ORIGINAL ARTICLE

Aqabamycins A–G: novel nitro maleimides from a marine *Vibrio* species: II. Structure elucidation*

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The structures of secondary metabolites with antibacterial and cytotoxic activities produced by a marine *Vibrio* strain from the Red Sea were elucidated. Aqabamycin A (1a) and seven further nitro-substituted maleimide derivatives named aqabamycins B–G (1b–f and 2) were obtained together with 12 known metabolites, 3-nitro-1*H*-indazole (3), indazole-3-carbaldehyde (4), 3-nitro-4-hydroxycinnamic acid, 4-hydroxycinnamic acid, 3-nitro-4-hydroxybenzaldehyde, phenyl-2-*bis*-indolylmethane (5a), turbomycin B (5b), vibrindole A (6), phenylacetic acid, 3-hydroxybenzoic acid, benzoic acid and 1,4-dithiane (7). Some of the known metabolites (for example, 3, 4 and 7) are described in this study for the first time as natural products. Their structures were elucidated based on 1D and 2D NMR, MS spectra and by comparison with synthetic material.

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INTRODUCTION

Besides marine streptomycetes, marine *Bacillus*, *Alteromonas*, *Pseudoalteromonas* and *Vibrio* species represent the most prolific producers of secondary metabolites.^{1,2} The latter bacteria colonize a large number of aquatic plants and sedentary animals and produce diverse chemical compounds that have a role in protection against pathogenic and fouling microorganisms.³ In a screening of bacterial strains isolated from living marine surfaces, extracts obtained from submerged cultures of the *Vibrio* strain WMBA showed antimicrobial and cytotoxic activities. Bioassay-guided isolation yielded 19 compounds, the majority of them with novel structures or for the first time isolated from nature. In this paper we report the chemical properties and the structure elucidation of these metabolites. The taxonomy of strain WMBA, its fermentation, the isolation and purification of the compounds as well as their biological activities were reported in a previous paper.⁴

RESULTS AND DISCUSSION

Maleimides are forming a discrete group of natural products with a variety of biological activities such as fungicidal, antibacterial and cytotoxic properties.⁵ So far, only a few members have been reported, such as the bisindolylmaleimide derivatives arcyrarubins B and C, or arcyriflavins B and C from the myxomycete *Arcyria denudate*,⁶ and the diarylmaleimide derivatives himanimides A, B and C from *Serpula himantoides* (*Coniophoraceae*).⁵ In most cases, these pyrrole-2,5-diones are substituted at the nitrogen or both in positions 3 and 4.

A number of unusual new 3,4-biaryl-maleimides has now been obtained from a marine *Vibrio* sp., isolated from the surface of the soft coral *Sinularia polydactyla* in the Red Sea at Aqaba (Jordan).⁴ Extracts of this strain yielded 19 compounds, among them eight new pyrrole-2,5-diones. Most of these metabolites are diphenyl-maleimides containing 16 carbon atoms; aqabamycin G (2) is an exception with 18 carbon atoms due to an indole residue. All aqabamycins are yellow with UV absorptions in the range of 360–400 nm. Besides 1a, all aqabamycins are nitrated, but unexpectedly, the influence of the nitrophenol unit on the long wavelength absorption is low. The ¹H NMR spectra are remarkably simple and showed only signals in the aromatic region.

The metabolite 1a was obtained as a yellow solid, which yielded a molecular formula of C₁₆H₁₁NO₃ by electrospray ionization HRMS (ESI HRMS) of the pseudomolecular ion at *m/z* 264 ([M–H][–]). The ¹H NMR spectrum indicated an A₂B₂ pattern of a *para*-disubstituted benzene with signals at δ 7.32 and 6.72. In addition, two multiplets integrating for three and two protons, respectively, appeared at δ 7.36 and 7.42, which were attributed to a monosubstituted benzene, based on the H,H COSY spectrum. The ¹³C NMR spectrum indicated two carbonyls of an acid or amide at δ 173.6 and 173.5 (C-2, 5), and additional five quaternary *sp*² carbons, including an oxygenated *sp*² carbon at δ 160.5 (C-4'), and nine *sp*² methines. The HBMBC spectrum confirmed the mono- and disubstituted benzene rings, accounting for eight double bond equivalents and nine *sp*² methines. Two carbonyls, a ring and a double bond contributed the remaining four double bond equivalents. The combination of these data led to the structure of

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3-(4-hydroxyphenyl)-4-phenylpyrrole-2,5-dione (**1a**), for which the name aqabamycin A is suggested.

Only two related 3,4-diphenylpyrrole-2,5-diones had been described before, the brominated polycitrins A and B from the marine ascidian *Polycitor* sp.⁷

HPLC separation of the extract, as described in the previous paper,⁴

	R ¹	R ²	R ³		R ¹	R ²	R ³	R ⁴
1a	H	H	H	1e	NO ₂	OH	H	H
1b	H	OH	NO ₂	1e'	H	H	OH	NO ₂
1c	NO ₂	H	H	1f	NO ₂	OH	OH	NO ₂
1d	NO ₂	OH	NO ₂					

Structures **1a-f**.

delivered a series of six further diphenylmaleimides, which were, however, all nitrated: aqabamycin B (**1b**) had a mass of m/z 326 (EI-MS) and presented similar chemical properties as **1a**. The molecular formula, C₁₆H₁₀N₂O₆, derived from ESI HRMS, indicated 13 double bond equivalents, one more than in **1a**. The ¹H NMR spectrum showed a *para*-substituted phenol unit as in **1a**; however, ¹H signals at δ 8.29 (d, J 2.2 Hz), 7.61 (dd, J 8.7, 2.2 Hz) and 7.09 (d, J 8.8 Hz) indicated an additional 1,2,4-trisubstituted aromatic ring. The ¹³C NMR data of **1b** were also similar to those of **1a** (Table 1), except for the sp^2 carbon signals at δ 156.2 (C-4'') and 136.1 (C-3''). The H,H COSY spectrum confirmed the two fragments derived from the proton NMR spectrum. The difference in the molecular formula between **1a** and aqabamycin B (**1b**) can be interpreted as additional OH and NO₂ groups. The HMBC spectrum confirmed this assumption by correlations between H-2'' and C-4, H-2' and C-3, and cross-signals between H-2'', H-4'' and C-6'' (Figure 1).

According to the chemical and spectroscopic similarities with **1a** and **1b**, the third compound was also a diphenylmaleimide; the molecular formula C₁₆H₁₀N₂O₅ of aqabamycin C (**1c**) indicated, however, one oxygen atom less than in **1b**. The major difference in the ¹H NMR spectra of **1c** in comparison with those of **1a** and **1b** was a phenyl residue instead of the *para*-substituted phenol. The ¹³C NMR spectrum indicated two carbonyls at δ 169.9 and 169.8, an oxygenated sp^2 carbon at δ 156.2, five further quaternary sp^2 and eight sp^2 methine signals. Further COSY and HMBC correlations confirmed aqabamycin C as the new 3-(4-hydroxy-3-nitrophenyl)-4-phenylpyrrole-2,5-dione (**1c**).

The yellow powdery **1d** (C₁₆H₉N₃O₈) showed in methanol only three of the expected nine proton signals, with the typical pattern of a 1,3,4-trisubstituted benzene. The ¹³C NMR spectrum gave only eight signals and indicated a symmetrical molecule. The structure **1d** of aqabamycin D was proven by the HMBC data (Supplementary Figure 1), the similarity with **1b** and the empirical formula. The three acidic protons were exchanged in methanol.

Aqabamycin E (**1e**) showed similar ¹H NMR data as **1c**, although the spectrum showed a mixture of two closely related compounds in

Table 1 ¹H NMR (δ , J in Hz) data of compounds **1a-c** at 600 MHz

Position	1a ^a	1b ^a	1c ^c	1d ^a	1e/1e' ^a	1f ^a	1f ^b
1-NH	—	—	10.78 (s)	—	—	—	11.76 (br s)
2'	7.32 (d, 8.8)	7.35 (d, 8.8)	8.42 (d, 2.2)	8.32 (d, 2.2)	8.24 (d, 2.2), 8.20 (d, 2.2)	8.24 (d, 2.2)	8.02 (d, 2.2)
3'	6.72 (d, 8.8)	6.78 (d, 8.8)	—	—	—	—	—
4'	—	—	—	—	—	—	—
4'-OH	—	—	7.60 (s br)	—	—	—	10.98 (s)
5'	6.72 (d, 8.8)	6.78 (d, 8.8)	7.10 (d, 8.8)	7.12 (d, 8.8)	7.02 (d, 8.8), 7.03 (d, 8.8)	7.08 (d, 7.7)	7.05 (d, 7.7)
6'	7.32 (d, 8.8)	7.35 (d, 8.8)	7.61 (dd, 8.8, 2.2)	7.63 (dd, 8.8, 2.2)	7.52 (dd, 8.7, 2.2), 7.49 (dd, 8.7, 2.2)	7.59 (dd, 8.7, 2.2)	7.43 (dd, 7.7, 2.2)
1''	—	—	—	—	—	—	—
2''	7.42 (m)	8.29 (d, 2.2)	7.42 (m)	8.32 (d, 2.2)	—	8.22 (d, 2.2)	8.01 (d, 2.2)
3''	7.36 (m)	—	7.42 (m)	—	7.38 (m)	—	—
4''	7.36 (m)	—	7.42 (m)	—	7.38 (m)	—	—
5''	7.36 (m)	7.09 (d, 8.8)	7.42 (m)	7.12 (d, 8.8)	7.38 (m)	7.08 (dd, 7.7)	7.03 (d, 7.7)
6''	7.42 (m)	7.61 (dd, 8.7, 2.2)	7.42 (m)	7.63 (dd, 8.8, 2.2)	7.38 (m)	7.58 (dd, 8.7, 2.2)	7.40 (dd, 7.7, 2.2)

^aMeOH-d₄.

^bDMSO-d₆.

^cCDCl₃.

Figure 1 Selected HMBC correlations in aqabamycin B (**1b**).

Figure 2 Selected HMBC correlations in aqabamycin F (**1f**).

the ratio of 2:1. The molecular formula $C_{16}H_{11}N_3O_5$ (by (–)-ESI HRMS) indicated an additional nitrogen atom and a proton with respect to **1c**. As the ^{13}C NMR spectrum showed only one carbonyl group at δ 170.7 (170.5 for the minor isomer) instead of two as in **1a–c**, the additional signal at δ 149.3 (minor 149.7) in **1e** must be due to an oxime. The satellite peaks in the 1H and ^{13}C NMR spectra may indicate *E/Z* isomers, or be because of the isomeric 3-(4-hydroxy-3-nitrophenyl)-4-phenylpyrrole-2,5-dione 2- or 5-oxime (**1e** or **1e'**). As the related aqabamycin F (**1f**) was not obtained as an *E/Z* mixture, positional isomers **1e/1e'** are more likely. The position of the oxime group could not be assigned unambiguously.

Aqabamycin F had the molecular formula of $C_{16}H_{10}N_4O_8$. The 1H NMR spectrum of **1f** in DMSO- d_6 indicated three 2H signals, two pairs of doublets δ 8.01/8.02 (*J* 2.2 Hz) and 7.03/7.05 (*J* 7.7 Hz), and two overlapping doublets of doublets at δ 7.40/7.43. The coupling constants were characteristic of two sets of 1,2,4-trisubstituted benzene rings. In addition, two H/D exchangeable 1H singlets at δ 11.76 and 10.98 were visible, attributed to two hydrogen-bonding hydroxy groups. The only difference with respect to **1d** was the absence of the symmetry plane in **1f** and the higher mass due to an additional nitrogen and a hydrogen atom.

The ^{13}C NMR spectrum showed all 16 carbons signals between δ 180 and 110 as in **1a–d**, suggesting the same skeleton. The HMBC (Figure 2) and the H,H COSY spectra indicated the presence of two identically substituted benzene units, but instead of two carbonyls as in **1a–d**, only one was seen at δ 170.3 and an imide signal was found at 149.2 as in compound **1e**; for aqabamycin F, the oxime structure **1f** is therefore plausible. As the NMR spectra showed only a single set of signals, an *E/Z* mixture can be excluded, and aqabamycin exists probably as the more stable *Z* isomer.

Aqabamycin G (**2**) was obtained as a red solid, which gave a brown color with anisaldehyde/sulfuric acid. It was nearly insoluble in chloroform, but fairly soluble in methanol (MeOH) and DMSO. From the molecular mass, the formula $C_{18}H_{11}N_3O_5$ was derived. Accordingly, the ^{13}C NMR spectrum indicated 18 carbon signals, including two carbonyls at δ 173.2 and 172.6, eight sp^2 methines and eight quaternary sp^2 carbons.

Figure 3 Structure and H,H-COSY and HMBC correlations of aqabamycin G (**2**).

The proton NMR spectrum in DMSO- d_6 showed two broad singlets at δ 11.62 and 10.68 attributed to two acidic protons; the aromatic region showed two doublets and two triplets of a 1,2-disubstituted benzene unit and the pattern of a 1,2,4-trisubstituted benzene with shifts similar as for **1d**; both fragments were confirmed by COSY correlations (Figure 3). The doublet at δ 7.73 (DMSO, Table 1) collapsed to a singlet in methanol, and the HMBC spectrum indicated correlations with the signals of quaternary carbons, C-3'', C-3a'' and C-7a'', confirming the existence of a 3-substituted indole moiety.

The proton signals at δ 8.22 (2'-H) showed a weak HMBC correlation with the sp^2 carbon at 130.6 (C-3), but the respective cross-signal of the proton at δ 7.73 (2''-H) with the quaternary carbon at δ 125.3 (C-4) was missing. The remaining four double bond equivalents and the residual atoms (C_4HNO_2), together with the two carbonyl signals at δ 173.2 and 172.6, made a further maleimide plausible. As a result, the structure of **2** was determined as 3-(4-hydroxy-3-nitrophenyl)-4-(1H-indol-3-yl)-pyrrole-2,5-dione (Figure 3).

Among >200 000 natural products, approximately 150 are maleimides, ca. 60 are unsubstituted at the imide nitrogen and only around 10 of them are 3,4-bisarylmaleimides, if about 20 staurosporine-like metabolites are not taken into account. According to the literature, natural nitro maleimides were unknown so far.

The closest similarity with the aqabamycins is shown by the *Arcyria* pigments; ongoing from 1980, the bisindolylmaleimide derivatives arcyciarubins B and C in addition to arcyciaflavins B and C were isolated by Steglich *et al.*⁶ from the myxomycete *Arcyria denudata*. Several other maleimide derivatives are reported from basidiomycetes; for example, himanimides A and C.⁵

Aqabamycins E (**1e/1e'**) and F (**1f**) are new natural products with the unusual pyrrole-2,5-dione-2-oxime system. Maleimide-mono-oximes are very rare in natural products, in particular in bacteria.⁸ The only example, 3-methyl-pyrrole-2,5-dione-5-oxime, was isolated in 2005 from the marine sponge *Pseudoceratina purpurea* and showed inhibitory activity on the growth of human cancer cells (MCF-7 (breast), NCI-H460 (lung) and SF-268 (central nervous system)).⁹ Therefore, it is obvious that the aqabamycins are forming a unique class of compounds.

Indazoles

Among the additional fermentation products, 3-nitroindazole (**3**) was isolated as a pale yellow solid. The ^{13}C NMR spectrum showed seven carbon signals, the proton spectrum indicated a 1,2-disubstituted benzene and (–)-ESI HRMS afforded the molecular formula $C_7H_5N_3O_2$. Among the hits obtained by a literature search, **3** was the most plausible one. 3-Nitroindazole (**3**) can be obtained easily by nitration of indazole,¹⁰ and direct comparison with authentic material indeed proved its identity.

Indazole-3-carbaldehyde (**4**) was obtained as a white solid with a blue fluorescence at 366 nm. The 1H NMR spectrum was very similar to that of **3** and showed six signals, the typical pattern of a 1,2-disubstituted benzene, and at δ 14.12 the broad singlet of an acidic

proton. The carbon spectrum was nearly superimposable with that of **3**, but showed an additional aldehyde signal at δ 10.20, and differences for C-3. ESI HRMS data delivered the molecular formula $C_8H_6N_2O$, which confirmed the expected aldehyde **4**.

Structures **3** and **4**.

Both indazoles had been obtained previously by synthesis,^{10,11} but are described in this study as new natural products for the first time. Indazoles are very rare in nature, and only three further derivatives had been known as alkaloids from *Nigella sativa* (black cumin).¹² In our tests, indazole **4** showed antibacterial activity against Gram-positive bacteria as well as cytotoxic activity against mouse lymphocytic leukemia and Jurkat-T-cell leukemia. Derivatives of **4** have found broad medical applications because of their androgen receptor modulating activity, for the treatment of arrhythmia and as modulators of glutamate receptors, etc.

The 5-, 6- and 7-nitroindazoles were examined in spinal column therapy as inhibitors for N_7 -nitro-L-arginine methyl ester, which is responsible for the elevation of blood pressure by spinal injury.¹³ It remains an open question whether 3-nitroindazole is formed by nitration of indazole, which then should also be a natural product.

Bis-indolylmethanes

A second smaller fermentation in M1 medium showed a large number of peaks with indole-like UV/visible spectroscopy (Vis) spectra by analytical HPLC in the non-polar range.⁴ After various separation steps on normal-phase and reversed-phase silica gel, the bis-indole derivatives vibrindole A (**6**), phenyl-2-bis-indolylmethane (**5a**) and its cationic form, the red turbomycin B (**5b**), were isolated.

Compound **5a** was obtained as reddish oil (containing some **5b**) from fraction B,⁴ which gave a red color with anisaldehyde/sulfuric acid. The proton NMR spectrum indicated a broad 2H singlet at δ 10.90 and a further 1H singlet at δ 5.82. The sp^2 region indicated signals of a monosubstituted benzene, and additionally two doublets and two triplets of four consecutive protons, each of 2H intensity. In addition, a doublet at δ 6.82 attributed to the proton in position 2 of an indole system was observed.

EI MS indicated a molecular ion at m/z 322. However, the (+)-ESI MS delivered surprisingly a *quasimolecular* ion at m/z 321 (instead of the expected 323), corresponding to an ion formula $C_{23}H_{17}N_2$ by high resolution. In the ^{13}C NMR spectrum, only 13 of the expected 23 carbon signals were visible, pointing to a symmetrical molecule.

From the H,H COSY and HMBC spectra, a 3-substituted indole unit and a monosubstituted benzene ring were easily derived (Figure 4). The HMBC spectrum indicated a coupling between the aliphatic methine proton at δ 5.82 (CH-1''') and the quaternary carbons C-1'' and C-3, indicating that the indole and benzene fragments were connected through that carbon atom. A database search with this substructure led to turbomycin B (**5b**), and it follows that the compound isolated here is the neutral parent compound phenyl-2-bis-indolylmethane (**5a**). Tris-arylmethanes are easily oxidized; this explains the red tailing on TLC of **5a** and the molecular weight of 322 by EI MS, but a value of 321 by (+)-ESI MS for the contaminating charged **5b**.

Figure 4 HMBC correlations in **5a** (partial structure).

Structures **5a**, **b** and **6**.

Turbomycin B (**5b**) had been obtained previously from metagenomic DNA expressed in *Escherichia coli* and can be easily synthesized from indole and benzaldehyde.¹⁴ The 1H NMR spectrum of synthetic **5b** was indeed different from that of compound **5a**, which is described in this study as a new natural product. It showed cytotoxic activity because of an inhibition of the macromolecule biosynthesis in L1210 cells with IC_{50} values of 10–15 $\mu g\ ml^{-1}$.

A further colorless oily bis-indolylmethane, **6**, also gave a red color with anisaldehyde/sulfuric acid and was fairly soluble in methanol and DMSO. The 1H NMR spectrum showed signals of an indole system with double intensity, with respect to a methine quartet at δ 4.55 and a methyl doublet at δ 1.75. A search in AntiBase with the molecular formula ($C_{18}H_{16}N_2$ by (–)-ESI HRMS) and substructure revealed vibrindole A (**6**), which was first isolated from the marine strain *Vibrio parahaemolyticus* in 1994 by Carmeli *et al.*¹⁵

1,4-Dithiane

The proton NMR spectrum of the colorless oily **7** showed only one singlet at δ 2.18, and EI MS gave a molecular ion at m/z 120. On the basis of this evidence, the metabolite was identified as 1,4-dithiane.

Structure **7**.

Whereas the isomeric 1,3-dithiane is known as a garlic constituent, **7** is new as a natural product. It is, however, a widespread groundwater contaminant associated with the production and storage of ammunition at certain military installations.¹⁶

Further by-products of *Vibrio* sp. strain WMB4 were 3-nitro-4-hydroxybenzaldehyde,^{17,18} 3-nitro-4-hydroxycinnamic acid,¹⁹ 4-hydro-

xyccinnamic acid, phenylacetic acid, 3-hydroxybenzoic acid and benzoic acid. They were identified by comparison with AntiBase data; some values are listed in the supplementary information.

METHODS

Materials and methods were the same as described previously.²⁰

Taxonomy, fermentation and isolation

Details on the producing strain, its cultivation in Erlenmeyer flasks and in a fermentor as well as the isolation of the compounds have been published previously.⁴

Aqabamycin A (1a)

Yellow solid, R_F 0.41 (CH₂Cl₂/MeOH 9:1), R_T 14.30 min (LC MS); UV/Vis (MeOH) $\lambda_{\max}$ (log ϵ) 232 nm (4.21), 384 nm (3.81); IR (KBr) $\nu_{\max}$ 2295, 1799, 1709, 1607, 1514, 1351, 1276, 1175, 1020 cm⁻¹; ¹H NMR (MeOH-*d*₄, 300 MHz) and ¹³C NMR (125 MHz, MeOH-*d*₄; see Tables 1 and 2 and Supplementary Figures 2 and 3); (–)-ESI MS m/z (%) 264 ([M–H][–], 100), 528 ([2M–H][–], 25); (–)-ESI HRMS m/z 264.06654 ([M–H][–], calcd 264.06661 for C₁₆H₁₀N₃O₅).

Aqabamycin B (1b)

Yellow solid, R_F 0.56 (CH₂Cl₂/MeOH 9:1), R_T 13.82 min (LC MS); UV/Vis (MeOH) $\lambda_{\max}$ (log ϵ) 232 nm (4.33), 253 nm (4.30), 386 nm (3.95); IR (KBr) $\nu_{\max}$ 2352, 1766, 1715, 1625, 1607, 1536, 1513, 1422, 1346, 1320, 1279, 1250, 1175, 1024, 995 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆ and MeOH-*d*₄) and ¹³C NMR (125 MHz, DMSO-*d*₆ and MeOH-*d*₄; see Tables 1 and 2 and Supplementary Figures 4 and 5). EI MS (70 eV) m/z (%) 326 ([M]^{•+}, 80), 255 (20); (–)-ESI MS m/z (%) 325 ([M–H][–], 100), 528 ([2M–H][–], 25); (–)-ESI HRMS m/z 325.04653 ([M–H][–], calcd 325.04660 for C₁₆H₉N₂O₆).

Aqabamycin C (1c)

Yellow solid, R_F 0.62 (CH₂Cl₂/MeOH 9:1), R_T 15.99 min (LC MS); UV/Vis (MeOH) $\lambda_{\max}$ (log ϵ) 228 nm (4.43), 265 nm (4.24), 364 nm (3.96); IR (KBr) $\nu_{\max}$ 2849, 2159, 1771, 1717, 1625, 1537, 1537, 1485, 1445, 1421, 1344, 1322, 1255, 1183, 1126, 1079, 1020 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) and ¹³C NMR (125 MHz, CDCl₃; see Tables 1 and 2 and Supplementary Figures 6 and 7); EI MS (70 eV) m/z (%) 310 ([M]^{•+}, 100), 277 (18), 263 (10), 239 (32); (–)-ESI MS m/z (%) 309 ([M–H][–], 45), 641 ([2M+Na–2H][–], 30); (–)-ESI HRMS m/z 309.05172 ([M–H][–], calcd 309.05169 for C₁₆H₉N₂O₅).

Aqabamycin D (1d)

Yellow solid, R_F 0.60 (CH₂Cl₂/MeOH 9:1), R_T 15.02 min (LC MS); UV/Vis (MeOH) $\lambda_{\max}$ (log ϵ) 232 nm (4.49), 265 nm (4.37), 362 nm (4.05) nm; IR (KBr) $\nu_{\max}$ 2219, 2308, 1724, 1690, 1613, 1564, 1514, 1481, 1415, 1341, 1250, 1151, 1102, 1078, 1012, 974 cm⁻¹; ¹H NMR (MeOH-*d*₄, 600 MHz) and ¹³C NMR (125 MHz, MeOH-*d*₄; see Tables 1 and 2 and Supplementary Figures 8 and 9). EI MS (70 eV) m/z (%) 371 ([M]^{•+}, 100), 300 (20), 254 (2); (–)-ESI MS m/z (%) 370 ([M–H][–], 100), 762 ([2M+Na–2H][–], 70); (–)-ESI HRMS m/z 370.03155 ([M–H][–], calcd 370.03168 for C₁₆H₈N₃O₈).

Aqabamycin E (1e/1e')

Yellow solid, R_F 0.58 (CH₂Cl₂/MeOH 9:1), R_T 15.58 min (LC MS). UV/Vis (MeOH) $\lambda_{\max}$ (log ϵ) 312 nm (4.23). IR (KBr) $\nu_{\max}$ 3267, 2924, 2288, 1704, 1627 cm⁻¹ (oxime), 1535, 1484, 1420, 1342, 1320, 1251, 1177, 1135, 1079, 1026, 1008, 960 cm⁻¹. ¹H NMR (MeOH-*d*₄, 600 MHz) and ¹³C NMR (125 MHz, MeOH-*d*₄; see Tables 1 and 2 and Supplementary Figures 10 and 11); EI MS (70 eV) m/z (%) 325 ([M]^{•+}, 100), 308 (15), 278 (10), 262 (12); (–)-ESI MS m/z (%) 324 ([M–H][–], 40), 671 ([2M+Na–2H][–], 30); (–)-ESI HRMS m/z 324.06254 ([M–H][–], calcd 324.06259 for C₁₆H₁₀N₃O₅).

Aqabamycin F (1f)

Yellow solid, R_F 0.44 (CH₂Cl₂/MeOH 9:1), R_T 14.79 min (LC MS); UV/Vis (MeOH) $\lambda_{\max}$ (log ϵ) 321 nm (3.99); IR (KBr) $\nu_{\max}$ 1712, 1626 (oxime), 1529, 1484, 1422, 1342, 1320, 1252, 1178, 1135, 1082, 1023, 964 cm⁻¹; ¹H NMR

Table 2 ¹³C NMR (125 MHz) data of aqabamycins A–F (1a–f)

Position	1a ^{a,b}	1b ^a	1c ^{b,c}	1d ^a	1e/1e' ^{a,b}	1f ^d
2	173.6	173.3	169.9	172.5	149.3, 149.7	149.2
3	133.5	138.5	134.1	135.6	142.5, 142.5	139.4
4	138.4	133.1	133.9	135.6	135.8, 134.5	131.6
5	173.5	173.1	169.8	172.5	170.7, 170.5	170.3
1'	133.5	120.8	127.9	121.6	123.2, 123.7	123.3
2'	128.8	132.8	127.4	128.3	129.5, 128.5	128.9
3'	116.9	116.6	137.8	136.3	138.8, 138.8	136.0 ^e
4'	160.5	160.8	156.2	156.6	155.9, 155.4	156.0
5'	121.1	116.6	120.7	121.5	120.8, 120.8	121.0
6'	135.9	132.8	138.6	138.5	139.2, 139.1	139.0
1''	121.0	122.5	121.0	121.5	123.2, 123.7	122.9
2''	130.2	128.1	129.8	128.3	129.6, 130.0	127.7
3''	130.1	136.1	129.3	136.3	130.9, 130.4	135.9 ^e
4''	130.9	156.2	130.9	156.6	131.4, 131.2	155.6
5''	128.8	121.2	129.3	121.5	130.9, 130.4	121.1
6''	132.3	138.8	129.8	138.5	129.6, 130.0	138.8

^aMeOH-*d*₄.

^bSignals tentatively assigned.

^cCDCl₃.

^dDMSO-*d*₆.

^eValues may be exchanged.

Table 3 ¹H (600, MHz) and ¹³C NMR (125, MHz) data of aqabamycin G (2) in DMSO-*d*₆ and MeOD

Position	¹ H (DMSO)	¹³ C (DMSO)	¹ H (MeOD)	¹³ C (MeOD)
1-NH	10.68 (br s)	—	—	—
2	—	173.2	—	NV
3	—	130.6	—	127.2
4	—	125.3	—	134.2 ^a
5	—	172.6	—	NV
1'	—	109.0	—	124.2
2'	8.22 (d, 2.5)	129.2	8.26 (d, 2.2)	127.7
3'	—	136.1	—	135.5
4'	—	168.1	—	155.5
4'-OH	—	—	—	—
5'	6.10 (d, 9.1)	126.9	7.01 (d, 8.0)	120.5
6'	7.01 (dd, 9.2, 2.2)	133.9	7.68 (dd, 8.0, 2.2)	139.0
1''-NH	11.62 (s)	—	—	—
2''	7.73 (d, 2.5)	128.9	7.99 (s)	132.3
3''	—	105.0	—	105.8
3a''	—	124.6	—	125.2
4''	6.93 (d, 8.1)	121.3	6.50 (dbr, 7.0)	122.6
5''	6.84 (t, 7.9)	119.3	6.80 (td, 7.0, 1)	121.3
6''	7.05 (t, 8.1)	121.5	7.10 (td, 7.0, 1)	123.6
7''	7.41 (d, 8.1)	111.8	7.42 (dbr, 7.0)	113.2
7a''	—	136.3	—	138.5

Abbreviation: NV, signal not visible.

^aValue and assignment uncertain.

(600 MHz, DMSO-*d*₆ and MeOH-*d*₄) and ¹³C NMR (125 MHz, DMSO-*d*₆ and MeOH-*d*₄; see Tables 1 and 2 and Supplementary Figures 12 and 13); EI MS (70 eV) m/z (%) 386 ([M]^{•+}, 25), 370 (5), 328 (5); (–)-ESI MS m/z (%) 385 ([M–H][–], 100), 792 ([2M+Na–2H][–], 100); (–)-ESI HRMS m/z 385.04244 ([M–H][–], calcd 385.04258 for C₁₆H₉N₄O₈).

Aqabamycin G (2)

Yellow solid, R_F 0.50 (CH₂Cl₂/MeOH 9:1), R_T 16.02 min (LC MS); IR (KBr) $\nu_{\max}$ 3380, 1707, 1608, 1535, 1423, 1346, 1246, 1165, 1024 cm⁻¹; ¹H NMR

(600 MHz, DMSO- d_6 and MeOH- d_4) and ^{13}C NMR (125 MHz, DMSO- d_6 and MeOH- d_4 ; see Table 3 and Supplementary Figures 14 and 15); (–)-ESI MS m/z (%) 348 ([M–H] $^-$, 95), 719 ([2M+Na–2H] $^-$, 100), 1090 ([3M+2Na–3H] $^-$, 60); (–)-ESI HRMS m/z 348.06250 ([M–H] $^-$, calcd 348.06259 for $\text{C}_{18}\text{H}_{10}\text{N}_3\text{O}_5$).

3-Nitro-1H-indazole (3)

Pale yellow needles, R_F 0.56 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1); UV/Vis (MeOH) λ_{max} (log ϵ) 326 nm (3.5), 234 nm (3.6); IR (KBr): ν_{max} 1679, 1620, 1530, 1484, 1286, 1161 cm^{-1} ; ^1H NMR (MeOH- d_4 , 600 MHz) δ 8.20 (d, J 8.5 Hz, 1H, 4-H), 7.69 (d, J 8.5 Hz, 1H, 7-H), 7.57 (t, J 8.5 Hz, 1H, 6-H), 7.48 (t, J 8.5 Hz, 1H, 5-H; see Supplementary Figure 16); ^{13}C NMR (150 MHz, MeOH- d_4) δ 149.4 (C_q -3), 143.4 (C_q -7a), 129.5 (CH-4), 126.4 (CH-6), 121.6 (C_q -3a), 116.9 (CH-5), 112.6 (CH-7; see Supplementary Figure 17); (synthetic) 11 , 125 MHz, MeOH- d_4) δ 149.9 (C_q -3), 143.1 (C_q -7a), 129.3 (CH-4), 126.2 (CH-6), 121.4 (C_q -3a), 116.7 (CH-5), 112.4 (CH-7); (125 MHz, DMSO- d_6) δ 148.0 (C_q -3), 141.3 (C_q -7a), 127.9 (CH-4), 125.2 (CH-6), 119.8 (C_q -3a), 115.1 (CH-5), 111.8 (CH-7); (–)-ESI MS m/z (%) 162 ([M–H] $^-$, 18), 346 ([2M+Na–2H] $^-$, 8), 532 ([3M+2Na–3H] $^-$, 20), 727 ([4M+3Na–4H] $^-$, 60), 913 ([5M+4Na–5H] $^-$, 100); (–)-ESI HRMS m/z 162.03081 ([M–H] $^-$, calcd 162.03079 for $\text{C}_7\text{H}_4\text{N}_3\text{O}_2$).

Indazole-3-carbaldehyde (4)

Colorless oil, R_F 0.44 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1); UV/Vis (MeOH) λ_{max} (log ϵ) 298 nm (4.07), 236 nm (4.02), 242 nm (4.02); IR (KBr) ν_{max} 3192, 2854, 1697, 1670, 1465, 1257, 1138, 1088, 920, 801, 740 cm^{-1} ; ^1H NMR (DMSO- d_6 , 300 MHz): δ 14.12 (br, 1H, 1-NH), 10.20 (s, 1H, CHO), 8.18 (d, J 8.0 Hz, 1H, 4-H), 7.72 (d, J 8.0 Hz, 1H, 7-H), 7.54 (t, J 8.5 Hz, 1H, 6-H), 7.38 (t, J 8.5 Hz, 1H, 5-H; see Supplementary Figure 18); ^{13}C NMR (125 MHz, DMSO- d_6): δ 187.1 (CHO), 143.3 (C_q -3), 141.0 (C_q -7a), 127.1 (CH-6), 123.6 (CH-5), 120.5 (C_q -4), 120.2 (CH-3a), 111.0 (CH-7); EI MS (70 eV) m/z (%) 146 ([M] $^{+\bullet}$, 100), 145 (55), 118 (32), 91 (30), 63 (18). (+)-ESI HRMS m/z 169.0372 ([M+Na] $^+$, calcd 169.03725 for $\text{C}_8\text{H}_6\text{N}_2\text{ONa}$). (–)-ESI HRMS m/z 145.0407 ([M–H] $^-$, calcd 145.04072 for $\text{C}_8\text{H}_5\text{N}_2\text{O}$).

Phenyl-2-bis-indolylmethane (5a)

Reddish solid, R_F 0.57 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1); UV/Vis (MeOH) λ_{max} (log ϵ) 223 nm (4.82), 281 nm (4.00), 290 nm (3.96); IR (KBr) ν_{max} 3412, 2944, 2833, 1619, 1455, 1417, 1338, 1217, 1095, 1227, 744, 701 cm^{-1} ; ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.90 (br, 2H, 2-H), 7.34 (d, J 7.1 Hz, 2H), 7.33 (t, J 7.2 Hz, 2H), 7.26 (d, J 7.1 Hz, 2H), 7.25 (d, J 7.6 Hz, 2H), 7.18 (t, J 7.2 Hz, 1H), 7.03 (t, J 8.1 Hz, 2H), 6.85 (t, J 8.0 Hz, 2H), 6.82 (d, J 2.5 Hz, 2H), 5.82 (s, 1H; see Supplementary Figure 19); ^{13}C NMR (125 MHz, DMSO- d_6) δ 144.9 (C_q), 136.5 (2 C_q), 128.3 (2 CH), 128.0 (2 CH), 126.6 (2 C_q), 125.7 (CH), 123.5 (2 CH), 120.8 (2 CH), 119.1 (2 CH), 118.1 (2 CH), 118.0 (2 C_q), 111.4 (2 CH), 39.6 (CH); (–)-ESI MS (5b, neutral form) m/z (%) 319 ([M–H] $^-$, 100), 683 ([2M+HCOO] $^-$, 40); (+)-ESI MS (5b, protonated form) m/z (%) 321 ([M+H] $^+$, 10), 663 ([2M+Na] $^+$, 10); (–)-APCI MS (5b, neutral form) m/z (%) 319 ([M–H] $^-$, 100); EI MS (70 eV) (5a) m/z (%) 322 ([M] $^{+\bullet}$, 100), 245 (55), 243 (18), 206 (55), 204 (27), 144 (10), 122 (10); (–)-ESI (5a) HRMS m/z 321.13866 ([M–H] $^-$, calcd. 321.13970 for $\text{C}_{23}\text{H}_{17}\text{N}_2$).

1,4-Dithiane (7)

Colorless oil, R_F 0.51 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1); IR (KBr) ν_{max} 3430, 2923, 1634, 1399, 1057, 616 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 2.18 (s); EI MS (70 eV) m/z (%) 120 ([M] $^{+\bullet}$, 100), 74 (30), 61 (90).

For data of vibrindole A (6) and 4-hydroxy-3-nitrocinnamic acid, see Supplementary Information.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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