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Naquihexcin A, a S-Bridged Pyranonaphthoquinone Dimer Bearing an Unsaturated Hexuronic Acid Moiety from a Sponge-Derived *Streptomyces* sp. HDN-10-293

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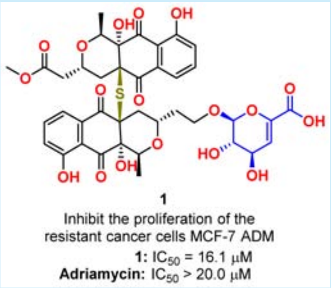
Ⓢ *Supporting Information*

ABSTRACT: S-Bridged pyranonaphthoquinone dimers, naquihexcins A and B (**1** and **2**), together with a related analogue (−)-BE-52440A (**3**) were obtained from the culture of a sponge-derived *Streptomyces* sp. HDN-10-293. Naquihexcin A (**1**) bears a rare unsaturated hexuronic acid moiety, and (−)-BE-52440A (**3**) has been discovered from natural resources. Compound **3** showed cytotoxicity against NB4 and HL-60 cells, while **1** could inhibit the proliferation of the adriamycin resistant human breast cancer cell line MCF-7 ADM.



Pyranonaphthoquinones (PNQs) are a class of secondary metabolites produced by various species of microorganisms.[1−7] With the core structure of the naphtha[2,3-*c*]pyran-5,10-dione ring, the structural diversity of PNQs results from not only the modification of core ring but also the formation of dimers with various dimeric patterns.[1−4] In a typical PNQ dimeric structure, the two PNQ monomers are directly connected by a C−C bond or sometimes via an oxygen bridge. Comparatively, the natural S-bridged dimeric PNQs were rare with only (+)-BE-52440A and BE-52440B (cytotoxic agents obtained from *Streptomyces* sp. A52440), frenolicin G (obtained from *Streptomyces* sp. RM-4-15), and hypogeamicin A (cytotoxic agent isolated from *Nonomuraea* sp.) reported.[5−7]

During our searching for bioactive molecules from marine-derived actinomycetes,[8] three S-bridged dimeric PNQs (Figure 1), naquihexcins A (**1**) and B (**2**), and a related known one (−)-BE-52440A (**3**) were isolated from the culture of a sponge derived *Streptomyces* sp. HDN-10-293. Among them, **1** is the first PNQ bearing a rare unsaturated hexuronic acid unit which has been discovered only in 5 compounds among the over 3400 naturally occurring glycosylated bacterial metabolites.[10,11] Compound **3** showed cytotoxicity against NB4 and HL-60 cells (IC_{50} values: 1.7 and 1.8 μM, respectively), while **1** could inhibit the proliferation of an adriamycin resistant human breast cancer cell line MCF-7 ADM with IC_{50} = 16.1 μM (adriamycin as positive control, IC_{50} > 20.0 μM).

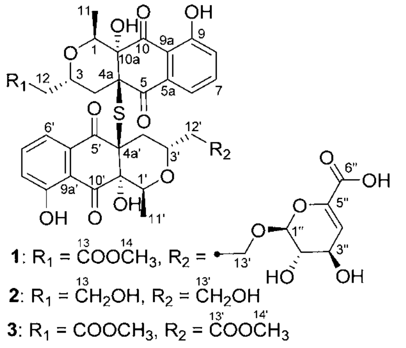


Figure 1. Structures of compounds **1**−**3**.

The *Streptomyces* sp. HDN-10-293 was incubated for 7 days at 28 °C on a rotatory shaker, and the whole broth (60 L) was extracted with EtOAc. The organic extract (9.0 g) was fractionated by repeated column chromatography including silica gel, LH-20, and HPLC ODS column leading to the yield of compounds **1** (28.0 mg), **2** (18.0 mg), and **3** (15.0 mg).

Naquihexcin A (**1**) was obtained as a yellow oil with molecular formula $C_{39}H_{40}O_{18}S$ established on the basis of the sodiated HRESIMS peak at *m*/*z* 851.1822 (calcd for 851.1828). The analysis of the $^1H/^{13}C$ NMR and gHSQC data suggested the presence of three methyls including one methoxy, five methylenes with one oxygenated, 14 methines (seven aromatic/olefinic and seven oxygenated ones), and 17 non-protonated carbons (six carbonyls, seven aromatic/olefinic carbons, two oxygenated and two sulfur-linked ones). The 1D and 2D NMR data of **1** were similar to those of BE-52440A and BE-52440B[5] revealing the existence of the S-bridged pyronaphthoquinone dimer moiety, which was further confirmed by the COSY and HMBC correlations (Figure 2). The additional signals were assigned as a hexuronic acid moiety on the basis of the key COSY correlations of H-1″ (δ_H 4.87)/H-2″ (δ_H 3.49)/H-3″ (δ_H 3.98)/H-4″ (δ_H 5.87), H-2″/2″-OH

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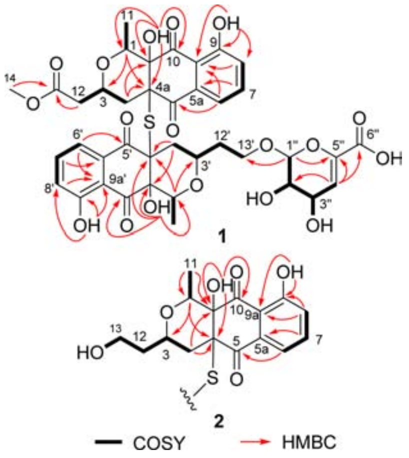

Figure 2. Key COSY and HMBC correlations of **1** and **2**.

(δ_H 5.40), and H-3″/3″-OH (δ_H 4.97) and the HMBC correlations from H-1″ to C-5″ (δ_C 140.9) and from H-4″ to C-2″ (δ_C 71.1), C-5″ and C-6″ (δ_C 163.8) (Figure 2, Table 1). Finally, the planar structure of **1** was constructed by connecting C-1″ to C-13′ via an oxygen atom based on the HMBC correlation from H-1″ to C-13′ (δ_C 66.3) and the chemical shifts.

The relative configuration of **1** was established mainly by analyzing NOESY correlations (Figure 3). The NOESY correlations of H-1 (H-1′)/10a-OH (10a′-OH)/H-4 (H-4′) indicated that they were on the same face of the pyran ring, and H_3-11 (H_3-11′) and H-3 (H-3′) were assigned as orientating to another side based on the NOESY correlation between them. The relative configuration of (1″*S**, 2″*S**, 3″*R**) was also deduced by the NOESY correlations between H-1″ (δ_H 4.87) and H-3″ (δ_H 3.98), between H-3″ and 2″-OH (δ_H 5.40), and the large coupling constant ($^3J_{\text{H-1}'',\text{H-2}''}$ = 6.2 Hz) of H-1″ and H-2″. The stereochemistry of C-4a (C-4a′) could not be deduced according to current data.

The absolute configuration of **1** was deduced by Electronic Circular Dichroism (ECD) data. The Cotton effects around 300 and 350 nm indicated that the benzene rings are the major chromophore dominating the ECD curves, which was also supported by the similar ECD spectra between compounds **1**–**3** (Figure 4). The absolute configuration of the S-bridged pyronaphthoquinone dimer moiety in **1** was determined to be 1*S*, 3*S*, 4a*S*, 10a*R*, 1′*S*, 3′*S*, 4a′*S*, 10a′*R* by comparing its ECD spectrum with that of the coisolated compound **3** whose absolute configuration was determined by X-ray diffraction with Cu Kα irradiation (Figure S18, Supporting Information). It is worth noting that the crystallographic data of **3** agree with those of the chemical synthetic (−)-BE-52440A.[9]

Due to no evidence to establish the stereo relationship between the PNQ dimer core and the unsaturated hexuronic acid moieties, the absolute configurations of C-1″, C-2″, and C-3″ were deduced by using the *in situ* dimolybdenum ECD method.[12,13] By forming a metal complex of a vicinal diol (cyclic or acyclic 1,2-diols) and $Mo_2(OAc)_4$ which works as an auxiliary chromophore, the additional ECD curve will be induced around 310 nm and the observed sign of the Cotton effect induced by the O–C–C–O torsion angle allows assignment of the absolute configuration. The negative Cotton effect at 310 nm observed in the ECD spectrum (Figure 5) of the complex of **1** and $Mo_2(OAc)_4$ in anhydrous DMSO permitted the assignment of the 2″*S* and 3″*R* configurations. By combining the relative configuration established by NOESY data, the absolute configuration of C-1″ was assigned as *S*. Thus, the absolute configuration of **1** was established as 1*S*, 3*S*, 4a*S*, 10a*R*, 1′*S*, 3′*S*, 4a′*S*, 10a′*R*, 1″*S*, 2″*S*, 3″*R*.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for **1** in DMSO-d_6

no.	δ_H (*J* in Hz)	δ_C, type
1	3.95 (q, 7.0)	72.9, CH
3	3.64 (m)	62.8, CH
4	2.09 (1H, dd, 14.0, 13.7) 1.79 (1H, t, 14.0)	27.8, CH_2
4a		62.0, C
5		190.4, C
5a		133.4, C
6	7.59 (d, 7.3)	120.0, CH
7	7.76 (t, 8.0)	137.7, CH
8	7.33 (overlap)	124.5, CH
9		160.8, C
9-OH	11.10 (s)	
9a		114.6, C
10		197.5, C
10a		76.4, C
10a-OH	7.33 (s)	
11	1.05 (d, 7.0)	15.4, CH_3
12	2.38 (m)	39.5, CH_2
13		170.8, C
14	3.63 (s)	51.8, CH_3
1′	3.95 (q, 7.0)	73.1, CH
3′	3.18 (m)	62.9, CH
4′	2.12 (1H, dd, 14.0, 13.7) 1.80 (1H, t, 14.0)	28.7, CH_2
4a′		62.1, C
5′		190.2, C
5a′		133.5, C
6′	7.53 (d, 7.3)	119.5, CH
7′	7.82 (t, 8.0)	137.8, CH
8′	7.34 (overlap)	124.5, CH
9′		160.8, C
9′-OH	11.12 (s)	
9a′		114.7, C
10′		197.7, C
10a′		76.5, C
10a′-OH	7.31 (s)	
11′	1.07 (d, 7.0)	15.2, CH_3
12′	1.70 (m), 1.64 (m)	34.6, CH_2
13′	3.65 (m) 3.57 (m)	66.3, CH_2
1″	4.87 (d, 6.2)	101.8, CH
2″	3.49 (m)	71.1, CH
3″	3.98 (m)	67.6, CH
4″	5.87 (d, 3.3)	113.6, CH
5″		140.9, C
6″		163.8, C
2″-OH	5.40 (d, 4.5)	
3″-OH	4.97 (d, 7.0)	

Naquihexcin B (**2**) was assigned with the molecular formula $C_{32}H_{34}O_{12}S$ evidenced by the protonated HRESIMS peak at *m/z* 643.1831. The NMR spectra show signals for 17 protons and 16 carbons, suggesting compound **2** to be a symmetrical structure. Careful comparison of its ^{1}H and ^{13}C NMR data (Table 2) with those of BE-52440B[5] indicated that they shared

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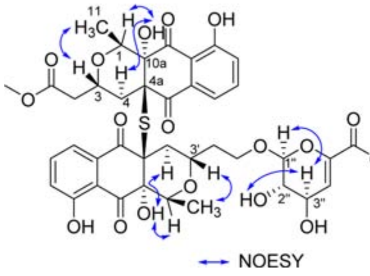

Figure 3. Key NOESY correlations of **1**.

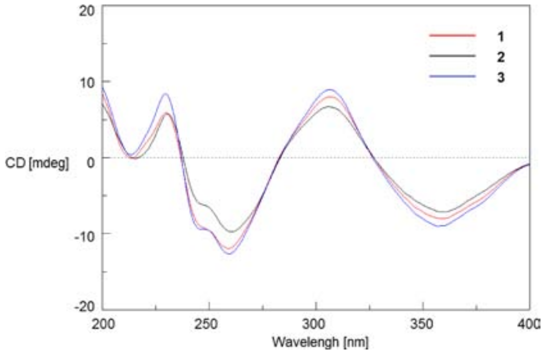

Figure 4. Experimental ECD spectra of compounds **1**−**3**.

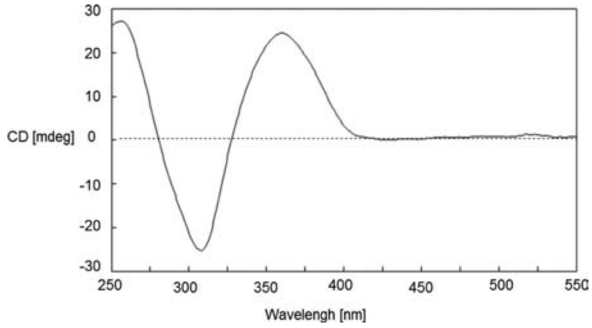

Figure 5. ECD spectrum of **1** in DMSO containing $Mo_2(OAc)_4$ with the inherent ECD spectrum subtracted.

the same skeleton with the sole difference being the presence of a $-CH_2-CH_2-OH$ moiety at C-3 (δ_C 62.6), which was further confirmed by the contiguous correlations from H-3 (δ_H 3.49) to 13-OH (δ_H 4.46) in the COSY spectrum (Figure 2). The relative and absolute configurations of **2** were also determined to be the same as those for **3** according to the NOESY (Figure S19, Supporting Information) and ECD spectra (Figure 4).

Compounds **1**−**3** were evaluated *in vitro* for their cytotoxicity against HL-60 and NB4 cells by the MTT method, and the MCF-7 ADM, Hela, A549, and HCT-116 cells, by the SRB method.[14] Among them, **3** showed the best cytotoxic activities against NB4 and HL-60 cell lines, with IC_{50} values of 1.7 and 1.8 μM, respectively, while other compounds showed no significant cytotoxicities against the tested cell lines ($IC_{50} > 10.0$ μM) (Table S1, Supporting Information). It is worth noting that compound **1** could inhibit the proliferation of an adriamycin resistant human breast cancer cell line MCF-7 ADM with $IC_{50} = 16.1$ μM (adriamycin as positive control, $IC_{50} > 20.0$ μM), indicating that the unsaturated hexuronic acid moiety could enhance the activity against the resistant cancer cells.

Table 2. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data for **2** in DMSO-d_6

no.	δ_H (J in Hz)	δ_C, type
1/1′	3.96 (q, 7.0)	72.8, CH
3/3′	3.49 (m)	62.6, CH
4/4′	2.09 (dd, 11.8, 11.5) 1.80 (d, 14.0)	28.8, CH_2
4a/4a′		62.0, C
5/5′		190.3, C
5a/5a′		133.7, C
6/6′	7.62 (d, 7.7)	119.9, CH
7/7′	7.77 (t, 7.7)	137.6, CH
8/8′	7.32 (d, 7.7)	124.2, CH
9/9′		160.7, C
9/9′-OH	11.11 (s)	
9a/9a′		114.7, C
10/10′		197.8, C
10a/10a′		76.5, C
10a/10a′-OH	7.26 (s)	
11/11′	1.02 (d, 7.0)	15.4, CH_3
12/12′	1.56 (m)	39.7, CH_2
13/13′	3.47 (m)	57.9, CH_2
13/13′-OH	4.46 (t, 4.9)	

The PNQ antibiotics have been proven to be biosynthesized by a polyketide pathway,[2] while the formation of S-bridged pyranonaphthoquinone dimers remains uncharacterized. With only four natural occurred S-bridged dimeric PNQs reported to date, the discovery of **1**−**3** is a notable expansion to this subset. Among them, naquihexcin A (**1**) represents the first example of PNQs containing an unsaturated hexuronic acid moiety, and the cytotoxic compound **3**, which had been synthesized previously but had no bioassay data reported,[9] has been discovered from natural resources.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01485.

Experimental details, 1D and 2D NMR spectra of **1**−**3** (PDF)
Crystallographic data (CIF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Sperry, J.; Bachu, P.; Brimble, M. A. *Nat. Prod. Rep.* **2008**, *25*, 376−400.

(2) Brimble, M. A.; Nairn, M. R.; Duncalf, L. J. *Nat. Prod. Rep.* **1999**, *16*, 267−281.

(3) Salaski, E. J.; Krishnamurthy, G.; Ding, W. D.; Yu, K.; Insaf, S. S.; Eid, C.; Shim, J.; Levin, J. I.; Tabei, K.; Toral, B. L.; Zhang, W. G.; Leonard, A. M.; Erick, H.; Cilien, H.; Ayako, Y.; Bernard, J.; Zhong, L.; Leif, L.; Dennis, P.; Tarek, S. M. *J. Med. Chem.* **2009**, *52*, 2181−2184.

(4) Sperry, J.; Lorenzo-Castrillejo, I.; Brimble, M. A.; Machin, F. *Bioorg. Med. Chem.* **2009**, *17*, 7131−7137.

(5) Tsukamoto, M.; Nakajima, S.; Murooka, K.; Naito, M.; Suzuki, H.; Hirayama, M.; Hirano, K.; Kojiri, K.; Suda, H. J. *J. Antibiot.* **2000**, *53*, 687−693.

(6) Wang, X. C.; Shaaban, K. A.; Elshahawi, S. I.; Ponomareva, L. V.; Sunkara, M.; Zhang, Y. N.; Copley, G. C.; Hower, J. C.; Morris, A. J.; Kharel, M. K.; Thorson, J. S. *J. Nat. Prod.* **2013**, *76*, 1441−1447.

(7) Derewacz, D. K.; McNees, C. R.; Scalmani, G.; Covington, C. L.; Shanmugam, G.; Marnett, L. J.; Polavarapu, P. L.; Bachmann, B. O. *J. Nat. Prod.* **2014**, *77*, 1759−1763.

(8) Che, Q.; Zhu, T.; Qi, X.; Attila, M.; Tibor, K.; Mo, X. M.; Li, J.; Gu, Q. Q.; Li, D. *Org. Lett.* **2012**, *14*, 3438−3441.

(9) Tatsuta, K.; Suzuki, Y.; Toriumi, T.; Furuya, Y.; Hosokawa, S. *Tetrahedron Lett.* **2007**, *48*, 8018−8021.

(10) Elshahawi, S. I.; Shaaban, K. A.; Kharel, M. K.; Thorson, J. S. *Chem. Soc. Rev.* **2015**, *44*, 7591−7697.

(11) Wang, X. C.; Reynolds, A. R.; Elshahawi, S. I.; Shaaban, K. A.; Ponomareva, L. V.; Saunders, M. A.; Elgumati, I. S.; Zhang, Y. N.; Copley, G. C.; Hower, J. C.; Sunkara, M.; Morris, A. J.; Kharel, M. K.; Van Lanen, S. G.; Prendergast, M. A.; Thorson, J. S. *Org. Lett.* **2015**, *17*, 2796−2799.

(12) Frelek, J.; Ikekawa, N.; Takatsuto, S.; Snatzke, G. *Chirality* **1997**, *9*, 578−582.

(13) Wang, W. J.; Li, D. Y.; Li, Y. C.; Hua, H. M.; Ma, E. L.; Li, Z. L. *J. Nat. Prod.* **2014**, *77*, 1367−1371.

(14) Du, L.; Zhu, T. J.; Liu, H. B.; Fang, Y. C.; Zhu, W. M.; Gu, Q. Q. *J. Nat. Prod.* **2008**, *71*, 1837−1842.

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