (4; white powder, 4.7 mg). Finally, fractionation and purification of fraction FIV using Sephadex LH-20, PTLC and HPLC afforded pyridamycin D (5.7 mg, 2,6-dihydroxyisoflavonane (7; yellow solid, 3.3 mg), 2-deoxy-thymidine (white solid, 3.8 mg), 2-deoxy-uridine (white solid, 5.1 mg) and adenosine (white solid, 10.3 mg) in pure forms (Figure 2).

2-Aminobenzamide; anthranilamide (1). Pale-yellow solid; UV absorbing (254 nm), blue fluorescence under long UV (365 nm); Rf 0.59 (70% MeOH/CH2Cl2). Yellow coloration with anisaldehyde/sulfuric-acid spraying reagent; UV (MeOH) λmax (log ε) 214 (4.14), 256 (3.66), 330 (3.53) nm; 1H NMR (DMSO-d6, 500 MHz) and 13C NMR (DMSO-d6, 125 MHz), see Tables 3 and 4; (–)→APCI MS m/z 135 [M–H]+; (+)→ESI MS m/z 135 [M–H]+.

Pyridamycin A (2). Physicochemical properties, see Table 1; 1H NMR (DMSO-d6, 500 MHz) and 13C NMR (DMSO-d6, 125 MHz), see Tables 3 and 4; 1H NMR (CDCl3, 500 MHz) δ 12.48 (1H, br s, 2-OH), 7.25 (1H, d, 9.0, H-6), 6.45 (1H, d, 2.5, H-5), 6.40 (1H, dd, 9.5, 2.5, H-5), 5.75 (2H, br s, NH2), 3.80 (3H, s, 4-OCH3); 13C NMR (CDCl3, 125 MHz) δ 172.5 (1-CO), 165.2 (Cq-4), 164.6 (Cq-2), 127.9 (CH-6), 107.5 (CH-5), 101.7 (CH-3), 55.7 (3-OCH3).

2,6-Dihydroxyisoflavonane; daidzein (7). Yellow solid; Rf 0.43 (70% MeOH/CH2Cl2); UV absorbing (254 nm); 1H NMR (DMSO-d6, 500 MHz) δ 10.84 (1H, br s, 4′-OH), 9.54 (1H, br s, 7-OH), 8.28 (1H, s, H-2), 7.96 (1H, d, 8.5, H-6), 6.93 (1H, dd, 8.5, 2.5, H-6), 6.86 (1H, d, 2.0, H-8), 7.38 (2H, dd, 6.5, 2,5, 2′H, 2,5′-H), 6.80 (2H, dd, 7.0, 2, 3′H, 3,4′-H) p.p.m.; 13C NMR (DMSO-d6, 125 MHz), δ 152.8 (CH-2), 123.5 (Cq-3), 174.7 (CO-4), 116.6 (Cq-4a), 127.3 (CH-5), 113.9 (CH-6), 162.6 (Cq-7), 102.1 (CH-8), 157.4 (Cq-8a), 122.5 (Cq-1′), 130.1 (CH-2′/CH-2″), 115.0 (CH-3′/CH-3″), 157.2 (Cq-4′); (–)→APCI MS m/z 253 [M–H]+.

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