Published in final edited form as:

J Antibiot (Tokyo). 2012 December ; 65(12): 615–622. doi:10.1038/ja.2012.81.

Pyramidamycins A-D and 3-Hydroxyquinoline-2-carboxamide; Cytotoxic Benzamides from *Streptomyces* sp. DGC1

Khaled A. Shaaban[1], **Micah D. Shepherd**[1], **Tamer A. Ahmed**[1], **S. Eric Nybo**[1], **Markos Leggas**[1,2], and **Jürgen Rohr**[*,1,2]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536-0596, U.S.A

[2]Markey Cancer Center, University of Kentucky, 800 Rose Street, Lexington, KY 40536-0293, U.S.A

Abstract

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 HRMS 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 hrs and against several bacterialstrains. Pyramidamycin C (4) was the most active compound against both PC3 and H460 cell lines (GI_{50} = 2.473 μM and GI_{50} = 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 mm 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.

Keywords

benzamides; anticancer agents; cytotoxicity; antibacterial; taxonomy; streptomycetes

Correspondence: J. Rohr, Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536-0596, U.S.A. jrohr2@email.uky.edu.

Supplementary Information accompanies this paper on the Journal of Antibiotics website (http>//www.nature.com/ja) including the partial 16S rDNA sequence of DGC1 strain, HPLC analysis of the crude extract obtained from the terrestrial *Streptomyces* sp. DGC1, some work-up procedure photographs, and HRMS and NMR spectra.

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 due to 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.[4–7] Diverse habitats, e.g., 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 play a significant role in the production of bioactive metabolites. *Streptomyces* spp. produce many classes of secondary metabolites with great bio-functional diversity (antibiotics, antifungal, antiviral, anticancer, immunosuppressants, insecticides, herbicides etc.) and diverse chemical structures, which makes them useful as pharmaceuticals and agricultural agents.[1,9,10]

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, California, 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 *S. atrovirens* strain NRRL B-16357. The extract obtained from the small scale fermentation of *Streptomyces* sp. DGC1 on SG-Medium,[12,13] exhibited several unusual green fluorescent bands under long UV (365 nm), which stained to yellow with anisaldehyde/sulphuric 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 & 2D) spectroscopy and mass spectrometry (ESI and HRESI) studies (Figure 1). Benzamides are of increased interest, since Ning and co-workers have demonstrated recently that the synthetic benzamide chidamide is a potent histone deacetylase inhibitor in T-cell lymphoma cell lines.[14] 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 was cultivated on ISP4-agar plates at 28 °C for 3 days. After grown over, small agar pieces (circa 1 cm^3) of the strain were used to inoculate twelve 2 L Erlenmeyer flasks each containing 670 mL of SG-medium.[12,13] 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.

A 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, Figure 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 (-$CONH_2$). In addition, the five known compounds 2-aminobenzamide (anthranilamide, **1**),[15,16] 4′,7-dihydroxyisoflavanone (daidzein, **7**),[17,18] 2′-deoxy-thymidine,[19] 2′-deoxy-uridine[20] and adenosine,[19,21,22] were also isolated and characterized.

Structure elucidation

The physicochemical properties of compounds **1**~**6** are summarized in tables 1 and 2. The known compounds were identified from their NMR and mass data, by comparison with literature data. Structures **1** and **7** were determined by 1D and 2D NMR studies, 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 HRESIMS as $C_8H_9NO_3$ (Table 1). The proton NMR spectrum of **2** in DMSO-d_6 (Table 3) displayed one chelated broad signal for a hydroxyl group at δ 13.40 along with two broad singlets at δ 8.21 and 7.68, typical for an amide group (-$CONH_2$), which converted to a broad signal (as known from anthranilamide **1**) of 2H at δ 5.75 when measured in $CDCl_3$ solvent. Additionally, the ^{1}H 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 ^{13}C 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 ^3J correlations from the doublet proton H-6 (δ 7.76) to the amide-carbonyl (δ 172.4), to C-2 (δ 163.6) and to 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 to C-4 (δ 164.0). Furthermore, NOESY correlations between this methoxy group and H-3 as well asH-5 were observed, all of which confirmed structure **2** as 2-hydroxy-4-methoxybenzamide, (Figure 3, Tables 3, 4). A data base 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 Daltons corresponding to a molecular formula of $C_8H_9NO_4$, as deduced by HRESIMS (table 1). The proton NMR spectrum (Table 3) and the ^{13}C NMR/HSQC spectra(Table 4) of **3** showed that it contains the samebenzamide core as compound **2** with Δm/z =16 *amu* higher than **2** corresponding to an additional oxygen atom. An additional broad signal at δ 9.86 in the proton NMR spectrum and the absence of the meta-coupled aromatic proton at (at C-3 of compound **2**) suggested that the extra OH group (δ 9.86) might be located at C-3 (like in the

hypothetical structure **8**, Figure 5). However, based on the full 2D-NMR studies, the methoxy group (δ 3.68) showed an HMBC correlation to C-3 (δ 135.1), confirming its linkage at C-3, rendering the OH group at 4-position (structure **3**). All of the remaining HMBC correlations (Figure 3) and NMR data (Tables 3, 4) are in full agreement with structure **3**. Compound **3** is a new structural analogue of **2**, 2,4-dihydroxy-3-methoxybenzamide, and was named pyramidamycin B.

Closely related to pyramidamycin B (**3**) compound **4** was obtained as a white powder from the same fraction FIII, exhibiting a molecular formula of $C_8H_{10}N_2O_3$ (HRESI MS), which is 1 *amu* smaller than **3**, indicating that one of the OH groups was replaced by an NH_2 group (Table 1). The ^1H and ^{13}C NMR data of **4** were similar to those of **3** (Tables 3 and 4), giving two alternative possible structures (**4** and **9**, see Figure 5 for alternative structures **8~10**) depending on the positions of the methoxy and the amino groups. In the HMBC spectra, a ^3J correlation was observed from the methoxy group (δ 3.79) to C-4 (δ 149.7) confirming its linkage to C-4 as in compound **2**, and not to C-3 (δ 124.9) as in **3**. All the remaining HMBC correlations (Figure 3) and NMR data (Tables 3, 4) are in full agreement with structure **4**. Therefore, structure **4** was determined as 3-amino-2-hydroxy-4-methoxybenzamide, and consequently named pyramidamycin C.

Structurally related to pyramidamycin C (**4**) compound **5** was obtained as an orange solid, with a molecular formula of $C_{10}H_{12}N_2O_4$ (HREIMS), i.e. by 42 *amu* (typical for an acetyl group) higher than **4** (for physico-chemical properties see tables 1, 2). The comparison of the NMR data of compound **5** with those of pyaramidamycin C (**4**) confirmed that **5** contains an additional acetyl group (-$COCH_3$) and the chemical shift of its carbonyl (δ 168.3) suggested an amide or ester connectivity, leaving the two alternative structures **5** and **10**. The proton NMR spectra of **5** showed a chelated broad signal at δ 13.52, indicating a free hydroxyl group at C-2 and a broad signal of an NH group at δ 8.87, thus excluding the isomeric structure **10**. Compound **5** was further subjected to 2D NMR (HSQC and HMBC) experiments, and ^2J correlationswere observed from the NH (δ 8.87) and CH_3 (δ 1.96) to the carbonyl at δ 168.3 confirming the acetamide moiety. The observed $^3J_{C\text{-}H}$ HMBC coupling from the methoxy group (δ 3.80) to C-4 (δ 159.4) also confirmed its linkage to C-4. Also the remaining HMBC couplings (Figure 3) and the other NMR data (tables 3, 4) are in full agreement with structure **5**. Thus, compound **5** was identified as 3-acetamido-2-hydroxy-4-methoxybenzamide, and named pyramidamycin D.

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 $C_{10}H_8N_2O_2$ determined by HRESIMS. The ^1H NMR spectrum revealed signals for a di-substituted 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 (-$CONH_2$) as in the above discussed compounds (**1**~**5**, Figure 3). The ^{13}C NMR/HMQC spectra revealed ten carbons, five sp^2 methine (δ 129.9, 129.6, 128.3, 127.2 and 120.5, Table 5) and four quaternary sp^2 carbon atoms (δ 171.8, 153.9, 141.6, 135.8 and 132.2), of which the first one is the carbonyl amide. In the HMBC spectrum (Figure 3), the di-substituted 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) to C-5 (δ 127.2) and from H-5 (δ 7.82) to C-3 (δ 120.5). Additionally, $^{3}J_{C\text{-}H}$ HMBC correlations (Figure 3) were observed from the amide protons (-$CONH_2$) and from the singlet methine H-4 (δ 7.75) to C-2 (δ 135.8) along with the $^{2}J_{C\text{-}H}$ 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 et al. as one of the intermediates of their synthesis of 3-hydroxyquinoline derivatives, however no NMR and MS data were reported.[23]

Biological activity

Cytotoxicity assays—The cytotoxic activity of anthranilamide (**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 landomycin A, using PC3 (Prostate cancer) and H460 (non small cell lung cancer) cell lines (Figure 4A 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 μM and GI_{50} = 7.339 μM, respectively). It exhibited cytostatic activity at all tested concentrations, but was less active than landomycin A (GI_{50} = 0.5505 μM and GI_{50} = 4.109 μM, 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 4A, Table 6).

Antibacterial activity—The antibacterial activity of compounds **1**~**7** were also determined against the Gram-negative bacterium *Escherichia coli* DH5α (Invitrogen) and the Gram-positive bacteria *Micrococcus luteus* NRRL B-2618 and *Kocuria rosea* B-1106 (Table 7). *Kocuria 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 *Kocuria 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* DH5α (**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 SECTION

General experimental procedures

UV spectra were recorded on a Shimadzu UV-1800 (Model TCC-240A) UV spectrometer. NMR spectra were measured on a Varian VnmrJ 500 (^{1}H, 500 MHz; ^{13}C, 125.7 MHz) spectrometer; the δ-values were referenced to the solvent signals (δ 2.5 and 40.6 ppm, respectively, for DMSO-d_6, and δ 7.21 and 77.06 ppm, respectively, for $CDCl_3$). ESI mass spectra were recorded on a Finnigan LCQ ion trap mass spectrometer. ESIHR mass spectra

were recorded on an Agilent LC/MSD TOF (Resolution: 10,000; 3 ppm mass accuracy; Inlet Systems: Agilent Technologies 1200 Series LC pumps) Mass Spectrometer, Manufacturer: Agilent Palo Alto, CA, USA. Samples were introduced by means of a syringe pump. Prep C_{18} 7μm column (7.8 × 300 mm) on a binary LC system (Solvent A: H_2O/0.2%/formic acid, solvent B: acetonitrile; flow rate: 2.0 mL min^{-1}; 0–15 min, 75-0% 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} 5μm column (4.6 × 250 mm) on a binary LC system. Flash chromatography was carried out on silica gel MN 60 (140–270 mesh ASTM). R_f values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). Size exclusion chromatography was performed on Sephadex LH-20 (GE Healthcare).

Taxonomy

A soil sample containing DGC1 was collected from the Devil's Golf Course salt pan (Death Valley National Park, California, USA). Approximately 1 g of soil sample was suspended in 25 mL sterile H_2O and was subsequently heated to 80 °C water 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}, 10^{-8}) onto oatmeal agar plates supplemented with nalidixic 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,[24] and ISP4[24] 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 tryptic soy broth (TSB) 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 landomycin A. Experiments were performed in four replicates and conducted as previously described.[25]

Antibacterial Activity Test

The Gram-negative bacterium *Escherichia coli* DH5α (Invitrogen) and the Gram-positive bacteria *Micrococcus luteus* NRRL B-2618 and *Kocuria rosea* B-1106 were maintained in lysogeny broth (LB) liquid media and Mueller-Hinton agar (beef infusion, 2 g L^{-1}, casein peptone, 17.5 g L^{-1}, starch, 1.5 g L^{-1}, Difco granulated agar, 17 g L^{-1}, one liter of double distilled H_2O, autoclaved). A sterile loopful of each organism was inoculated into a 7 mL culture of LB broth and incubated in a 37 °C orbital shaker at 200 RPM for 10 hours. Each test organism was streaked on a sterile Mueller-Hinton agar plate with a sterile cotton swab.

Compounds **1~7** were dissolved in methanol and were aliquoted in 100 μg amounts per each 6 mm sterile filter disc and were allowed to dry in a laminar flow hood. The discs were placed on the plates, which were then incubated for 24 hours at 37 °C.[26,27] The resulting diameter halos were measured (table 7).

Culture material, fermentation and isolation

SG-Medium—Glucose (20 g, Sigma-Aldrich), yeast extract (5 g, Acros Organics), Soytone (10 g, Becton, Dickinson & Co), $CoCl_2$ x 6 H_2O (1 mg, Acros Organics) and calcium carbonate (2 g, Sigma-Aldrich) were dissolved in 1 liter of demineralized water. The suspension (pH 7.2) was sterilized by autoclaving for 33 min at 121 °C.

Oatmeal-Agar Medium—Oatmeal (60 g) and agar (12.5 g, Becton, Dickinson & Co) were dissolved in 1 liter of demineralized water. The pH of the medium was adjusted to 6.0 with 1M NaOH and 1M HCl before sterilization

M2-Agar Medium—Glucose (4.0 g, Sigma-Aldrich), yeast extract (4.0 g, Acros Organics), malt extract (10.0 g, MP Biomedicals, LLC) and agar (15.0 g, Becton, Dickinson & Co) were dissolved in 1 liter of demineralized water.

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 of 2 Lflasks each containing 670 mL of SG-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 × 2L) and the biomass was extracted with EtOAc (4 × 500 mL), then with acetone (1 × 300 mL). The organic extracts were evaporated *in vacuo* at 38 °C, and 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 × 50 cm, 120 g), using a stepwise MeOH/CH_2Cl_2 gradient (0.4 L 0% MeOH → fraction FI, then 0.2 L 3% MeOH and 0.2 L 5% MeOH combined → fraction FII, then 0.2 L 10%MeOH and 0.2 L 20%MeOH combined → fraction FIII, then 0.3 L 50% MeOH and 0.2 L 100% MeOH combined → fraction FIV), yielded four fractions, FI (350 mg, yellow-oil), FII (40.8 mg, yellow solid), FIII (700 mg, yellow solid) and FIV (400 mg, yellow solid). Fraction FI was identified as fats based on TLC, HPLCMS and anisaldehyde/sulfuric acid spraying reagent. Fraction FII was further purified using Sephadex LH-20 (2× 50 cm, 50% MeOH/CH_2Cl_2) 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× 50 cm, 50% MeOH/CH_2Cl_2) followed by HPLC to yield anthranilamide (**1**; pale yellow solid, 3.8 mg), pyramidamycin 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 usingSephadex LH-20, PTLC and HPLC afforded pyramidamycin D (**5**, 7.2 mg), 4′,7-dihydroxyisoflavanone (**7**; yellow solid, 5.3 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); R_f 0.59 (7% MeOH/CH_2Cl_2), 0.31 (CH_2Cl_2); yellow coloration with anisaldehyde/sulfuric acid spraying reagent; UV (MeOH) λ_{max} (log ε) 214 (4.14), 256 sh (3.66), 330 (3.53) nm; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see tables 3 and 4; (−)-APCI MS *m/z* 135 $[M-H]^-$; (−)-ESI MS *m/z* 135 $[M-H]^-$; (+)-ESI MS *m/z* 137 $[M+H]^+$.

Pyramidamycin A (2): Physico-chemical properties, see table 1; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 3 and 4; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.48 (1H, brs, 2-OH), 7.25 (1H, d, 9.0, H-6), 6.45 (1H, d, 2.5, H-3), 6.40 (1H, dd, 9.5, 2.5, H-5), 5.75 (2H, brs, NH_2), 3.80 (3H, s, 4-OCH_3); ^{13}C NMR ($CDCl_3$, 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 (4-OCH_3).

4′,7-Dihydroxyisoflavanone; Daidzein (7): Yellow solid; R_f 0.43 (7% MeOH/CH_2Cl_2); UV absorbing (254 nm); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.84 (1H, brs, 4′-OH), 9.54 (1H, brs, 7-OH), 8.28 (1H, s, H-2), 7.96 (1H, d, 8.5, H-5), 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″-H), 6.80 (2H, dd, 7.0, 2.0, 3′-H, 3″-H) ppm; ^{13}C NMR (DMSO-d_6, 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 $[M-H]^-$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

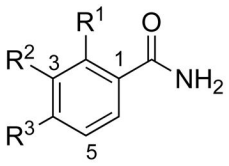
Acknowledgments

We thank Dr. Jack Goodman (University of Kentucky) for the ESIMS measurements. The mass spectrometry department, University of Wisconsin Biotechnology Center is acknowledged for the HRMS data. This work was supported by grants CA102102 and CA 091901 from the US National Institutes of Health to J. R.

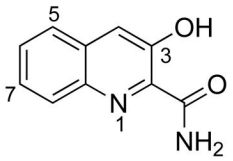
References

1. Bérdy J. Bioactive Microbial Metabolites. J Antibiot. 2005; 58:1–26. [PubMed: 15813176]
2. Kelecom A. Secondary Metabolites from Marine Microorganisms. An Acad Bras Cienc. 2002; 74:151–170. [PubMed: 11960184]
3. Wiedemann B. GIT Labor Medizin. 1996; 5:217–226.
4. Bernan VS, Greenstein M, Maiese WM. Marine Microorganisms as a Source of New Natural Products. Adv Appl Microbiol. 1997; 43:57–90. [PubMed: 9097412]
5. Faulkner DJ. Marine Natural Products. Nat Prod Rep. 2002; 19:1–48. [PubMed: 11902436]
6. Moore BS. Biosynthesis of Marine Natural Products: Macroorganisms (Part B). Nat Prod Rep. 2006; 23:615–629. [PubMed: 16874392]
7. Fenical W. New Pharmaceuticals from Marine Organisms. Trends Biotechnol. 1997; 15:339–341. [PubMed: 9293031]
8. Pelaez F. The Historical Delivery of Antibiotics from Microbial Natural Products - Can History Repeat? Biochem Pharmacol. 2006; 71:981–990. [PubMed: 16290171]

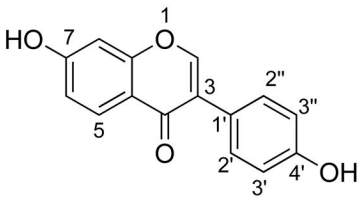
9. Laatsch, H. Antibase, a Data Base for Rapid Structural Determination of Microbial Natural Products. Weinheim: 2010.
10. Anderson AS, Wellington EM. The Taxonomy of *Streptomyces* and Related Genera. Int J Syst Evol Microbiol. 2001; 51:797–814. [PubMed: 11411701]
11. Abdelfattah MS, Kharel MK, Hitron JA, Baig I, Rohr J. Moromycins A and B, Isolation and Structure Elucidation of C-Glycosylangucycline-type Antibiotics from *Streptomyces* sp KY002. J Nat Prod. 2008; 71:1569–1573. [PubMed: 18666798]
12. Shaaban KA, Srinivasan S, Kumar R, Damodaran C, Rohr J. Landomycins P-W, Cytotoxic Angucyclines from *Streptomyces cyanogenus* S-136. J Nat Prod. 2011; 74:2–11. [PubMed: 21188999]
13. Shaaban KA, Stamatkin C, Damodaran C, Rohr J. 11-Deoxylandomycinone and Landomycins X-Z, New Cytotoxic Angucyclin(on)es from a *Streptomyces cyanogenus* K62 Mutant Strain. J Antibiot. 2011; 64:141–150. [PubMed: 20978514]
14. Ning ZQ, et al. Chidamide (CS055/HBI-8000): A New Histone Deacetylase Inhibitor of the Benzamide Class with Antitumor Activity and the Ability to Enhance Immune Cell-Mediated Tumor Cell Cytotoxicity. Cancer Chemother Pharmacol. 2012; 69:901–909. [PubMed: 22080169]
15. Meier RM, Tamm C. Studies Directed Towards the Biosynthesis of the C_7N-Unit of Rifamycin B: Incorporation of [^{14}C(G)]Quinic Acid and [1,2-$^{13}C_2$]Glycerol. J Antibiot. 1992; 45:400–410. [PubMed: 1577667]
16. Naito J, Sasaki E, Ohta Y, Shinohara R, Ishiguro I. Anthranilic Acid Metabolism in the Isolated Perfused Rat Liver: Detection and Determination of Anthranilic Acid and Its Related Substances Using High-Performance Liquid Chromatography with Electrochemical Detection. Biochem Pharmacol. 1984; 33:3195–3200. [PubMed: 6548385]
17. Hosny M, Rosazza JP. Novel Isoflavone, Cinnamic acid, and Triterpenoid Glycosides in Soybean Molasses. J Nat Prod. 1999; 62:853–858. [PubMed: 10395502]
18. Hosny M, Rosazza JP. New Isoflavone and Triterpene Glycosides from Soybeans. J Nat Prod. 2002; 65:805–813. [PubMed: 12088420]
19. Pathak T, Bazin H, Chattopadhyaya J. Synthesis of 2′-Deoxy-2′(S)-Deuterio and 2′-Deoxy-2′(R)-Deuterio-Beta-D-Nucleosides. Tetrahedron. 1986; 42:5427–5441.
20. Cao SG, et al. Marine Sesquiterpenoids That Inhibit the Lyase Activity of DNA Polymerase beta. J Nat Prod. 2004; 67:1716–1718. [PubMed: 15497946]
21. Evidente A, Iacobellis NS, Vellone R, Sisto A, Surico G. 2′-Deoxyzeatin Riboside and Other Cytokinins in Culture Filtrates of *Pseudomonas amygdali*. Phytochemistry. 1989; 28:2603–2607.
22. Jones AJ, Grant DM, Winkley MW, Robins RK. Carbon-13 Magnetic Resonance. XVII Pyrimidine and Purine Nucleosides. J Am Chem Soc. 1970; 92:4079–4087. [PubMed: 5419046]
23. Kaneko C, Yameda S. The Isomerization of 1aH-Oxazirino[2,3-a]quinoline la-carbonitrile and Its Substituted Derivatives to the Corresponding 3-Hydroxyquinoline Derivatives. Chem Pharm Bull. 1967; 15:663–669. [PubMed: 6076767]
24. Shirling EB, Gottlieb D. Methods for Characterization of *Streptomyces* Species. Int J Syst Bacteriol. 1966; 16:313–340.
25. Shaaban KA, Ahmed TA, Leggas M, Rohr J, Saquayamycins G-K. Cytotoxic Angucyclines from *Streptomyces* sp. Including Two Analogues Bearing the Aminosugar Rednose. J Nat Prod. 2012; 75 accepted.
26. Drautz H, Zähner H, Rohr J, Zeeck A. Metabolic products of microorganisms. 234 Urdamycins, new angucycline antibiotics from Streptomyces fradiae I Isolation, characterization and biological properties. J Antibiot. 1986; 39:1657–1669. [PubMed: 3818439]
27. Atlas, RM. Handbook of Microbiological Media. CRC Press; 2004. p. 1226



1: $R^1 = NH_2$, $R^2 = H$, $R^3 = H$; Anthranilamide
2: $R^1 = OH$, $R^2 = H$, $R^3 = OCH_3$; Pyramidamycin A
3: $R^1 = OH$, $R^2 = OCH_3$, $R^3 = OH$; Pyramidamycin B
4: $R^1 = OH$, $R^2 = NH_2$, $R^3 = OCH_3$; Pyramidamycin C
5: $R^1 = OH$, $R^2 = NHCOCH_3$, $R^3 = OCH_3$; Pyramidamycin D



3-Hydroxyquinoline-2-carboxamide (**6**)



4´,7-Dihydroxyisoflavanone (**7**)

Figure 1.
Chemical structures of compounds **1**–**7**.

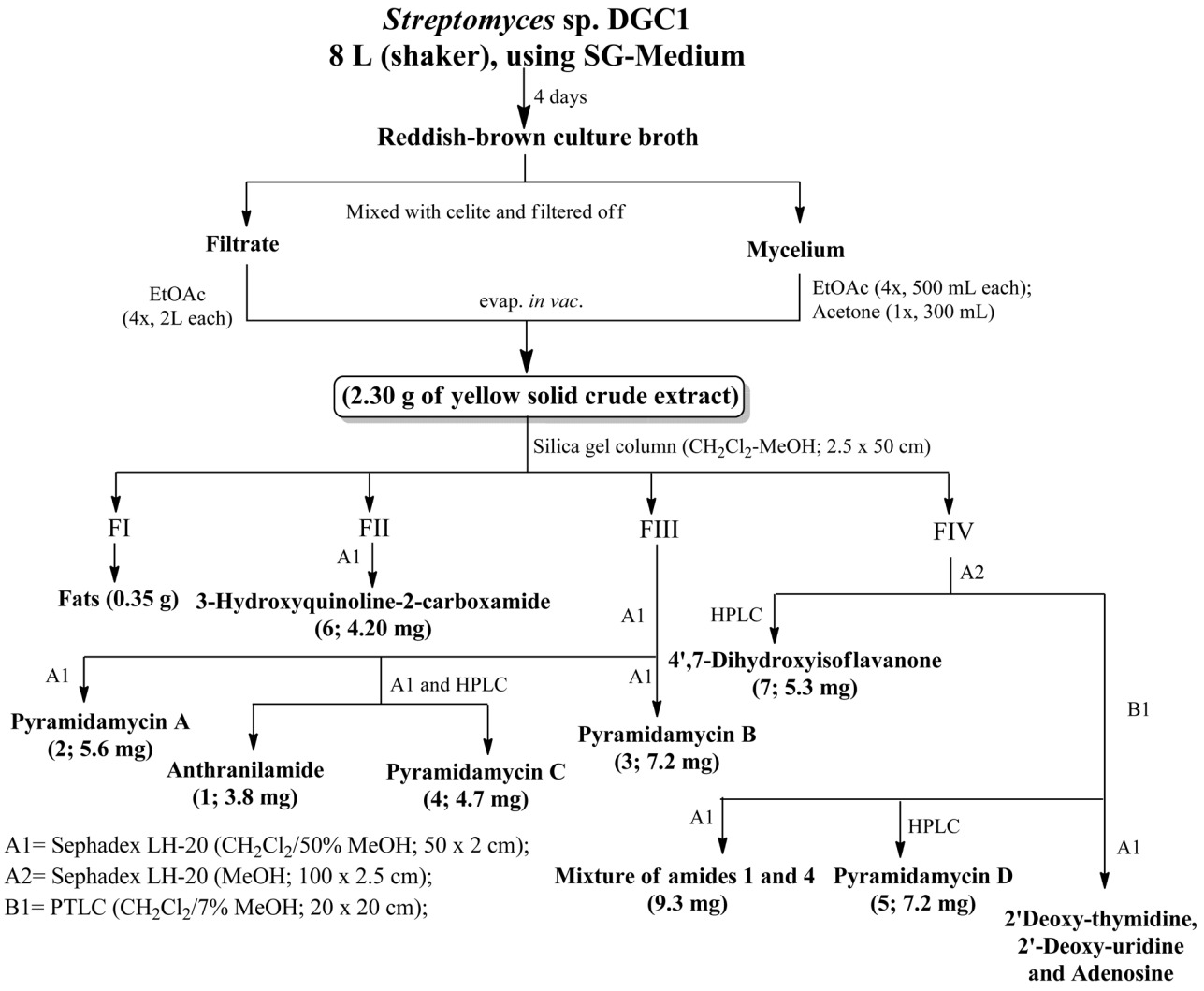


Figure 2.
Work-up procedure of extracts from *Streptomyces* sp. DGC1

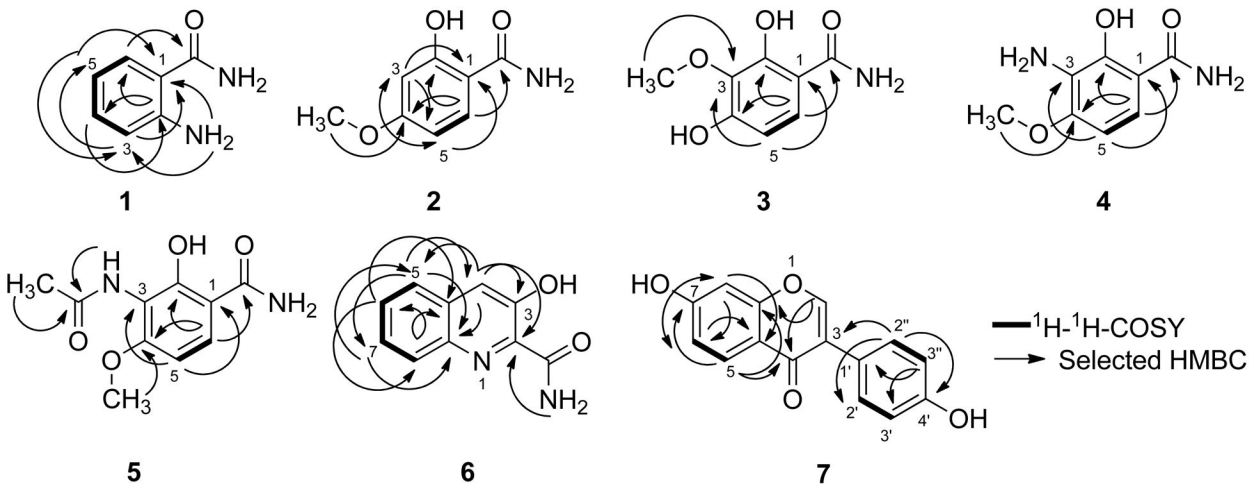


Figure 3.
^{1}H-^{1}H-COSY (bold lines) and selected HMBC (→) correlations in compounds **1**–**7**.

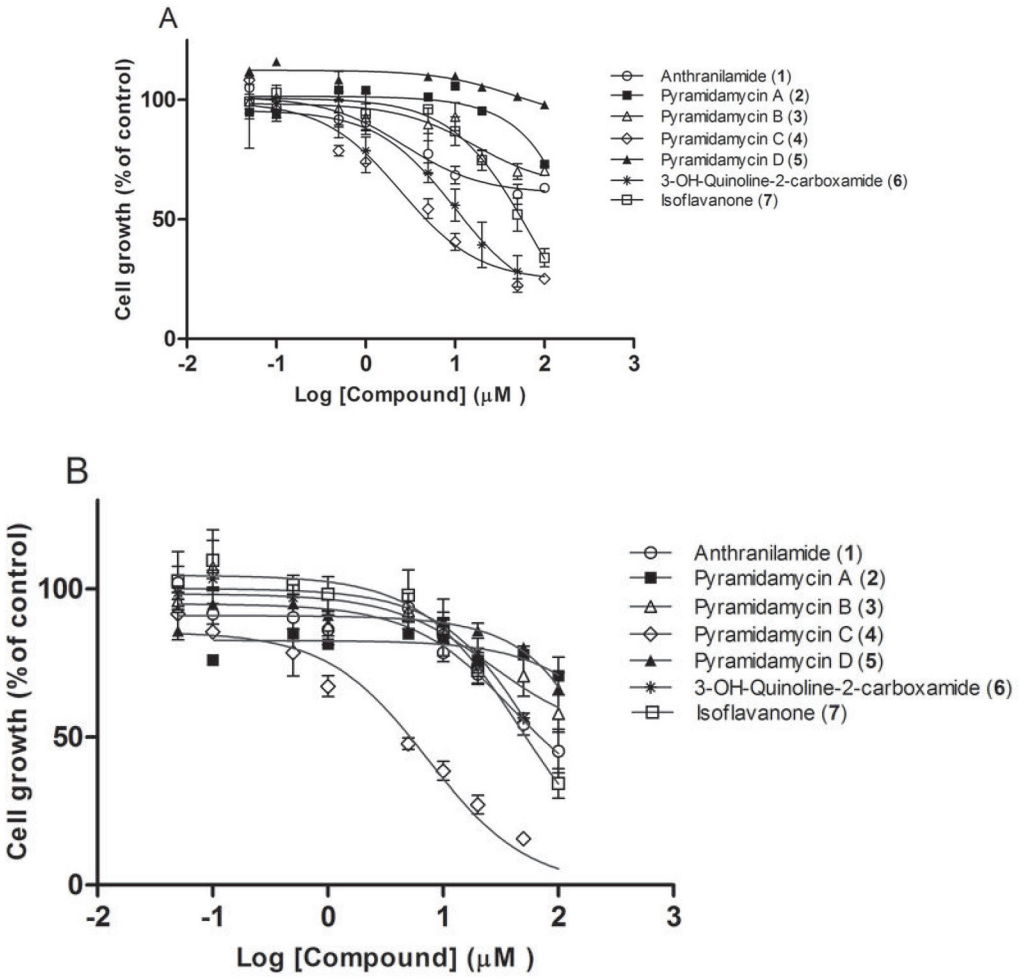


Figure 4.

Dose response curve of anthranilamide, pyramidamycins A-D, 3-hydroxy-Quinoline-2-carboxamide and isoflavanone in PC3 (**A**) and H460 (**B**) cell lines at 48h.

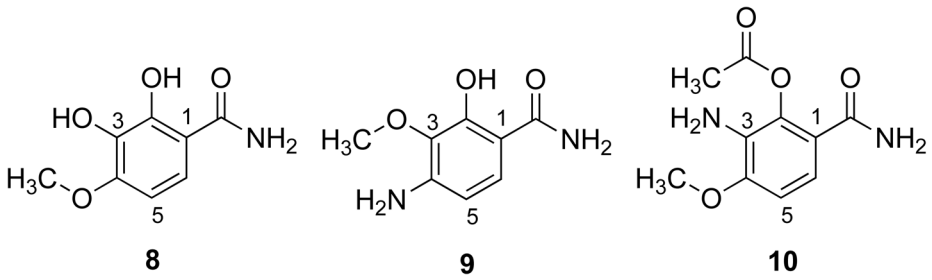


Figure 5.
Chemical structures of compounds **8**–**10**.

Table 1

Physico-chemical properties of Pyramidamycins A-C (**2**–**4**).[a)]

	Pyramidamycin A (2)	**Pyramidamycin B (3)**	**Pyramidamycin C (4)**
Appearance	White solid, UV absorbing and blue fluorescence under long UV (365 nm)	Colourless solid, UV absorbing	White powder, UV absorbing
Anisaldehyde/H_2SO_4[b)]	Pale yellow	Pale yellow	Yellow
R_f	0.32 (CH_2Cl_2/5%MeOH)	0.22 (CH_2Cl_2/5%MeOH)	0.57 (CH_2Cl_2/2%MeOH), 0.30 (CH_2Cl_2)
Molecular formula	$C_8H_9NO_3$	$C_8H_9NO_4$	$C_8H_{10}N_2O_3$
(−)-ESI MS: *m/z*	166 $[M-H]^-$	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 $[M+Na]^+$
(+)-HRESI MS (*m/z*)			
Found	168.0654 $[M+H]^+$ and 190.0489 $[M+Na]^+$	184.0609 $[M+H]^+$, 206.0435 $[M+Na]^+$ and 389.0963 $[2M+Na]^+$	183.0774 $[M+H]^+$, 205.0600 $[M+Na]^+$ and 387.1292 $[2M+Na]^+$
Calcd.	168.0655 for $C_8H_{10}NO_3$ and 190.0475 for $C_8H_9NO_3Na$	184.0604 for $C_8H_{10}NO_4$, 206.0424 for $C_8H_9NO_4Na$ and 389.0955 for $C_{16}H_{18}N_2O_8Na$	183.0770 for $C_8H_{11}N_2O_3$, 205.0589 for $C_8H_{10}N_2O_3Na$, 387.1275 for $C_{16}H_{20}N_4O_6Na$
UV/VIS (MeOH): λ_{max} (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.

[a)] See also Figures S4, S9–11, S23, and S28–30 (for comparison);

[b)] Colouration with anisaldehyde/sulfuric acid spraying reagent and heating.

Table 2

Physico-chemical properties of Pyramidamycin D (**5**) and 3-Hydroxyquinoline-2-carboxamide (**6**).[a)]

	Pyramidamycin D (5)	**3-Hydroxyquinoline-2-carboxamide (6)**
Appearance	White powder, UV absorbing	Pale yellow solid, UV absorbing, green fluorescence under long UV (365 nm)
Anisaldehyde/Sulfuric acid [b)]	Pale yellow	-
R_f	0.31 (CH_2Cl_2/7%MeOH)	0.30 (CH_2Cl_2)
Molecular formula	$C_{10}H_{12}N_2O_4$	$C_{10}H_8N_2O_2$
(−)-ESI MS: *m/z*	223 $[M-H]^-$	187 $[M-H]^-$
(+)-ESI MS: *m/z*	225 $[M+H]^+$	189 $[M+H]^+$
(+)-HRESI MS (*m/z*)		
Found	225.0875 $[M+H]^+$, 247.0704 $[M+Na]^+$ and 263.0438 $[M+K]^+$	189.0653 $[M+H]^+$ and 211.0473 $[M+Na]^+$
Calcd.	225.0870 for $C_{10}H_{13}N_2O_4$, 247.0689 for $C_{10}H_{12}N_2O_4Na$ and 263.0429 for $C_{10}H_{12}N_2O_4K$	189.0658 for $C_{10}H_9N_2O_2$ and 211.0478 for $C_{10}H_8N_2O_2Na$
UV/VIS (MeOH): λ_{max} (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.

[a)] See also Figures S4, S35 and S40–42 (for comparison);

[b)] Colouration with anisaldehyde/sulfuric acid spraying reagent and heating.

Table 3

^{1}H NMR (500 MHz) data of the benzamides **1**–**5** in DMSO-d_6, δ in ppm relative to TMS, multiplicities (*J*/Hz).

Position	Anthra. (1) [a)]	Pyram. A (2) [a,b)]	Pyram. B (3) [a)]	Pyram. C (4) [a)]	Pyram. D (5) [a)]
	δ_H (*J*/Hz)	δ_H (*J*/Hz)	δ_H (*J*/Hz)	δ_H (*J*/Hz)	δ_H (*J*/Hz)
1-CO<u>NH$_2$</u>	7.70 (1H, br s), 7.03 (1H, br s)	8.21 (1H, br s), 7.68 (1H, br s)	8.29 (1H, br s), 7.63 (1H, br s)	8.16 (1H, br s), 7.59 (1H, br s)	8.30 (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-NH$_2$	6.54 (2H, br s)	-	-	-	-
3	6.66 (1H, dd, 8.5, 1.0)	6.39 (1H, d, 2.5)	-	-	-
3-<u>NH</u>	-	-	-	-	8.87 (1H, br s)
3-<u>NH$_2$</u>	-	-	-	13.0-11.0 (2H, br s)	-
3-OCH$_3$	-	-	3.68 (3H, s)	-	-
3-NHCO<u>CH$_3$</u>	-	-	-	-	1.96 (3H, s)
4	7.12 (1H, td, 7.0, 1.5)	-	-	-	-
4-OH	-	-	9.86 (1H, br s)	-	-
4-OCH$_3$	-	3.75 (3H, s)	-	3.79 (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)

a) See also Figures S5–8, S12–22, S24–27, S31–S34, S36–39 and S43–47 for comparison;

b) For ^{1}H NMR data in CDCl3, see the experimental part.

Table 4

^{13}C NMR (125 MHz) data of benzamides **1**–**5** in DMSO-d_6, (δ_C, mult.).

Position	Anthra. (1) [a)]	Pyram. A (2)[a,b)]	Pyram. B (3) [a)]	Pyram. C (4) [a)]	Pyram. D (5) [a)]
	δ_C, mult.	**δ_C, mult.**	**δ_C, mult.**	**δ_C, mult.**	**δ_C, mult.**
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	-	-	-	-	168.3 s
3-NHCOCH$_3$	-	-	-	-	22.7 q
3-OCH$_3$	-	-	59.8	-	-
4	131.9 d	164.0 s	154.9 s	149.7 s	159.4 s
4-OCH$_3$	-	55.5 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

a) See also Figures S6, S13, S25, S32 and S37 for comparison;

b) For ^{13}C NMR data in CDCl$_3$, see the experimental part.

Table 5

^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of 3-Hydroxyquinoline-2-carboxamide (**6**) in DMSO-d_6, δ in ppm relative to TMS.

Position	3-Hydroxyquinoline-2-carboxamide (6) [a]	
	δ_C, mult.	δ_H (*J*/Hz)
2	135.8 s	-
2-CO	171.8 s	-
2-CONH_2	-	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 t, 6.5)
7	128.3 d	7.59 (1H, br t, 7.0)
8	129.9 d	7.99 (1H, br d, 7.5)
8a	141.6 s	-

[a] See also Figures S43–47 for comparison.

Table 6

Cytotoxic activity of Pyramidamycin C (**4**), 3-Hydroxy-Quinoline-2-carboxamide (**6**) and 4′,7-Dihydroxyisoflavanone (**7**) in comparison with landomycin A (GI_{50} values, μM)

Compound	PC3 cells- 48hr		H460 cells- 48hr	
	GI_{50} (μM)	95% Confidence Intervals	GI_{50} (μM)	95% Confidence Intervals
Pyramidamycin C (**4**)	2.473	1.349 to 4.533	7.339	4.456 to 12.09
3-OH-quinoline-2-carboxamide (**6**)	9.812	2.823 to 34.11	---	---
4′,7-Dihydroxyisoflavanone (**7**)	69.93	29.23 to 167.3	46.54	16.77 to 129.2
Landomycin A	0.5505	0.4982 – 0.6081	4.109	2.548 – 6.626

--- denotes no measurable GI50.

Table 7

Diameter halo measurements (in millimeters) of compounds **1–7** tested against gram positive (*K. rosea* NRRL B-1106 and *M. luteus* NRRL B-2618) and gram negative bacteria (*E. coli* DH5α) at 100 μg/disc.

Compound	*E. coli* DH5α	*K. rosea* NRRL B-1106	*M. luteus* NRRL B-2618
Anthranilamide (**1**)	---	13±2	---
Pyramidamycin A (**2**)	---	10±2	10±2
Pyramidamycin B (**3**)	---	10±2	---
Pyramidamycin C (**4**)	---	---	---
Pyramidamycin D (**5**)	10±2	---	---
3-Hydroxyquinoline-2-carboxamide (**6**)	8±2	---	9±2
4′,7-Dihydroxyisoflavanone (**7**)	---	---	---

--- denotes no measurable halo.