Mansouramycins A−D, Cytotoxic Isoquinolinequinones from a Marine Streptomycete[1]

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Chemical screening of the ethyl acetate extract from the marine-derived *Streptomyces* sp. isolate Mei37 resulted in five isoquinolinequinones, four new derivatives, mansouramycin A−D (**1**, **3**−**5**), and the known 3-methyl-7-(methylamino)-5,8-isoquinolinedione (**2**). Their structures were elucidated by NMR and MS techniques and by comparison with related compounds. Cytotoxicity profiling of the mansouramycins in a panel of up to 36 tumor cell lines indicated significant cytotoxicity of several derivatives, with pronounced selectivity for non-small cell lung cancer, breast cancer, melanoma, and prostate cancer cells.

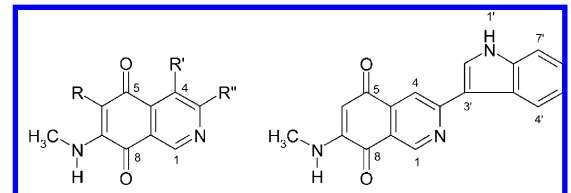
A number of isoquinolinequinones, including the cribrostatins, renierone, and *O*-demethylrenierone,[2,3] have been obtained as natural products from the marine sponges *Cribrochalina* sp.[4] and *Petrosia* sp.[5] and have generated interest due to their anticancer properties. The related caulibugulones A−F were isolated from the marine bryozoan *Caulibugula intermis*.[6] Isoquinolinequinones[7,8] have also been obtained from *Calothrix*[9] and an associated *Streptomyces lavendulae* strain,[10,11] but have not been reported from any other microbial source.

During our search for marine bacterial antibiotics with cytotoxic activity, we isolated a new isoquinolinequinone (mansouramycin A, **1**) together with antimycin A, albaflavenol,[12] 5-(6′-methyl-7′-oxooctyl)-(5*H*)-furan-2-one, pantolactone, and genistein from the Atlantic streptomycete isolate B3497. The same quinone was later isolated as a trace component also from other marine streptomycetes,[13,14] including the North Sea isolate Mei37.

The strain Mei37 was derived from the muddy sediment of Jade Bay on the southern German North Sea coast. The samples were taken from the boundary area of the littoral zone, where the land is regularly flooded by seawater, creating small natural pools within the saline meadows near the dike. This area is a refuge for sea birds, and is covered by a unique assemblage of salt tolerant plants. An initial fermentation of strain Mei37 provided **1** and two further new mansouramycins B (**3**) and D (**5**), along with the known 3-methyl-7-(methylamino)-5,8-isoquinolinequinone (**2**), uracil, N^{β}-acetyltryptamine,[15] and 2,3-dihydroxy-1-(indole-3-yl)propanone.[16] A subsequent fermentation of the same strain delivered mansouramycin C (**4**) as the main quinone, in addition to the sesquiterpene albaflavenone,[17] indole-3-acetic acid, indole-3-carboxylic acid, and uracil. The isolation of these sponge-related quinones from marine-derived streptomycetes further supports the assumption that bacteria may be involved in the biosynthesis of sponge constituents.

Results and Discussion

The *Streptomyces* sp. isolate Mei37 was incubated as a shaker culture on a calcium carbonate medium and worked up by solid-phase and EtOAc extraction. The oily extract delivered a red fraction on silica gel, which by PTLC yielded five well-separated orange and red zones containing the quinolinequinones. Uracil, N^{β}-acetyltryptamine,[12] and 2,3-dihydroxy-1-(indole-3-yl)propan-1-one[13] were isolated from the colorless polar fractions and identified by comparison of their spectroscopic data with literature values.



	R	R′	R″
1	H	Me	Me
2	H	H	Me
3	Cl	H	Me
4	H	H	COOMe

5

Further purification by preparative HPLC delivered the isoquinolinequinones as red powders. Their color change with sodium dithionite from orange to nearly colorless indicated quinones, but *peri*-hydroxyquinones were excluded by the missing bathochromic shift with sodium hydroxide. Their molecular weights and formulas were deduced from the high-resolution ESI and EI mass spectra.

Mansouramycin A (**1**) was obtained as a trace component in a yield of only 0.025 mg/L, but isolation was strongly facilitated by the conspicuous color. The molecular formula was determined as $C_{12}H_{12}N_2O_2$; the proton NMR spectrum showed CH singlets at δ 9.01 (H-1) and 5.72 (H-6) and a broadened NH signal at δ 5.82. In addition, a methyl doublet at δ 2.93, pointing to a CH_3NH fragment, and two further 3H singlets at δ 2.76 and 2.70, indicative of aromatic methyl groups, were detected. The ^{13}C and APT NMR spectra indicated two aromatic methine and three methyl carbon signals. Furthermore, signals of seven quaternary carbon atoms were observed, of which two, at δ 185.0 and 181.9, represented carbonyl groups of a quinone.

HMBC spectra, along with ^{13}C and ^{1}H shifts, pointed to an *N*-methylamino-isoquinolinequinone: The proton signal at δ 9.01 was established as H-1 in an isoquinoline and not in a quinoline, as the low-field ^{13}C shift of both CH-1 and C_q-3 (δ 145.1 and 167.4, respectively) cannot be explained by the latter. HMBC couplings of the *N*-methyl doublet at δ 2.93 and the NH signal allowed C-6, C-7, and C-8 to be assigned. In addition, H-6 showed couplings with both carbonyls at C-5 and C-8. All further couplings and chemical shifts (Figure 1) are in full agreement with structure **1**.

Interpretation of the HMBC and HMQC couplings with COCON[18] delivered an additional isomeric *ortho*-quinone (3,4-dimethyl-5-methylamino-7,8-isoquinolinequinone) as an alternative structure. However, the missing NOE cross-peaks between the

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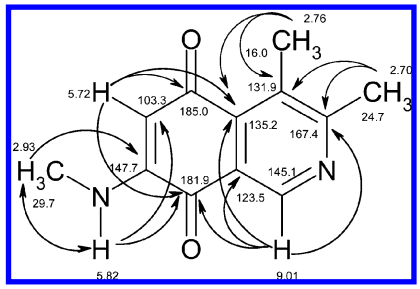

Figure 1. Selected HMBC (→) and H,H COSY (↔) correlations of mansouramycin A (**1**) in $CDCl_3$.

4-CH_3 group (δ 2.76) and NH (δ 5.82) or NCH_3 (δ 2.93) made this structure less plausible. Structure **1** has been confirmed since by synthesis in the group of v. Zezschwitz.[19]

From the second orange zone, compound **2**, with the formula $C_{11}H_{10}N_2O_2$, was obtained. The proton NMR spectrum was very similar to that of **1**; however, the methyl singlet at δ 2.76 was missing and instead a further 1H singlet at δ 7.75 was detected. In the ^{13}C and 2D spectra, not all expected signals and correlations were visible due to the small available amount. The structure was, however, easily elucidated as 3-methyl-7-(methylamino)-5,8-isoquinolinedione (**2**) by comparison with compound **1** and with predicted NMR values:[20] The proton signal at δ 7.75 agrees very well with H-4 in quinaldine, whose protons in *ortho* position to nitrogen are found beyond δ 8.45, while H-4 appears at an average experimental value of δ 7.61.[20] Compound **2** had been obtained by synthesis[21] and UV and IR, but no NMR data were accessible.

Mansouramycin B was obtained as a further trace component with a slightly higher R_f value than **2**. The chemical shifts of the NH signal and of H-1, H-4, and CH_3-3 were nearly identical to those of **2**, and according to the molecular formula $C_{11}H_9ClN_2O_2$, it was obviously a monochlorinated **2**. As the signal of H-6 was missing, and the N-Me signal showed a strong downfield shift, mansouramycin B has structure **3** and is therefore a new C-methyl derivative of caulibugulone C.[6]

A further fermentation of *Streptomyces* sp. isolate Mei37, using the same cultivating conditions as before, afforded a modified metabolite pattern with further isoquinolinequinone trace components. Mansouramycin C (**4**) was elucidated by spectroscopic methods; however synthetic approaches will be required to determine the structures of further quinones. Two less polar subfractions led to the isolation of albaflavenone,[14] (4*S*)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide, indole-3-acetic acid, and indole-3-carboxylic acid, while further fractions of higher polarity yielded uracil, 2,3-dihydroxy-1-(indole-3-yl)propan-1-one,[13] 2-deoxythymidine, and 2-deoxyuridine.

The dark red mansouramycin C (**4**) turned blue with anisaldehyde/sulfuric acid on TLC. HRESIMS suggested the molecular formula $C_{12}H_{10}N_2O_4$. The ^1H NMR spectrum showed a pattern similar to that of **2**, with signals at δ 9.36 (H-1), 8.71 (H-3 or -4), 5.90 (H-6), and 6.13/2.99 (NH-Me). These NMR data, particularly the methoxy signal at δ 4.07 and the respective carbon data, indicated 7-methylamino-3-carbomethoxyisoquinolinequinone (**4**) or its 4-substituted isomer. The methoxy group (δ 4.07) exhibited a $^3J_{CH}$ coupling with the quaternary carbon signal at δ 153.3, which was assigned to C-3 due to the shift value and observed couplings with H-1 (δ 9.36) and H-4 (δ 8.71) (Figure 2). So, mansouramycin C must have structure **4**.

Mansouramycin D (**5**) was isolated as a dark red solid, which turned red with Ehrlich's reagent and violet with concentrated sulfuric acid. The molecular weight was established by ESI and EI-MS as *m/z* 303. HRESIMS gave the molecular formula $C_{18}H_{13}N_3O_2$, which entails 14 double-bond equivalents.

As in **1/2**, the ^1H NMR spectrum showed the same broadened quartet at δ 7.82 (7-NH) and a methyl doublet at δ 2.83, which

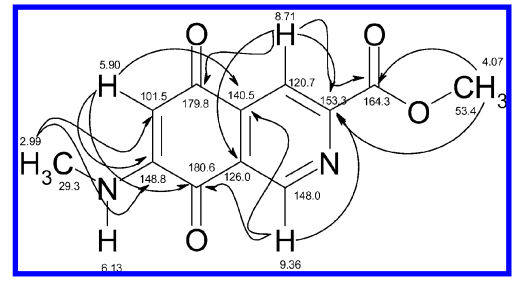

Figure 2. HMBC (→) correlations of mansouramycin C (**4**).

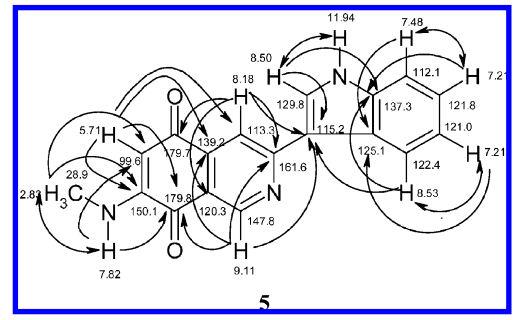

Figure 3. H, H COSY (↔) and selected HMBC (→) couplings in mansouramycin D (**5**).

gave rise to a ^{13}C signal at δ 28.9. Again, the HMBC data (Figure 3) confirmed a monosubstituted 7-methylamino-isoquinolinequinone skeleton. The H-6 signal (singlet at δ 5.71) showed long-range couplings with the *N*-methyl group and was attached to a carbon with a high-field chemical shift (δ 99.6), as was seen in **1**. The singlets at δ 9.11 and 8.18 were assigned to H-1 and H-4, respectively. Observed couplings indicated that a further substituent must occupy position 3.

The proton NMR spectrum exhibited a second broad NH singlet at δ 11.94, along with a singlet at δ 8.50 (H-2′) and four aromatic protons showing the pattern of a 1,2-disubstituted benzene. The residual atoms were shown by HMBC couplings (Figure 3) to be part of an indole system. This was obviously substituted in position 3′, due to the presence of a COSY correlation between NH and H-2′.

Four HMBC correlations of protons at δ 9.11 (H-1), 8.18 (H-4), 8.53 (H-4′), and 8.50 (H-2′) with C_q-3′ (δ 115.2) of the indole moiety confirmed the connection of indole and the isoquinolinequinone fragment via C-3/C-3′, as in **5**. Comparison with reference data confirmed further that the low-field shift of H-4′ must be due to a heteroaromatic substituent at position 3′. In addition, as all other indole C and H values are closely related to those of further 3-heteroaryl-indoles, but differ by up to $\Delta\delta_H$ 1 from 2-aryl-indoles,[20] mansouramycin D is clearly the 3-(3-indolyl)isoquinolinequinone **5**.

In the agar diffusion test, at 20 μg/paper disk, mansouramycin A (**1**) exhibited moderate activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, with inhibition zones of 10−12 mm diameter. The activity against the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus* was, however, strong and comparable to that of 8-hydroxyquinolinium sulfate as a reference compound. Mansouramycin A (**1**) was inactive against the fungus *Mucor miehei* and the yeast *Candida albicans* (Table 1). The other mansouramycins showed similar profiles, but could not be tested in detail in this study due to lack of material.

The toxicity of mansouramycins A (**1**), B (**3**), and C (**4**) and 3-methyl-7-(methylamino)-5,8-isoquinolinedione (**2**) was determined in a monolayer cell proliferation assay using a panel of up to 36 human tumor cell lines, comprising 14 different solid tumor types. The high cytotoxicity of the mansouramycins is not unexpected.[22] Mansouramycin C (**4**) proved to be the most active

Table 1. Antibiotic Activity of Mansouramycin A (**1**) in the Agar Diffusion Test[a]

	BS	EC	SA	SV	CA	MM	CV	CS	SS
1	10	10	12	0	0	0	22	17	25
quinosol	15	15	20	0	11	30	25	28	27

[a] Diameters of inhibition zones were measured (in mm) at 20 μg **1**/paper disk (8 mm diameter). BS = *Bacillus subtilis,* EC = *Escherichia coli,* SA = *Staphylococcus aureus,* SV = *Streptomyces viridochromogenes,* CA = *Candida albicans,* MM = *Mucor miehei,* CV = *Chlorella vulgaris,* CS = *Chlorella sorokiniana,* SS = *Scenedesmus subspicatus,* quinosol = potassium 8-hydroxyquinoliniumsulfate.

compound, with an overall potency of 0.089 μM (mean IC_{50} value of 36 tumor cell lines tested). It was followed by mansouramycin B (**3**, mean IC_{50} = 2.7 μM) and then 3-methyl-7-(methylamino)-5,8-isoquinolinedione (**2**, mean IC_{50} = 3.49 μM) (Table S1). Mansouramycin A (**1**) showed moderate concentration-dependent cytotoxicity, with a mean IC_{50} value of 13.44 μM. Mansouramycin C (**4**) displayed significant *in vitro* tumor cell selectivity toward 10 of the 36 tumor cell lines tested (using an individual IC_{50} value < 1/2 of the mean IC_{50} value as the threshold). Above average activity was pronounced in tumor cell lines of bladder cancer (T-24), glioblastoma (SF-268), lung cancer (LXFA 629L), mammary cancer (MCF-7), melanoma (MEXF 276L, MEXF 514L, MEXF 520L), ovarian cancer (OVCAR-3), renal cancer (RXF 944L), and uterus cancer (UXF 1138L), with IC_{50} values ranging from 0.008 to 0.02 μM (Table S1). Cytotoxic selectivity of the other isoquinolinequinones was observed in 6/36 (**1**, **2**) or 1/18 (**3**) cell lines (Table S1). Thus, antitumor potency of the isoquinolines differed according to their substitution pattern (Supporting Information, Table S3). The quinones **2** and **3** displayed comparable overall cytotoxicity against human cancer cell lines, indicating that substitution at position C-6 of the isoquinolinequinone moiety is not crucial for their cytotoxic potency, apart from the chlorination of **3**, which obviously influenced cytotoxic selectivity. By contrast, variation of the C-4 (**1**) or C-3 position (**4**) of the isoquinolinequinone greatly influenced cytotoxicity. Due to the paucity of material, further studies concentrated on 3-methyl-7-(methylamino)-5,8-isoquinolinedione (**2**), which showed selective cytotoxicity toward six out of the 36 tested cell lines, i.e., T-24 (bladder cancer), HNXF 536L (head and neck cancer), MCF-7 (mammary cancer), MEXF 276L and MEXF 520L (melanoma), and DU-145 (prostate cancer), with IC_{50} values ranging from 0.24 to 1.11 μM (Table S1).

Inhibition of clonogenicity of tumor cells by 3-methyl-7-(methylamino)-5,8-isoquinolinedione (**2**) was evaluated in additional tumor models using a clonogenic assay. The antiproliferative activity was evaluated in cell suspensions prepared from human tumor xenografts of eight different tumor types, which were cultured as solid tumors in serial passage on immune-deficient nude mice. In addition, compound **2** was tested in two preparations of hematopoietic stem cells as a model system for nonmalignant tissue. The overall potency of **2** in the clonogenic assay (mean IC_{50} = 2.94 μM) confirmed the results observed in cell lines. IC_{50} values ranged from 0.05 to 10.32 μM (Table S2). Cytotoxic selectivity of **2** for lung cancer (LXFA 629, IC_{50} = 1.63 μM) and melanomas (MEXF 276 and MEXF 514, IC_{50} = 0.33 and 0.05 μM, respectively) was confirmed. Colony formation of hematopoietic stem cells derived from cord blood was inhibited by **2**, with IC_{50} values of 4.58 and 4.53 μM, respectively (Table S2). Thus, on the basis of IC_{50} values, the sensitive tumor models mentioned above were on average around 15-fold more sensitive than hematopoietic stem cells as representative model systems for nonmalignant tissue, confirming the tumor-specific nature of the activity.

Experimental Section

General Experimental Procedures. UV/vis spectra were recorded on a Perkin-Elmer Lambda 15 UV/vis spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer from KBr pellets. NMR spectra were measured on Varian Unity 300 (^{1}H, 300.145 MHz) and Varian Inova 600 (^{1}H, 599.744 MHz; ^{13}C, 150.7 MHz) spectrometers. ESIMS was recorded on a Finnigan LCQ ion trap mass spectrometer. HRMS was recorded by ESIMS on an Apex IV 7 T Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA). EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV). Flash chromatography was carried out on silica gel (230−400 mesh). TLC was performed on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex, Amersham Biosciences Ltd.; purchased from Sigma-Aldrich Chemie, Steinheim, Germany). XAD-16 resin was obtained from Rohm and Haas, France.

Cytotoxicity Tests. Cytotoxicity testing of mansouramycins against cell lines 1218L, T24, 498NL, SF268, HCT116, HT29, 251L, 536L, 1121L, 289L, 526L, 529L, 629L, H460, 401NL, MCF7, DA231, 276L, 394NL, 462NL, 514L, 520L, 1619L, 899L, OVCAR3, 1657L, PANC1, 22RV1, DU145, LNCAP, PC3M, 1752L, 1781L, 393NL, 486L, 944L, and 1138L was carried out using the MTT method as described previously.[23] Human tumor cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice or obtained from American Type Culture Collection, Rockville, MD, National Cancer Institute, Bethesda, MD, or Deutsche Sammlung von Mikroorganismen and Zellkulturen, Braunschweig, Germany.

Taxonomy of the Producing Strains. Strain B3487. The taxonomy of the marine *Streptomyces* sp. isolate B3497 has been described previously.[24]

Strain Mei37. The almost complete 16S rRNA gene sequence of Mei37 shows a 99% similarity to the type strain of *Streptomyces intermedius* (DSM 40372). The strain forms yellow aerial mycelia on yeast extract−malt extract agar (YMA)[25] with straight to flexible spore chains (*Rectiflexibiles*). Spores are cylindrical and 0.5 to 0.6 μm in diameter and about 0.7 to 0.8 μm in length with a smooth surface (Figure S1). The vegetative mycelium is olive-beige on YMA. On some media olive-beige diffusible pigment is formed. Growth temperatures were tested in yeast extract−malt extract medium. The optimal growth temperature is about 30 °C. Growth is obtained at 10 °C but not at 4 and 46 °C. The strain develops in the range 0% to 7% (w/v) NaCl but not with 10% or 13% NaCl in yeast extract−malt extract medium. Melanin pigment is not produced on peptone−yeast extract−iron agar nor on tyrosine agar.[26] Chitin is not cleaved, but starch is degraded.[27] The strain is catalase positive; nitrate reductase is not formed. A voucher specimen of the strain is deposited in the culture collection at the Institute of Organic and Biomolecular Chemistry, Göttingen, Germany.

Arginine Glycerol Medium. Arginine hydrochloride (1.0 g), glycerol (12.5 g), rifampicin (50 μg), and agar (18.0 g) were dissolved in 1 L of artificial seawater and sterilized by autoclaving for 33 min at 121 °C.

Modified M_2 Medium. Malt extract (10 g), yeast extract (4 g), and glucose (4 g) were dissolved in 500 mL of tap water and 500 mL of artificial seawater. The solution was adjusted to pH 7.8 and sterilized by autoclaving for 33 min at 121 °C.

Calcium Carbonate Medium. To 1 L of modified M_2 medium was added 0.5 g of calcium carbonate prior to sterilization.

Fermentation, Extraction, and Isolation. (A) In a 20 L jar fermentor (Meredos, Göttingen), 20 L of M_2 medium was inoculated with 2 L of a well-grown B3497 shaker culture in the same medium. Fermentation was performed for 64 h at 28 °C/200 rpm with an aeration pressure of 0.5 bar. Mycelia (light yellow) and culture broth (red-brown, final pH 7.17) were homogenized (Ultraturrax) with 5 L of EtOAc and filtered after addition of 1 kg of Celite by means of a pressure filter. Filtrate and residue were extracted three times each with 5 L of EtOAc. The combined organic phases delivered 2.8 g of a dark brown, oily residue. The extract was dissolved in 50 mL of MeOH, and fats were removed by extraction with cHex. Chromatography on silica gel (column 25 × 400 mm) with a stepwise chloroform/MeOH gradient (1.5 L of $CHCl_3$/3% CH_3OH, 1.5 L of $CHCl_3$/5% CH_3OH, 3 L of $CHCl_3$/10% CH_3OH) delivered three fractions: I, R_f 0.95−0.50, 230 mg; II, R_f 0.60−0.30, 560 mg; III, $R_f \leq$ 0.30, 120 mg).

Fraction I yielded 200 mg of fatty acids and 1.8 mg of albaflavenol, which gave a pink spot with a violet border with anisaldehyde/sulfuric

acid. Flash chromatography (silica gel, column 10 × 200 mm, cHex/30% EtOAc) of fraction II afforded a further 480 mg of fatty acids, 12 mg of antimycin A (yellow color with anisaldehyde/sulfuric acid), 4 mg of 5-(6′-methyl-7′-oxooctyl)-(5*H*)-furan-2-on (R_f 0.15, C_6H_{12}/30% EtOAc, violet with anisaldehyde/sulfuric acid), and from a yellow subfraction 0.4 mg of red **1** (R_f 0.07, C_6H_{12}/30% EtOAc). In a similar way, fraction III yielded 50 mg of fatty acid mixture, 4 mg of genistein, and 20 mg of pantolactone. On evaporation of its solution, the latter sublimed as a 15 cm (!) long hair-like needle.

(B) The marine-derived *Streptomyces* sp. isolate Mei37 was precultivated on calcium carbonate medium agar plates at 28 °C for 7 days. With pieces of well-grown agar subculture of the strain, 160 1 L Erlenmeyer flasks, each containing 250 mL of calcium carbonate medium, were inoculated and cultivated at 28 °C (110 rpm) for 120 h. The culture broth was mixed with ca. 1.5 kg of Celite and filtered through a pressure filter. The filtrate was extracted by passing through an XAD-16 column (4 × 140 cm), which was finally eluted with MeOH. The MeOH phase was concentrated to the aqueous residue and extracted with EtOAc. The mycelium was separately extracted with EtOAc. Both extracts were combined and evaporated to dryness under vacuum at 40 °C.

The oily residue (1.5 g) was chromatographed on silica gel (column 3 × 60 cm, 200 g) using a stepwise MeOH/CH_2Cl_2 gradient (0−10% MeOH) to yield fractions I (70 mg of fats), II (120 mg, orange), and III (160 mg, colorless). PTLC (5% MeOH/CH_2Cl_2) and size exclusion chromatography (3 × 70 cm, 40% MeOH/CH_2Cl_2) of fraction II yielded 3-methyl-7-(methylamino)-5,8-isoquinolinedione (**2**, 2.2 mg) and the mansouramycins A (**1**, 1.0 mg), B (**3**, 0.8 mg), and D (**5**, 3 mg). In a similar way, fraction III gave uracil (18 mg), N$^{\beta}$-acetyltryptamine (6 mg), and 2,3-dihydroxy-1-(indole-3-yl)propan-1-one (4 mg).

A refermentation of *Streptomyces* sp. isolate Mei37 using the same conditions as above yielded 3.7 g of a dark red extract. The first low-polarity subfractions delivered albaflavenone (35.7 mg),[14] (4*S*)-4,10,11-trihydroxy-10-methyldodec-2-en-1,4-olide (58.0 mg), indole-3-acetic acid (10.7 mg), and indole-3-carboxylic acid (3.0 mg). Further purification of the intermediately polar fraction on Sephadex LH-20 (40% MeOH/CH_2Cl_2) led to uracil (20.7 mg) and 2,3-dihydroxy-1-(indole-3-yl)propan-1-one (3.0 mg).[13] In the same way, the polar fraction afforded 2-deoxythymidine (5.1 mg) and 2-deoxyuridine (4.3 mg), along with mansouramycin C (**4**, 2.3 mg) and further trace components with the UV spectra of mansouramycins.

Mansouramycin A (7-methylamino-3,4-dimethylisoquinoline-5,8-dione, 1): red solid; R_f 0.60 (5% MeOH/CH_2Cl_2), 0.11 (40% EtOAc/cHex), yellow coloration with anisaldehyde/H_2SO_4 or concentrated H_2SO_4; UV/vis (MeOH) λ_{max} (log ε) 227 (3.90), 232 (3.83), 258 (3.60), 273 (3.56), 312 (3.10), 338 (4.47), 380 (3.99), 441 nm (4.26); (MeOH/NaOH) 211, 231, 274, 340, 448 nm; (MeOH/HCl) 201, 239, 314, 472 nm; IR (KBr) ν_{max} 3420, 3270, 290, 2850, 1739, 1675, 1638, 1601, 1567, 1543, 1508, 1461, 1421, 1343, 1268, 1181, 1108, 850, 811, 704 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz; Figure S2) δ 9.01 (1H, s, H-1), 5.82 (s br, 1H, NH), 5.72 (1H, s, H-6), 2.93 (3H, d, J = 5.2 Hz, 7-NCH_3), 2.76 (3H, s, CH_3-4), 2.70 (3H, s, CH_3-3); ^{1}H NMR ($CDCl_3$, 300 MHz) of synthetic **2**,[19] δ 9.01 (1H, s, H-1), 5.82 (1H, s, NH), 5.72 (1H, s, H-6), 2.91 (3H, d, J = 4.1 Hz, $NHCH_3$), 2.74 (3H, s, CH_3-4), 2.69 (3H, s, CH_3-3); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.84 (1H, s, H-1), 7.55 (1H, s br, NH), 5.61 (1H, s, H-6), 2.76 (3H, d, J = 5 Hz, 7-NCH_3), 2.66 (3H, s, 4-CH_3), 2.61 (3H, s, 3-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 185.0 (C_q-5), 181.9 (C_q-8), 167.4 (C_q-3), 147.7 (C_q-7), 145.1 (CH-1), 135.2 (C_q-4a), 131.9 (C_q-4), 123.5 (C_q-8a), 103.3 (CH-6), 29.7 (NCH_3), 24.7 (CH_3-3), 16.0 (CH_3-4); (+)-ESIMS m/z 455 [2M + Na]$^+$, 217 [M + H]$^+$; (−)-ESIMS m/z 431 [2M − H]$^-$, 215 [M − H]$^-$; EIMS (70 eV) m/z (%) 216 [M]$^{\bullet+}$ (100), 188 (8), 158 (8), 107 (8), 82 (24); HREIMS (70 eV) m/z 216.0899 [M]$^{\bullet+}$ (calcd for $C_{12}H_{12}N_2O_2$, 216.08934).

7-Methylamino-3-methylisoquinoline-5,8-dione (2): dark red solid; R_f 0.60 (7% MeOH/CH_2Cl_2), 0.52 (5% MeOH/CH_2Cl_2), yellow with H_2SO_4; UV/vis (MeOH) λ_{max} 231, 279, 325, 456 nm; IR (KBr) ν_{max} 3412, 3298, 2361, 2300, 1734, 1684, 1653, 1617, 1559, 1506, 1457, 1419, 1341, 1280, 1080, 668, 620 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz; Figure S3) δ 9.12 (1H, s, H-1), 7.75 (1H, s, H-4), 6.07 (1H, s br, 7-NH), 5.78 (1 H, s, H-6), 2.95 (3H, d, J = 5.5 Hz, 7-NCH_3), 2.70 (3H, s, CH_3-3); (+)-HRESIMS m/z 203.08156 [M + H]$^+$ (calcd for $C_{11}H_{11}N_2O_2$, 203.08150).

Mansouramycin B (6-chloro-3-methyl-7-methylaminoisoquinoline-5,8-dione, 3): dark red solid; R_f 0.68 (7% MeOH/CH_2Cl_2), 0.52 (5% MeOH/CH_2Cl_2), yellow with H_2SO_4; UV/vis (MeCN) λ_{max} (%E) 228 (100), 287 (72), 298 (sh, 66) 330 (21), 480 nm (22); ^{1}H NMR ($CDCl_3$, 600 MHz; Figure S4) δ 9.14 (1H, s, H-1), 7.82 (1H, s, H-4), 6.08 (1H, s br, NH), 3.48 (3H, d, J = 5.5 Hz, NCH_3), 2.73 (3H, s, CH_3-3); (+)-ESIMS m/z (%) 239 [M′ + H]$^+$ (54), 237 [M + H]$^+$ (100); (+)-HRESIMS m/z 237.04260 [M + H]$^+$ (calcd for $C_{11}H_{10}ClN_2O_2$, 237.042532).

Mansouramycin C (3-carbomethoxy-7-methylaminoisoquinoline-5,8-dione, 4): dark red solid; R_f 0.55 (5% MeOH/CH_2Cl_2), blue-violet with anisaldehyde/H_2SO_4 or concentrated H_2SO_4; UV/vis (MeOH) λ_{max} (log ε) 258 (4.43), 284 (4.37), 464 nm (3.70); (NaOH) λ_{max} (log ε) 248 (4.44), 268 sh (4.41), 322 (4.01), 457 nm (3.91); (MeOH/HCl) λ_{max} (log ε) 257 (4.46), 283 (4.35), 466 nm (6.67); ^{1}H NMR ($CDCl_3$, 300 MHz; Figure S5) δ 9.36 (1H, s, H-1), 8.71 (1H, s, H-4), 6.13 (1H, m br, NH), 5.90 (1H, s, H-6), 4.07 (3H, s, 9-OCH_3), 2.99 (3H, d, J = 5.5 Hz, NCH_3); ^{13}C NMR ($CDCl_3$, 150 MHz; Figure S6) δ 180.6 (C_q-8), 177.4 (C_q-5), 164.3 (C_q-9), 153.3 (C_q-3), 148.8 (C_q-7), 148.0 (CH-1), 140.5 (C_q-4a), 126.0 (C_q-8a), 120.7 (CH-4), 101.5 (CH-6), 54.4 (9-OCH_3), 29.3 (NCH_3); EIMS (70 eV) m/z (%) 246 [M]$^+$ (6), 188 [M − $COOCH_3$]$^+$ (16), 82 (100), 76 (37), 63 (12), 57 (16), 50 (25), 43 (18), 41 (11); (+)-ESIMS m/z (%) 247 [M + H]$^+$ (4), 269 [M + Na]$^+$ (23), 514 [2M + Na]$^+$ (18); (+)-HRESIMS m/z 247.07136 [M + H]$^+$ (calcd for $C_{12}H_{11}N_2O_4$, 247.07188).

Mansouramycin D (3-(1*H*-indol-3-yl)-7-methylaminoisoquinoline-5,8-dione, 5): dark red solid; R_f 0.60 (7% MeOH/CH_2Cl_2), 0.43 (5% MeOH/CH_2Cl_2), blue-violet with anisaldehyde/H_2SO_4 or concentrated H_2SO_4, immediately red with Ehrlich's reagent, violet on heating; UV/vis (MeOH) λ_{max} (log ε) 274 (3.75), 330 (sh, 3.38), 428 (3.73), 510 nm (sh, 3.38); (MeOH/HCl) λ_{max} 268 (3.73), 292 (3.72), 454 nm (3.73); (MeOH/NaOH) λ_{max} 287 (3.77), 338 (sh, 3.32) 430 (3.65), 510 nm (sh, 3.38); IR (KBr) ν_{max} 3427, 2925, 1675, 1653, 1617, 1578, 1524, 1457, 1524, 1457, 1443, 1344, 1028, 986, 751 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz; Figure S8) δ 11.91 (1H, s br, 1′-NH), 9.11 (1H, s, H-1), 8.53 (1H, d, J = 7.5 Hz, H-4′), 8.50 (1H, s, H-2′), 8.18 (1H, s, H-4), 7.82 (1H, s br, 7-NH; q in pyridine), 7.48 (1H, d, J = 7.5 Hz, H-7′), 7.21 (2H, m, H-5′,6′), 5.71 (1H, s, H-6), 2.83 (3H, d, J = 5.1 Hz, N-Me); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 179.8 (C_q-8), 179.7 (C_q-5), 161.6 (C_q-3), 150.1 (C_q-7), 147.8 (CH-1), 139.2 (C_q-4a), 137.3 (C_q-7′a), 129.8 (CH-2′), 125.1 (C_q-3′a), 122.4 (CH-4′), 121.8 (CH-6′), 121.0 (CH-5′), 120.3 (C_q-8a), 115.2 (C_q-3′), 113.3 (C_q-4), 112.1 (CH-7′), 99.6 (CH-6), 28.9 (NCH_3); EIMS (70 eV) m/z (%) 303 [M]$^+$ (100), 262 (14), 172 (18), 142 (16), 114 (12), 72 (50), 59 (88), 41 (56); (+)-ESIMS m/z 629 [2M + Na]$^+$, 304 [M + H]$^+$; (+)-HRESIMS m/z 304.10805 [M + H]$^+$ (calcd for $C_{18}H_{14}N_3O_2$, 304.10807).

Antitumor Test. A modified propidium iodide assay was used to examine the cytotoxic activity of the compounds against human tumor cell lines. The test procedure is described elsewhere.[28,29] Cell lines tested were derived from patient tumors, engrafted as a subcutaneously growing tumor in NMRI nu/nu mice, or obtained from the American Type Culture Collection, Rockville, MD, National Cancer Institute, Bethesda, MD, or Deutsche Sammlung von Mikroorganismen and Zellkulturen, Braunschweig, Germany.

The effect of the test compounds on clonogenicity of tumor cells was investigated in a colony formation assay. Tumor xenografts were derived from patient tumors, engrafted as a subcutaneously growing tumor in NMRI nu/nu mice obtained from Oncotest's breeding facility. Cell suspensions were prepared, and embedded and incubated in cell culture medium containing soft agar. Hematopoietic stem cells were isolated from cord blood, and embedded and incubated in cell culture medium containing methyl cellulose. Details of the test procedure have been described previously.[29,30]

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Note Added after ASAP Publication: The version of this paper published on November 18, 2009, contained errors in the paragraphs immediately preceding the Experimental Section. The corrected version was published on November 23, 2009.

Supporting Information Available: High-resolution SEM image of *Streptomyces* sp. Mei37; ^{1}H NMR spectra of isoquinolinequinones

1−**5** and 2D NMR spectra of mansouramycin C (**4**); tables of cytotoxic activities of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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