


Article

Mansouramycins E–G, Cytotoxic Isoquinolinequinones from Marine Streptomycetes

Mohamed Shaaban ^{1,2,†}, Khaled A. Shaaban ^{1,†,‡} , Gerhard Kelter ³, Heinz Herbert Fiebig ⁴ and Hartmut Laatsch ^{1,*}

¹ Institute of Organic and Biomolecular Chemistry, University of Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany; mshaaba@gmail.com or ms.attia@nrc.sci.eg (M.S.); khaled_shaaban@uky.edu (K.A.S.)

² National Research Centre, Chemistry of Natural Compounds Department, Pharmaceutical and Drug Industries Research Institute, El-Behoos St. 33, Giza 12622, Egypt

³ Oncotest GmbH, Charles River Discovery Germany, Am Flughafen 14, D-79108 Freiburg, Germany; Gerhard.Kelter@crl.com

⁴ Oncotest GmbH, Biotec GmbH, Am Flughafen 14, D-79108 Freiburg, Germany; fiebig@4hf.eu

* Correspondence: hlaatsc@gwdg.de; Tel.: +49-551-393-211; Fax: +49-551-399-660

† Authors contributed equally to this work.

‡ Current address: Center for Pharmaceutical Research and Innovation, Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA.

Abstract: Chemical investigation of the ethyl acetate extract from the marine-derived *Streptomyces* sp. isolate B1848 resulted in three new isoquinolinequinone derivatives, the mansouramycins E–G (**1a–3a**), in addition to the previously reported mansouramycins A (**5**) and D (**6**). Their structures were elucidated by computer-assisted interpretation of 1D and 2D NMR spectra, high-resolution mass spectrometry, and by comparison with related compounds. Cytotoxicity profiling of the mansouramycins in a panel of up to 36 tumor cell lines indicated a significant cytotoxicity and good tumor selectivity for mansouramycin F (**2a**), while the activity profile of E (**1a**) was less attractive.

Keywords: mansouramycins; isoquinolinequinones; marine-derived *Streptomyces* sp.; cytotoxicity



Citation: Shaaban, M.; Shaaban, K.A.; Kelter, G.; Fiebig, H.H.; Laatsch, H. Mansouramycins E–G, Cytotoxic Isoquinolinequinones from Marine Streptomycetes. *Mar. Drugs* **2021**, *19*, 715. <https://doi.org/10.3390/md19120715>

Academic Editor: Ipek Kurtboke

Received: 11 November 2021

Accepted: 8 December 2021

Published: 20 December 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The first natural isoquinolinequinone isolated from bacteria were reported by Fukum et al. in 1977 [1] and then by Kubo et al. in 1988 [2]. A few others were isolated from porifera, including cribrostatis (produced by the blue marine sponge *Cribochalina* sp.) [3], renierones (from *Reniera*, *Petrosia*, and *Haliclona* spp.) [4,5], and caulibugulones (found in the marine bryozoon *Caulibugula inermis*) [6]. These isoquinolinequinones showed a potent antimicrobial activity against Gram positive bacteria and yeast (*Candida albicans*) and a pronounced cytotoxicity against L1210 and other cell lines with IC₅₀ values as low as 30 ngmL⁻¹ [7,8]. In 1998, we isolated mansouramycin A (**5**) as a trace component from the marine derived *Streptomyces* sp. B3497 [9]. Mansouramycin A (**5**) and the synthetic analogue 3-methyl-7-(methylamino)-5,8-isoquinolinedione (**4a**) were re-isolated from the marine-derived *Streptomyces* sp. isolate Mei37, together with three new mansouramycins B–D (**1a–3a**) [10]. These compounds showed a pronounced selectivity for non-small cell lung cancer, breast cancer, melanoma, and prostate cancer cells. Recently, mansouramycin A (**5**) was also obtained from the marine-derived *Streptomyces albus* J1074 and found to be a potent inhibitor of the methicillin-resistant *Staphylococcus aureus* ATCC 43300 with an MIC of 8 µgmL⁻¹. *S. albus* J1074 produced additionally the novel isoindoloquinone albumycin [11].

While the marine-derived *Streptomyces* sp. isolate B1848 was previously noted as a producer of 6-hydroxy-isatine and several other known compounds [12–14], further fermentations led now to the isolation and characterization of three unusual mansouramycins

E–G (**1a–3a**) along with mansouramycins A (**5**), D (**6**) (Figure 1) and 13 known metabolites [12–14]. The chemical structures of **1a–3a** were elucidated by NMR (1D, 2D) and HRMS, by comparison with related compounds and by computer-assisted methods. The cytotoxic activity of the isolated isoquinolinequinones was determined.

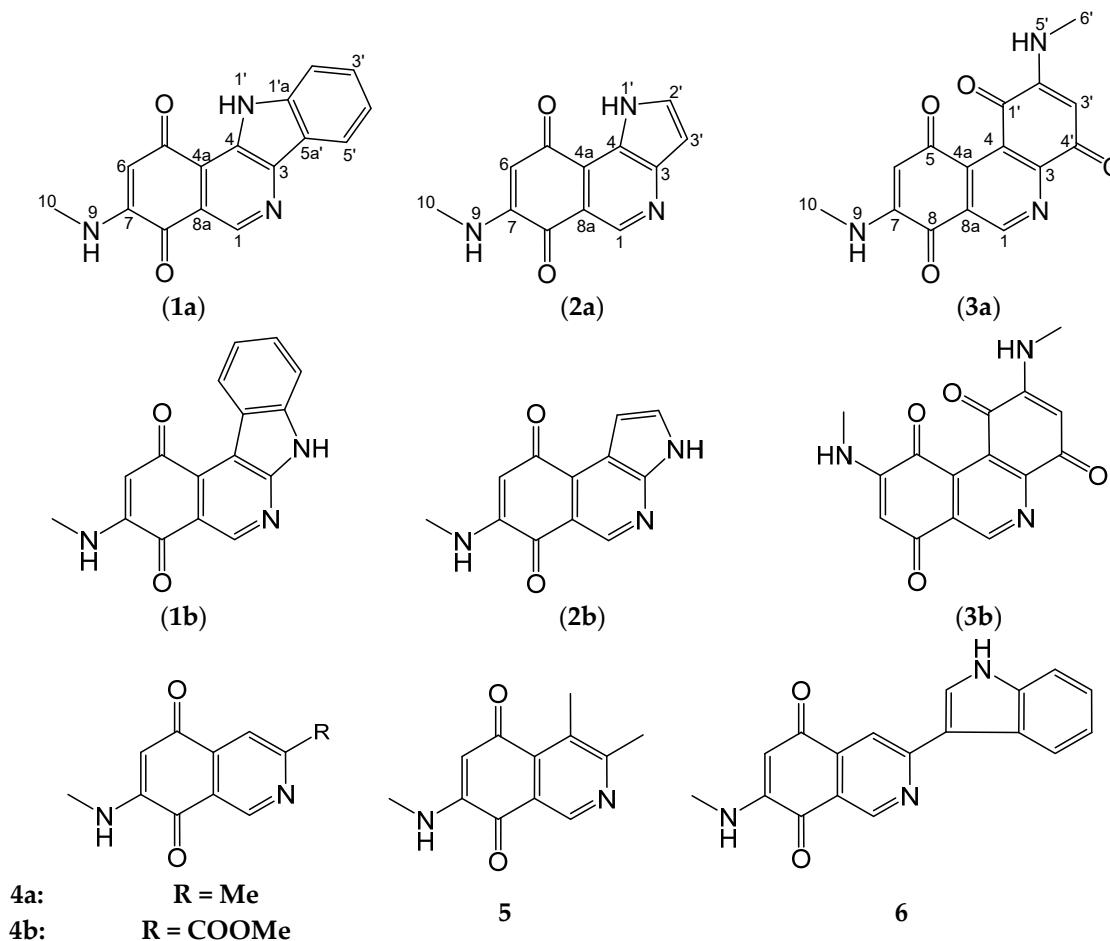


Figure 1. Chemical structures of isoquinolinequinones **1–6** produced by *Streptomyces* sp. B1848, and alternative structures **1b–3b**.

2. Results and Discussion

With a malt extract medium with 50% synthetic seawater (M_2^+ medium), the marine-derived *Streptomyces* sp. isolate B1848 produced only traces of mansouramycins A (**5**) and D (**6**), along with the zizaene derivative albaflavenol [9], 6-hydroxy-isatine [13,14], 2'-deoxythymidine, 2'-deoxuridine, 2'-deoxyadenosine, anthranilic acid, tyrosol, indolyl-3-acetic acid, phenyl acetamide, indolyl-3-carboxylic acid, N^β -acetyltryptamine, *N*-acetyltyramine, and *p*-hydroxybenzoic acid [12]. Better yields of the mansouramycins and further red pigments were obtained now on a meat extract medium in a fermentation with a 50 L shaker culture. After extraction and chromatographic separation, the strain B1748 afforded under these conditions the mansouramycins A (**5**) and D (**6**) and three new congeners, the mansouramycins E–G (**1a–3a**) as dark red solids. The isoquinolinequinones gave brown-red zones on TLC, with UV absorptions in solution similar as of *peri*-hydroxyquinones. Their reversible color change with sodium dithionite from orange to nearly colorless confirmed quinones; *peri*-hydroxyquinones were excluded, however, by the missing bathochromic shift with sodium hydroxide. Unlike the orange-red phenoxazinone chromophore of actinomycins and related pigments, which are becoming red with concentrated sulfuric acid, the isoquinolinequinones turned yellow. Further physicochemical properties of compounds **1a–3a** are summarized in Table 1.

Table 1. Physico-chemical properties of mansouramycins E–G (**1a–3a**).

Analytical Methods	Mansouramycin E (1a)	Mansouramycin F (2a)	Mansouramycin G (3a)
Appearance	Red powder	Dark red solid	Red solid
R _f ^a	0.76 (CH ₂ Cl ₂ /7% MeOH)	0.50 (CH ₂ Cl ₂ /7% MeOH)	0.23 (CH ₂ Cl ₂ /7% MeOH).
Anisaldehyde/H ₂ SO ₄ reagent	yellow	yellow	yellow
Staining with NaOH	no color change	no color change	no color change
Molecular Formula	C ₁₆ H ₁₁ N ₃ O ₂	C ₁₂ H ₉ N ₃ O ₂	C ₁₅ H ₁₁ N ₃ O ₄
UV/vis λ _{max} (log ε)	(MeOH): 244 (4.17), 264 (4.20), 287 sh (4.17), 314 sh (3.71), 377 (3.94), 448 sh (3.28), 509 sh (3.17); (MeOH + 1N NaOH): 243 (4.16), 263 (4.21), 287 sh (4.17), 313 sh (3.77) 378 (3.94), 449 sh (3.47), 508 sh (3.17); (MeOH + 1N HCl) 245 sh (4.04), 267 (4.14), 284 sh (4.02) 314 sh (3.31) 387 (3.91), 510 (3.17) nm	(MeOH): 234 (3.66), 288 (3.26), 373 (3.22), 481 sh (2.38); (MeOH+ 1N HCl): 237 (3.53), 313 (3.38), 378 (3.12), 485 sh (2.38) nm; (MeOH+1N NaOH): 233 (3.64), 289 (3.28), 375 (3.19), 485 sh (2.38) nm	(MeOH): 244 (4.13), 299 sh (3.65), 382 (3.42), 435 nm (3.47); (MeOH + 1N HCl): 243 (4.05), 303 (3.65), 377 (3.46), 436 (3.47); (MeOH + 1N NaOH): 245 (4.07), 302 sh (3.57), 384 (3.35), 437 (3.35) nm
IR (KBr) ν _{max} (KBr)	3434, 2925, 2855, 1672, 1625, 1598, 1510, 1491, 1412, 1384, 1354, 1311, 1268, 1208, 1050 cm ⁻¹	3419, 2926, 2856, 1669, 1595, 1543, 1515, 1489, 1420, 1384, 1336, 1264, 1097, 1028, 764, cm ⁻¹	3426, 2925, 2855, 1616, 1559, 1544, 1458, 1412, 1384, 1325, 1261, 1028 cm ⁻¹
CI-MS: m/z (%)		245.0 ([M+NH ₄] ⁺ , 5), 228.0 ([M+H] ⁺ , 100)	
(+)-ESI-MS: m/z (%)	278 ([M+H] ⁺)		320.2 ([M+Na] ⁺ , 31), 617.0 ([2M+Na] ⁺ , 100)
EI-MS: m/z (%)	277 [M] ⁺ (84), 256 (8), 249 (12), 236 (15), 220 (11), 195 (8), 192 (13), 179 (9), 166 (24), 138 (13), 102 (8), 97 (15), 82 (28), 73 (36), 69 (42), 57 (72), 43 (76), 44 (100)	227 ([M] ⁺ , 100), 199 ([M-CO] ⁺ , 8), 186 (16), 145 (9), 116 (8), 59 (12), 43 (8)	
(+)-ESI-HRMS: m/z		228.07663 [M+H] ⁺	298.08203 [M+H] ⁺
Calcd.	277.0846 for C ₁₆ H ₁₁ N ₃ O ₂	228.07667 for C ₁₂ H ₉ N ₃ O ₂ [M+H] ⁺	298.08223 for C ₁₅ H ₁₂ N ₃ O ₄ [M+H] ⁺
EI HRMS: m/z	277.0848		

^a Silica gel G/UV₂₅₄; **1b**, **2b**, **3b** (CH₂Cl₂/7% MeOH); sh = shoulder.

2.1. Structure Elucidation

Compound **1a** was obtained as red powder of moderate polarity. The molecular formula was determined as C₁₆H₁₁N₃O₂ by EI-HRMS, indicating 13 double bond equivalents (DBE). The color change to yellow with concentrated sulfuric acid and the characteristic UV curve with a flat absorption at λ_{max} 509 nm as for **5**, **6** pointed to an isoquinolinequinone moiety as well [10] (Table 1). The ¹³C NMR spectrum (Table 2) showed six aromatic/olefinic methines and one methyl signal. Furthermore, signals of nine non-protonated carbon atoms were observed, of which two at δ 182.8 and 181.4 pointed to carbonyl groups of a quinone. In the proton NMR spectrum (Table 2), the CH singlets at δ 9.01 (H-1) and 5.71 (H-6), in addition to a broadened NH signal at δ 7.83 and a methyl doublet at δ 2.85 of the CH₃NH fragment were typical for mansouramycins.

The proton H-6 showed HMBC correlations (Figure 2) with C-4 (⁴J), 4a, 5, 7, and C-8; correlations of the N-methyl signal with C-7, and of H-1 with C-3, 4a and 8 resulted in a 3,4-disubstituted 7-methylamino-isoquinolinequinone skeleton as in **4a–6**; unfortunately, NH HMBC correlations were not visible for **1a**.

A 1,2-disubstituted benzene ring was deduced from the typical signal pattern of four *o,m*-coupled protons at δ 8.23 (d), 7.79 (d), 7.61 (td), and 7.31 (td) ppm and from the expected HMBC correlations (Figure 2). A further broadened NH signal was seen at δ 11.98, which formed with the remaining atoms an aniline residue. With respect to the two open valencies in both fragments, the isoquinoline and the aniline unit can be merged only in two ways under formation of structures **1a** or **1b**. The more in-depth analysis of the NMR data by means of the structure elucidation program COCON [15] confirmed isomers **1a** and **1b** as allowed structures, but delivered >7600 additional alternatives! Most of them were highly strained (cyclobutenes, non-linear allenes, or bridged aromatic systems) and therefore excluded.

Table 2. ^{13}C (150 MHz) and ^1H NMR spectroscopic data of compounds **1a–3a** in $\text{DMSO-}d_6$ (δ in ppm, J in [Hz]).

Position	Mansouramycin E (1a)		Mansouramycin F (2a)		Mansouramycin G (3a)	
	δ_{C} , Type	δ_{H} (Mult, J in [Hz]) ^(a)	δ_{C} , Type	δ_{H} (Mult, J in [Hz]) ^(b)	δ_{C} , type	δ_{H} (Mult, J in [Hz]) ^(a)
1	139.0, CH	9.01 (s)	140.7, CH	8.90 (s)	150.4, CH	9.31 (s)
3	149.9, C		153.5, C		153.4, C	
4	127.6, C		122.4, C		127.0, C	
4a	120.3, C		121.9, C		141.5, C	
5	182.8, C		182.8, C		178.2, C	
6	99.6, CH	5.71 (s)	99.0, CH	5.62 (s)	100.8, CH	5.75 (s)
7	149.9 ^(c) , C		149.9, C		148.9, C	
8	181.4, C		181.2, C		179.8, C	
8a	120.4, C		117.3, C		126.7, C	
9		7.83 (brs)		7.81 (brq, 5.2)		7.84 (brq, 5.1)
10	29.0, CH_3	2.85 (d, 4.9)	28.9, CH_3	2.83 (d, 5.2)	28.9, CH_3	2.82 (d, 5.1)
1'		11.98 (brs)		11.88 (brs)	177.6, C	
1'a	144.7 ^(c) , C					
2'	113.3, CH	7.79 (d, 7.9)	137.6, CH	7.93 (t, 3.05)	99.6, CH	5.77 (s)
3'	129.9, CH	7.61 (td, 7.9, 1.3)	102.7, CH	6.70 (dd, 3.05, 1.83)	152.2, C	
4'	120.6, CH	7.31 (td, 8.1, 1.4)			180.9, C	
5'	120.8, CH	8.23 (d, 8.1)				
5'a	120.1, C					
5'						8.04 (brq, 4.9)
6'					29.0, CH_3	2.83 (brd, 4.9)

^(a) 300 MHz; ^(b) 600 MHz. See Supplementary Materials for NMR spectra. ^(c) Small signals; the assignment was confirmed by their HMBC correlations.

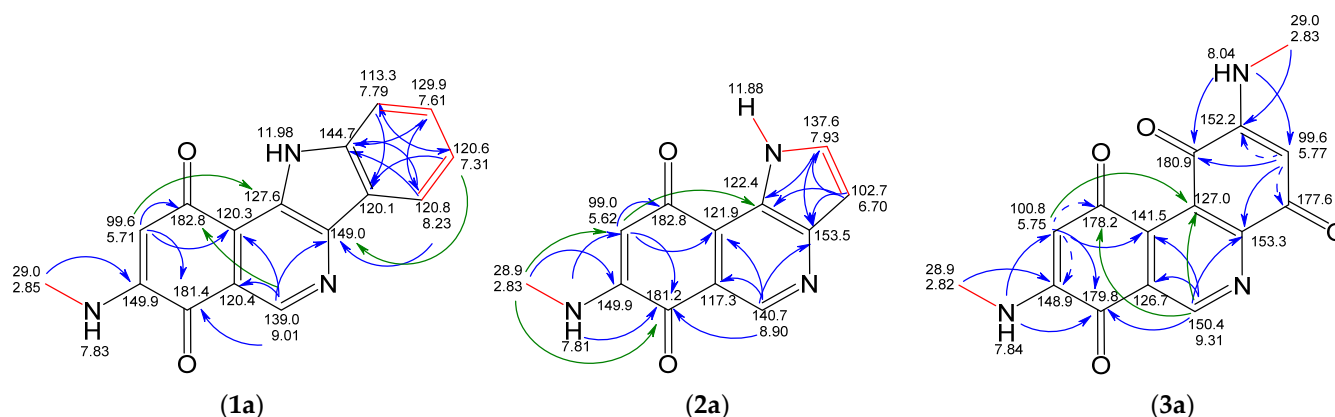


Figure 2. 2D NMR correlations of mansouramycins E–G (**1a–3a**). Blue arrows = 2J , 3J HMBC correlations; green arrows = 4J HMBC correlations, red bonds = COSY correlations.

Amongst 24 plausible indoloquinoline- and indoloisoquinoline-quinones, only 6 (**1a**, **1b**, **S1c**, **S1e**, **S1f**, **S1h**) * were found by COCON and therefore only these are in agreement with the COSY and HMBC correlations. For mansouramycin E, the isomer **1a** showed the best agreement of experimental NMR data with shifts calculated by SPARTAN'20 [16] using ab initio methods on a high level of theory. This structure was therefore assumed for mansouramycin E (see Supplementary Materials). Further applications of this technique have been described previously [17]. * Formula numbers with a leading bold letter “S” are referring to structures in Supplementary Materials.

For the dark red mansouramycin F (**2a**) the molecular formula $\text{C}_{12}\text{H}_9\text{N}_3\text{O}_2$ (ESI-HRMS) was determined, which entails 10 DBE. The ^1H and ^{13}C NMR shifts (Table 2), as well as the HMBC couplings, confirmed again an *N*-methyl-isoquinolinequinone substructure as in all other mansouramycins (Figure 1). According to the chemical shifts and 2D correlations, the unassigned residual atoms $\text{C}_2\text{H}_3\text{N}$ were belonging to an annulated pyrrole ring, which was confirmed by the ^1H triplet at δ_{H} 7.93 and the dd signal at 6.70 with the expected small coupling constants (~ 5 Hz). The pyrrole ring can be fused with the isoquinolinequinone core in three different ways, yielding structures **2a**, **2b**, **S2m**, and the respective isomers with the *N*-methyl group at C-6 instead at C-7 (see Supplementary Materials).

With COCON using atom types, 19 isomers were found. Four of them were quinones (**2a**, **2b**, **S2c**, **S2d**). The other structures were azepin-2-ones or highly strained bridged systems. Isomers of type **S2m** were excluded by COCON as well, and also *o*-quinones were not predicted for mansouramycin F.

H-3' in **2b** should show a 3J correlation with C-4a, which is missing in the experimental spectrum and therefore better fitting on **2a**. In **S2c/S2d**, the quinonoid proton H-7 ($\delta \sim 5.6$) should show a 3J correlation with C-8a at $\delta \sim 147$. However, this was also not observed, so that only structure **2a** was left. For further confirmation, we compared the experimental with calculated shifts of all possible pyrrolo-quinoline- and pyrroloisoquinoline-5,8-quinones. The results (Table S2) pointed again clearly to structure **2a** for mansouramycin F. This conclusion was further confirmed by comparison with similarly fused pyrrolo-pyridine skeletons [18,19].

Compound **3a** was obtained as a red solid as well, which displayed isoquinoline-quinone-like UV/vis and other physicochemical properties. The molecular formula of **3a** was established as $C_{15}H_{11}N_3O_4$ by ESI-HRMS and 1H and ^{13}C NMR analysis, entailing 12 DBE. The 1H and ^{13}C NMR spectra confirmed a further isoquinoline-quinone, which showed, however, remarkable differences compared with **1a** and **2a**. Instead of one *N*-methyl residue (7-NHCH₃) and one quinonoid proton (6-H), as in the other mansouramycins, the 1H NMR spectrum showed each two of these signals. In addition, the ^{13}C NMR spectrum displayed four carbonyl groups (δ_C 178.2, 179.8, 177.6, and 180.9) instead of two carbonyls as in **1a** and **2a** (Table 2). Interpretation of the HMBC spectrum of **3a** (Figure 2) revealed an isoquinoline-quinone and an *N*-methylaminobenzoquinone substructure, which can be connected in two different ways only, resulting in **3a** or **3b**, respectively (Figures 1 and 2). The alternative **3b** was excluded, however, based on the significant 3J HMBC correlations of H-6 (δ_H 5.75) and NH-9 (δ_H 7.84) with CO-8 (δ_C 179.8) and not with CO-5 (δ_C 178.2); the position of the second *N*-methyl group at C-3' was determined in a similar way. All the remaining HMBC and COSY correlations (Figure 2) were in full agreements with structure **3a**, a novel azaphenanthrene diquinone, which we named mansouramycin G (see also Supplementary Materials).

2.2. Biological Activities

Isolated compounds were evaluated in cytotoxicity assays against the same 36 cancer cell lines as published before [10]. Consistent with results previously reported herein for other members of the group, cytotoxicity profiling of the new mansouramycin **2a** revealed good anti-tumor activity in vitro with a mean IC₅₀ value of 7.92 μM (1.797 $\mu g mL^{-1}$). Furthermore, **2a** showed good tumor selectivity across the panel of 36 cell lines. Mansouramycin E (**1a**) was less active and selective [mean IC₅₀ = 23.10 μM (6.398 $\mu g mL^{-1}$)]. Previously reported mansouramycins C (**4b**) and A (**5**) exhibited mean IC₅₀ values of 0.089 μM (0.022 $\mu g mL^{-1}$) and 13.44 μM (2.902 $\mu g mL^{-1}$), respectively (Table 3). Mansouramycin G (**3a**) was not tested, due to a lack of material. In the agar diffusion test, crude extracts of *S. isolate* B1848 exhibited high bioactivity against *Mucor miehei* (Tü 284) and *Candida albicans*, and moderate activity against *Escherichia coli* and the alga *Chlorella vulgaris*. The samples of **1–6** were nearly consumed in the cytotoxicity assays and therefore not tested for their antimicrobial activity.

Table 3. In vitro cytotoxic activities of mansouramycins A (**5**), C (**4b**), E (**1a**), and F (**2a**).

Compound	Potency		Tumor Selectivity			Internal Code
	Mean IC ₅₀ μM ($\mu g mL^{-1}$)	Mean IC ₇₀ μM ($\mu g mL^{-1}$)	Selectivity */Total	% Selectivity	Rating **	
Mansouramycin A (5)	13.44 (2.902)	26.26 (5.671)	4/36	11%	++	MNSG078
Mansouramycin C (4b)	0.089 (0.022)	0.167 (0.041)	10/36	28%	+++	MNSG091
Mansouramycin E (1a)	23.10 (6.398)	33.95 (9.405)	0/18	0%	-	MNSG089
Mansouramycin F (2a)	7.92 (1.797)	15.19 (3.449)	7/36	19%	++	MNSG090

* individual IC₇₀ < 1/3 mean IC₇₀; e.g., if mean IC₇₀ = 2.1 μM the threshold for above average sensitivity was IC < 0.7 μM , ** - (% selective < 4%); + (4% > %selective >= 10%); ++ (10% > %selective >= 20%); +++ (% selective > 20%).

3. Materials and Methods

3.1. General Procedures

NMR spectra were measured on Varian Unity 300 and Varian Inova 600 spectrometers. The spectra were referenced to the signals of partially deuterated solvents (δ_{CH_2} 7.270, 77.000; δ_{DMSO} 2.500, 39.510). Electron spray ionization mass spectrometry (ESI HRMS): Finnigan LCQ ion trap mass spectrometer coupled with a Flux Instruments (Basel, Switzerland) quaternary pump Rheos 4000 and a HP 1100 HPLC (nucleosil column EC 125/2, 100-5, C 18) with autosampler (Jasco 851-AS, Jasco Inc., Easton, MD, USA) and a Diode Array Detector (Finnigan Surveyor LC System). High resolution mass spectra (HRMS) were recorded by ESI MS on an Apex IV 7 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). EI mass spectra (70 eV) were recorded on a Finnigan MAT 95 spectrometer (Thermo Electron Corp., Bremen, Germany) with perfluorokerosene as reference substance for EI HRMS. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer from KBr pellets. UV/vis spectra were recorded on a Perkin-Elmer Lambda 15 UV/vis spectrometer. Flash chromatography was carried out on silica gel (230–400 mesh). R_f -values were measured on Polygram SIL G/UV₂₅₄ (Macherey-Nagel & Co., Düren, Germany). Size exclusion chromatography was carried out on Sephadex LH-20 (Lipophilic Sephadex; Amersham Biosciences, Ltd., purchased from Sigma-Aldrich Chemie, Steinheim, Germany).

3.2. Isolation and Taxonomy of the Producing Strain

The marine *Streptomyces* sp. strain B1848 was isolated and deposited in the Actinomycetes culture collection of the Alfred-Wegener Institute for Polar- und Marine Research, Am Handelshafen, Bremen, Germany. The taxonomy of the strain has been described previously [12].

3.3. Fermentation and Working Up

The *S.* sp. isolate B1848 was previously cultivated on M_2^+ medium with 50% seawater in a 25 L jar fermenter (72 h at 28 °C) [12,13]. Optimization of the culture conditions has been performed now using six different media [14] at two pH values (6.5, 7.8), temperatures (28, 35 °C), and shaking rates (110, 95 rpm) for four days. TLC analysis and antimicrobial screenings indicated that medium C (meat extract medium: 10 g glucose, 2 g peptone, 1 yeast, 1 g meat extract, pH 7.8) gave the best yield of mansouramycins.

A 50-L jar fermenter with C-medium was inoculated with strain B1848 and stirred for 4 days at 28 °C with 120 rpm. The resulting pale yellow culture broth was mixed with diatomaceous earth (Celite, ca. 1.8 kg), and filtered-off under pressure. The mycelial cake was extracted with ethyl acetate (3×), and then with acetone (2×). The acetone extract was concentrated under reduced pressure, and the aqueous residue was extracted once more with ethyl acetate. The combined organic phases were concentrated in vacuo, yielding 4.8 g of reddish-orange residue. None of the compounds of interest were detected in the aqueous phases, and therefore they were discarded.

3.4. Isolation and Purification

The mycelial cake extract (4.8 g) was applied to flash silica gel column chromatography (3 × 60 cm) using a CH_2Cl_2 - CH_3OH gradient. After monitoring by TLC ($\text{CHCl}_3/5$; 10% MeOH), four fractions were obtained. Purification of fractions II-IV, using PTLC and Sephadex LH 20, led to isolation of five dark red compounds: mansouramycin A (**5**; 3.0 mg), D (**6**, 8.0 mg), E (**1a**; 4.1 mg), F (**2a**; 6.0 mg), and mansouramycin G (**3a**; 4.2 mg); for the physico-chemical properties and NMR spectral data of mansouramycins E–G (**1–3**), see Tables 1 and 2, respectively.

Mansouramycin C (3-Carbomethoxy-7-methylaminoisoquinoline-5,8-dione; **4b**): During this investigation, we realized two errors in the previously reported ^{13}C NMR data of mansouramycin D [10]: (CDCl_3 , 150 MHz): δ 180.6 (C_q -8), 179.8 (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), 53.4 (9-OCH₃), 29.3 (NCH₃).

Mansouramycin F (7-Methylamino-3*H*-pyrrolo [2,3-*c*]-isoquinoline-6,9-dione, **2a**): ¹H NMR (CDCl₃, 300 MHz): δ 10.25 (s br, 1H, NH-1'), 9.12 (s, 1H, 1-H), 7.79 (t, ³J = 2.6 Hz, 1H, 2'-H), 6.81 (t, ³J = 2.9 Hz, 1H, 3'-H), 6.25 (s br, 1H, 9-NH), 5.69 (s, 1H, 6-H), 2.98 (d, J = 5.1 Hz, 3H, CH₃-10).

Data of mansouramycins E–G (**1a–3a**) are listed in Tables 1–3, and spectra are depicted in the Supplementary Information. The working up and isolation of mansouramycins E–G (**1a–3a**) was carried out in August 2004. Spectral measurements, structural interpretation, and biological activity testing of **1a–3a** were achieved in the beginning of 2005.

3.5. Cytotoxicity Assays

A modified propidium iodide assay was used to examine the antiproliferative activity of the compounds against human tumor cell lines. 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, USA, National Cancer Institute, Bethesda, MD, USA, or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, and details of the test procedure have been described previously [20–22]. For the results, see Table 3.

3.6. DFT-Calculations

The calculation of NMR shifts was performed in a sequence of six calculation steps implemented in SPARTAN'20 [16]: (1) for all molecules of interest, the least energy conformers were determined using the “systematic approach” of the Merck Molecular Force Field program (MMFF). Up to 500 MMFF conformers within 40 kJ/mol above the global minimum were kept; in step (2), geometries were further optimized with a Hartree-Fock calculation (HF/3-21G); up to 200 conformers with <40 kJ/mol above the global minimum the energies were kept and (3) optimized (energies) with the DFT functional ωB97X-D and the 6-31G* basis set; (4) for up to 100 conformers within a window of 15 kJ/mol, the geometries were calculated now with the same functional and basis set; up to 50 conformers with <10 kJ/mol were kept for step (5); for the remaining conformers, energies and Boltzmann factors (300 K) were calculated with ωB97X-V/6-311+G(2df,2p) [6-311G*]; (6) for up to 30 resulting conformers with <10 kJ/mol the NMR data were calculated with ωB97X-D/6-31G* using the geometries from step (4). The conformer shifts were averaged with the Boltzmann factors from step five.

4. Conclusions

Isoquinoline-quinones from marine invertebrates and associated streptomycetes attracted scientific attention due to their strong anticancer activities [1–6]. From marine-derived *Streptomyces* spp., we isolated recently five isoquinoline-quinone derivatives, the mansouramycins A–D and 3-methyl-7-(methylamino)-5,8-isoquinolinedione (**4a**), which showed significant cytotoxicity in a panel of up to 36 tumor cell lines, with pronounced selectivity for non-small cell lung cancer, breast cancer, melanoma, and prostate cancer cells [10]. After a culture optimization, we succeeded now to isolate three further mansouramycins E–G (**1a–3a**) from the same marine *Streptomyces* sp. strain B1848, used optimized culture conditions. Their structures were elucidated by computer-assisted interpretation of 1D and 2D NMR spectra, high resolution mass spectrometry, by comparison with *ab initio*-calculated NMR data and by comparison with related compounds. Cytotoxicity profiling of the mansouramycins in a panel of up to 36 tumor cell lines indicated only a moderate cytotoxicity and tumor selectivity for the new quinones E (**1a**) and F (**2a**). The novel azaphenanthrene-diquinone mansouramycin G (**3a**) was not tested due to insufficient material.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/md19120715/s1>, NMR spectra and other supplementary data.

Author Contributions: Conceptualization, M.S., K.A.S. and H.L.; methodology, M.S., K.A.S. and G.K.; validation, M.S., K.A.S., G.K., H.H.F. and H.L.; formal analysis, M.S., K.A.S., G.K., H.H.F. and H.L.; investigation, M.S. and K.A.S.; resources, H.L.; data curation, M.S., K.A.S. and H.L.; writing—Original draft preparation, M.S. and K.A.S.; writing—Review and editing, M.S., K.A.S., G.K., H.H.F. and H.L.; visualization, M.S., K.A.S., G.K., H.H.F. and H.L.; supervision, H.L.; project administration, H.L.; funding acquisition, H.L. All authors have read and agreed to the published version of the manuscript.

Funding: The financial support of this work was funded by a grant from the Bundesministerium für Bildung und Forschung (BMBF, grant 03F0415A).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Further data are available on request from the corresponding author.

Acknowledgments: We thank R. Machinek for the NMR measurements, H. Frauendorf for the mass spectra, and F. Lissy and A. Kohl for technical assistance.

Conflicts of Interest: The authors have no competing interest to declare.

References

1. Fukumi, H.; Kurihara, H.; Hata, T.; Tamura, C.; Mishima, H.; Kubo, A.; Arai, T. Mimosamycin, a novel antibiotic produced by *Streptomyces lavendulae* No. 314: Structure and synthesis. *Tetrahedron Lett.* **1977**, *18*, 3825–3828. [[CrossRef](#)]
2. Kubo, A.; Kitahara, Y.; Nakahara, S.; Iwata, R.; Numata, R. Synthesis of mimocin, an isoquinolinequinone antibiotic from *Streptomyces lavendulae*, and its congeners. *Chem. Pharm. Bull.* **1988**, *36*, 4355–4363. [[CrossRef](#)] [[PubMed](#)]
3. Pettit, G.R.; Collins, J.C.; Herald, D.L.; Doubek, D.L.; Boyd, M.R.; Schmidt, J.M.; Hooper, J.N.A.; Tackett, L.P. Isolation and structure of cribrostatins 1 and 2 from the blue marine sponge *Cribrochalina* sp. *Can. J. Chem.* **1992**, *70*, 1170–1175. [[CrossRef](#)]
4. Pettit, G.R.; Knight, J.C.; Collins, J.C.; Herald, D.L.; Pettit, R.K.; Boyd, M.R.; Young, V.G. Antineoplastic agents 430. Isolation and structure of cribrostatins 3, 4, and 5 from the republic of maldives *Cribrochalina* species. *J. Nat. Prod.* **2000**, *63*, 793–798. [[CrossRef](#)] [[PubMed](#)]
5. Frincke, J.M.; Faulkner, D.J.J. Antimicrobial metabolites of the sponge *Reniera* sp. *J. Am. Chem. Soc.* **1982**, *104*, 265–269. [[CrossRef](#)]
6. Milanowski, D.J.; Gustafson, K.R.; Kelley, J.A.; McMahan, J.B. Caulibugulones A–F, novel cytotoxic isoquinoline quinones and iminoquinones from the marine bryozoan *Caulibugula intermis*. *J. Nat. Prod.* **2004**, *67*, 70–73. [[CrossRef](#)] [[PubMed](#)]
7. Kubo, A.; Nakahara, S.; Iwata, R.; Takahashi, K.; Arai, T. Mimocin, a new isoquinolinequinone antibiotic. *Tetrahedron Lett.* **1980**, *21*, 3207–3208. [[CrossRef](#)]
8. Mckee, T.C.; Ireland, C.M. Cytotoxic and antimicrobial alkaloids from the Fijian sponge *Xestospongia caycedoi*. *J. Nat. Prod.* **1987**, *50*, 754–756. [[CrossRef](#)] [[PubMed](#)]
9. Speitling, M. Vergleich der Metabolischen Kapazität Mariner und Terrestrischer Mikroorganismen—Isolierung und Strukturaufklärung von Branimycin, Brom-alterochromid A/B und weiteren Stoffwechselprodukten. Ph.D. Thesis, Georg-August University, Göttingen, Germany, 1998.
10. Hawas, U.W.; Shaaban, M.; Shaaban, K.A.; Speitling, M.; Maier, A.; Kelter, G.; Fiebig, H.H.; Meiners, M.; Helmke, E.; Laatsch, H. Mansouramycins A–D, Cytotoxic Isoquinolinequinones from a marine Streptomycete. *J. Nat. Prod.* **2009**, *72*, 2120–2124. [[CrossRef](#)] [[PubMed](#)]
11. Huang, C.; Yang, C.; Zhang, W.; Zhu, Y.; Ma, L.; Fang, Z.; Zhang, C. Albumycin, a new isoindolequinone from *Streptomyces albus* J1074 harboring the fluostatin biosynthetic gene cluster. *J. Antibiot.* **2019**, *72*, 311–315. [[CrossRef](#)] [[PubMed](#)]
12. Shaaban, M.; Schröder, D.; Shaaban, K.A.; Helmke, E.; Wagner-Döbler, I.; Laatsch, H. Flazin, Perlolyrin, and other new β -Carbolines from marine-derived Bacteria. *Rev. Latinoam. Quim.* **2007**, *35*, 58–67.
13. Shaaban, K.A.; Shaaban, M.; Nair, V.; Schuhmann, I.; Win, H.Y.; Lei, L.; Dittrich, B.; Helmke, E.; Schüffler, A.; Laatsch, H. Structure Elucidation and Synthesis of Hydroxylated Isatins from Streptomycetes. *Z. Naturforsch. B* **2016**, *71*, 1191–1198. [[CrossRef](#)]
14. Shaaban, M. Bioactive Secondary Metabolites from Marine and Terrestrial Bacteria: Isoquinolinequinones, Bacterial Compounds with a Novel Pharmacophor. Ph.D. Thesis, Georg-August University, Göttingen, Germany, 2004.
15. Lindel, T.; Junker, J.; Koeck, M. 2D-NMR-guided constitutional analysis of organic compounds employing the computer program COCON. *Eur. J. Org. Chem.* **1999**, *1999*, 573–577. [[CrossRef](#)]
16. SPARTAN'20; Wavefunction, Inc.: Irvine, CA, USA, 2020.
17. Shaaban, M.; Abou-El-Wafa, G.S.E.; Golz, C.; Laatsch, H. New haloterpenes from the marine red alga *Laurencia papillosa*: Structure elucidation and biological activity. *Mar. Drugs* **2021**, *19*, 35. [[CrossRef](#)] [[PubMed](#)]

18. Arzel, E.; Rocca, P.; Marsais, F.; Godard, A.; Quéguiner, G. First total synthesis of cryptomisine. *Tetrahedron* **1999**, *55*, 12149–12156. [[CrossRef](#)]
19. Kim, J.S.; Shin-ya, K.; Furihata, K.; Hayakawa, Y.; Seto, H. Structure of mescengricin, a novel neuronal cell protecting substance produced by *Streptomyces griseoflavus*. *Tetrahedron Lett.* **1997**, *38*, 3431–3434. [[CrossRef](#)]
20. Dengler, W.A.; Schulte, J.; Berger, D.P.; Mertelsmann, R.; Fiebig, H.H. Development of a propidium iodide fluorescence assay for proliferation and cytotoxicity assays. *Anticancer Drugs* **1995**, *6*, 522–532. [[CrossRef](#)] [[PubMed](#)]
21. He, J.; Roemer, E.; Lange, C.; Huang, X.; Maier, A.; Kelter, G.; Jiang, Y.; Xu, L.; Menzel, K.-D.; Grabley, S.; et al. Structure, derivatization, and antitumor activity of new griseusins from *Nocardioopsis* sp. *J. Med. Chem.* **2007**, *50*, 5168–5175. [[CrossRef](#)] [[PubMed](#)]
22. Fiebig, H.H.; Maier, A.; Burger, A.M. Clonogenic assay with established human tumour xenografts: Correlation of in vitro to in vivo activity as a basis for anticancer drug discovery. *Eur. J. Cancer* **2004**, *40*, 802–820. [[CrossRef](#)] [[PubMed](#)]