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Uroleuconaphins A_1 and B_1, two red pigments from the aphid *Uroleucon nigrotuberculatum* (Olive)

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Abstract—Two novel red pigments, uroleuconaphins A_1 (**1**) and B_1 (**2**) were isolated from the aphid *Uroleucon nigrotuberculatum* (Olive). The structures and the absolute configurations of **1** and **2** were determined by single-crystal X-ray analysis of their derivatives. These structures were constituted as dimeric compounds of two molecules of quinone A (**3**), which were linked via a dihydrofuran ring system.

1. Introduction

Aphids produce pigments such as rhododactynaphins,[1–5] xanthoaphins,[6–12] chrysoaphins,[6–14] erythroaphins,[1,6–10,15–27] protoaphins,[1,5–9,11,12,14,25,26,28–34] furanaphin,[35] and so on. It might be worthwhile to investigate these enchanting pigments, which were expected to possess interesting biological activities.[35] Unfortunately, however, the major and extraordinary works on aphids' pigments were discontinued in the early 1980s. Therefore, we started a chemical investigation on pigments in aphids', having an interest in biological activities and biological meaning of the aphids themselves. We recently isolated two red pigments, uroleuconaphins A_1 (**1**) and B_1 (**2**), from the red aphid *Uroleucon nigrotuberculatum* (Olive) (max. 4.0 mm long) (Fig. 1) feeding on *Solidago altissima* L. The structure of **2** (Fig. 2) was constituted as a dimeric compound of the C15 unit, quinone A (**3**) (Fig. 3).[2,3,14,29,32,36] Compound **1** was assigned as the dehydroxyl derivative of **2**. In this paper, we describe their structural elucidation in detail.

2. Results and discussion

The aphid *U. nigrotuberculatum* (Olive) was swept and collected with a soft paintbrush into a plastic Erlenmeyer flask equipped with a plastic funnel in Tokushima Prefecture, Japan in June. The aphid was squashed in ether, and then the ethereal supernatant solution was separated by decantation. The residue was washed with several portions of fresh ether. The combined ethereal solutions were evaporated and subjected to repeated silica-gel column chromatography to afford two yellowish pigments[37] and two red pigments **1** and **2** as major color components. The less polar compound **1** was obtained as red needles and its specific rotation was an extremely large value, $[\alpha]_D^{25}$ +2028.1 (*c* 0.01, $CHCl_3$). Its molecular formula was established as $C_{30}H_{28}O_{11}$ by HREIMS (*m/z* 564.1655).

Figure 1. *Uroleucon nigrotuberculatum* (Olive).

Keywords: Pigment; Structure determination; X-ray analysis; Aphid; *Uroleucon nigrotuberculatum* (Olive).
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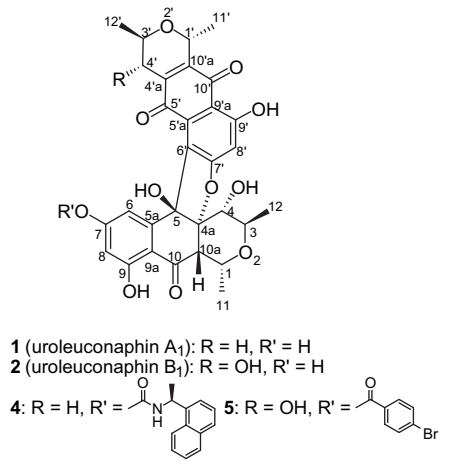

Figure 2. Structures of uroleuconaphins A_1, B_1 and their derivatives.

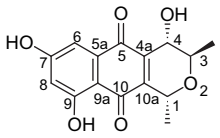

Figure 3. Structure of quinone A (**3**).

Detailed analyses of 1D and 2D NMR spectra involving ^1H–^1H COSY, NOESY, HMQC, and HMBC measurements revealed that **1** contained two component C15 unit (quinone A and dehydroxy quinone A). Unfortunately, however, the structure could not be elucidated clearly even by detailed analyses of the NMR spectra of **1** and its derivatives.

Finally, the structure and the absolute configuration of **1** could be established by a single-crystal X-ray diffraction analysis of the (*S*)-(+)-1-(1-naphthyl)ethyl carbamate **4** (Fig. 2), which was derived by carbamation employing (*S*)-(+)-1-(1-naphthyl)ethylisocyanate with 4-(*N*,*N*-dimethylamino)pyridine (DMAP) in CH_2Cl_2. The IR and ^1H NMR spectra of **4** suggested that the skeleton of **1** did not change in this carbamation. Figure 4 illustrated the molecular structure of **4** with the atomic numbering. The X-ray analysis revealed that a dihydrofuran ring system connected the two component C15 unit. Furthermore, two planes consisting each of the two C15 units in **1** were oriented in almost perpendicular arrangement.

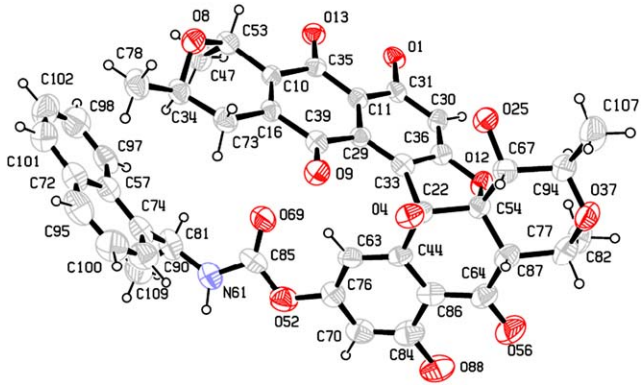

Figure 4. ORTEP drawing of **4** with 30% probability ellipsoids. Hydrogen atoms on hydroxy groups were not shown because of the difficulty to determine their positions.

The structure of compound **2** was confirmed as a hydroxy derivative at C4′ in **1** by detailed analyses of 1D and 2D NMR spectra. However, the stereochemistry of the additional hydroxyl group at C4′ could not be determined confidently even by NOE experiments of **2** and its monoacetate at C4′. Since compound **2** may simply consist of two units of quinone A (**3**), the hydroxy group at C4′ may be in α-orientation as illustrated. However, the extensive investigations of Todd, Cameron, and their colleagues revealed that quinone A′, the C4 hydroxy epimer of **3**, was also a component of aphids' pigments such as protoaphin-*sl* and erythroaphin-*sl*.[22] Therefore, the β-hydroxy epimer of **2** was also expected to be obtained from aphids.

The structural elucidation was accomplished again by a single-crystal X-ray diffraction analysis of the *p*-bromobenzoate **5** (Fig. 2), which was derived from **2** by esterification with *p*-bromobenzoyl chloride and DMAP in CH_2Cl_2. Disorder of the included AcOEt solvent molecules at the core of the benzoate **5** resulted in a loss of some resolution in the crystal structure, but the molecular structures and its stereochemistries were clearly refined from the diffraction data quickly obtained at 150 K. Figure 5 illustrates the molecular structure of **5** with the atomic numbering. The hydroxy group at C4′ was situated at trans position to the methyl group at C3′.

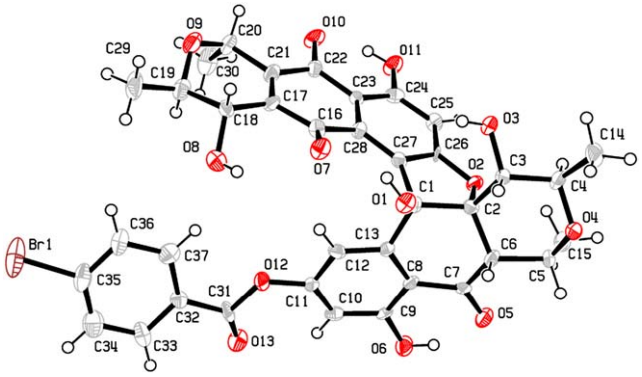

Figure 5. ORTEP drawing of **5** with 50% probability ellipsoids.

2.1. Cytotoxicity

Having an interest in biological activities of uroleuconaphins A_1 and B_1, we tested them for cytotoxicity against human promyelocytic leukemia HL-60 cells.[38] Compounds **1** and **2** were found to be active with ED_{50} of 45 μM and 20 μM, respectively. These results may suggest that the pigments are important for the aphids themselves in defense against viral infections. This finding encouraged us to continue the investigation of aphids' pigments.

3. Conclusion

Thus, the structures and absolute configurations of uroleuconaphins A_1 (**1**) and B_1 (**2**) were determined. Furthermore, they possessed cytotoxicity against HL-60 cells. Further work on the biological activities of **1** and **2**, and structure

determination of yellow pigments of the aphid *U. nigrotuberculatum* (Olive) is in progress.

4. Experimental

4.1. General

Melting points were determined on a Yanaco MP-3 apparatus and were uncorrected. IR spectra were measured on a JASCO FT/IR-410 spectrophotometer. UV–visible spectra were measured on a Shimadzu UV-1650pc spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Unity-600 (600 MHz) and a Varian Mercury-300 (300 MHz) with tetramethylsilane as an internal standard in acetone-d_6 and $CDCl_3$. ^{13}C NMR spectra were taken on a Varian Unity-600 (150 MHz) and a Varian Unity-200 (50 MHz), chemical shifts were referenced to the residual solvent signal (acetone-d_6: δ_C 29.8 ppm, $CDCl_3$: δ_C 77.0 ppm). Signal multiplicities were established by DEPT experiments. Mass spectra including high-resolution mass spectra were recorded on a JOEL JMX AX-500 spectrophotometer. Column chromatography was carried out with Silica gel 60N (Kanto Chemical Co. Inc., 63–210 μm). Acetyl chloride and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) were purchased from Nacalai Tesque Inc. (*S*)-(+)-1-(1-naphthyl)ethylisocyanate was purchased from Aldrich Chemical Co. Inc. *p*-Bromobenzoyl chloride was purchased from Acros Organics. They were used without any purification.

4.2. Material

The aphid *U. nigrotuberculatum* (Olive), which was feeding on *S. altissima* L. was collected in Tokushima Prefecture, Japan in June 1999.

4.3. Extraction and isolation

The aphid (200 g) was squashed in diethyl ether, and then the ethereal supernatant solution was separated by decantation. The residue was washed with several portions of fresh ether. The combined ethereal solutions were dried over Na_2SO_4 and were evaporated to give a crude extract (21.9 g). The reddish residue was subjected to silica-gel column chromatography (600 g) using hexane/AcOEt (3:1–1:3) as an eluent to afford two red pigments **1** (1.20 g) and **2** (755 mg), and two yellowish pigments (~780 mg).

4.3.1. Uroleuconaphin A_1 (1). Red needles (hexane/diethyl ether), mp 233 °C (dec). $[\alpha]_D^{25}$ +2028.1 (*c* 0.01, $CHCl_3$). UV (CH_3CN): λ_{max} 275 (log ε 4.38), 495 (log ε 3.68) nm. IR (KBr): ν_{max} 3436 (–OH), 1613 (C=O), 1442, 1406, 1282 cm^{-1}, NMR data: see Table 1, MS (EI) *m*/*z* 564

Table 1. ^{13}C (150 Hz) and ^{1}H (600 Hz) NMR data of **1** and **2**

Position	**1** In $CDCl_3$		**2** In acetone-d_6	
	δ_C	δ_H	δ_C	δ_H
1	68.5	4.74 (1H, qd, 6.9, 5.7)	68.5	4.66 (1H, qd, 7.0, 5.5)
3	64.3	4.00–4.08 (1H, overlap)	65.5	3.95–4.03 (1H, overlap)
4	73.9	4.00–4.08 (1H, overlap)	73.5	3.95–4.03 (1H, overlap)
4a	93.0		94.7	
5	79.5		80.1	
5a	146.8		148.2	
6	105.1	6.73 (1H, d, 2.3)	107.0	6.81 (1H, d, 2.4)
7	163.7		166.6	
8	103.7	6.26 (1H, d, 2.3)	103.6	6.21 (1H, d, 2.4)
9	164.3		165.1	
9a	108.0		108.0	
10	196.8		198.3	
10a	48.5	3.48 (1H, d, 5.7)	48.9	3.59 (1H, d, 5.5)
11	15.8	1.68 (3H, d, 6.9)	16.0	1.63 (3H, d, 7.0)
12	18.4	1.29 (3H, d, 5.8)	19.1	1.21 (3H, d, 6.2)
5-OH		8.20 (1H, s)		8.31 (1H, s)
9-OH		12.53 (1H, s)		12.55 (1H, s)
1′	67.1	4.96 (1H, qd, 6.9, 2.1)	66.9	4.86 (1H, br q, 7.0)
3′	62.5	4.00–4.08 (1H, overlap)	69.6	3.95–4.03 (1H, overlap)
4′	30.0	2.22 (1H, ddd, 19.2, 10.2, 2.1); 2.76 (1H, dd, 19.2, 3.3)	66.5	4.49 (1H, br d, 6.6)
4′a	141.8		142.9	
5′	188.8		187.3 or 190.6	
5′a	126.7		128.4	
6′	132.0		134.3	
7′	166.1		168.0	
8′	105.0	6.55 (1H, s)	104.7	6.60 (1H, s)
9′	166.8		167.3	
9′a	109.6		110.4	
10′	185.8		187.3 or 190.6	
10′a	148.8		150.4	
11′	19.8	1.56 (3H, d, 6.9)	19.4	1.60 (3H, d, 7.0)
12′	21.4	1.37 (3H, d, 6.0)	18.4	1.35 (3H, d, 6.2)
9′-OH		13.18 (1H, s)		13.13 (1H, s)
Other OH		4.23 (1H, br s)[a]; 9.64 (1H, br s)[a]		4.30 (1H, br d, 4.0); 5.04 (1H, br s); 9.69 (1H, br s)

[a] These signals were observed in acetone-d_6.

(M^+), HRMS (EI) calcd for $C_{30}H_{28}O_{11}$ 564.1632 (M^+), found 564.1655.

4.3.2. Uroleuconaphin B_1 (2). Red needles (hexane/$CHCl_3$), mp 223 °C (dec). $[\alpha]_D^{25}$ +2260.2 (*c* 0.01, $CHCl_3$). UV (CH_3CN): λ_{max} 276 (log ε 4.30), 497 (log ε 3.59) nm. IR (KBr): ν_{max} 3415 (–OH), 1613 (C=O), 1444, 1411, 1288 cm^{-1}. NMR data: see Table 1, MS (FAB) *m/z* 581 ([M+H]$^+$), HRMS (FAB) calcd for $C_{30}H_{29}O_{12}$ 581.1659 ([M+H]$^+$), found 581.1660.

4.3.3. (*S*)-(+)-1-(1-Naphthyl)ethyl carbamate of uroleuconaphin A_1 (4). To a suspension of **1** (20.5 mg) in CH_2Cl_2 (10 mL) were successively added (*S*)-(+)-1-(1-naphthyl)ethylisocyanate (22.3 mg) and DMAP (~5.0 mg). The resulting mixture was stirred at ambient temperature for 5.5 h and then was quenched with water (10 mL) and 1 N HCl (5 mL). The mixture was separated and the organic layer was dried over Na_2SO_4. After evaporation of the solvent, the crude residue was purified by silica-gel column chromatography (3.5 g, hexane/AcOEt=10:1–4:1) to give 17.8 mg of (*S*)-(+)-1-(1-naphthyl)ethyl carbamate **4** as red plates (AcOEt/hexane/$CHCl_3$), mp 166–168 °C (dec). $[\alpha]_D^{18}$ +2666.7 (*c* 0.01, $CHCl_3$). UV (CH_3CN): λ_{max} 271 (log ε 4.42), 493 (log ε 3.62) nm. IR (neat): ν_{max} 3310 (–OH), 1738 (C=O), 1638 (C=O), 1612 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 12 mg/mL): δ 0.82 (3H, d, *J*=6.2 Hz), 1.32 (3H, d, *J*=5.9 Hz), 1.37 (3H, d, *J*=6.6 Hz), 1.65 (3H, d, *J*=7.0 Hz), 1.69 (3H, d, *J*=7.0 Hz), 2.00 (1H, ddd, *J*=19.0, 10.3, 1.8 Hz), 2.37 (1H, d, *J*=5.1 Hz), 2.44 (1H, dd, *J*=19.0, 3.7 Hz), 3.37–3.41 (1H, m), 3.49 (1H, d, *J*=5.5 Hz), 4.05 (1H, dq, *J*=9.9, 5.9 Hz), 4.10 (1H, dd, *J*=9.9, 5.1 Hz), 4.67–4.73 (2H, overlapped), 5.41 (1H, d, *J*=7.7 Hz), 5.51 (1H, quint, *J*=7.0 Hz), 6.50 (1H, s), 6.51 (1H, d, *J*=2.2 Hz), 7.53 (1H, d, *J*=2.2 Hz), 7.50–7.57 (3H, m), 7.62 (1H, td, *J*=8.4, 1.5 Hz), 7.85 (1H, d, *J*=8.1 Hz), 7.93 (1H, d, *J*=8.1 Hz), 7.99 (1H, d, *J*=8.4 Hz), 8.54 (1H, s), 12.18 (1H, s), 13.04 (1H, s). ^{13}C NMR (150 MHz, $CDCl_3$): δ 15.8, 18.5, 19.7, 21.1, 21.8, 30.0, 47.5, 48.9, 62.3, 64.3, 66.6, 68.3, 73.5, 79.5, 92.6, 104.7, 108.8, 109.1, 109.8, 110.4, 122.0, 122.5, 125.4, 126.1, 126.8, 127.6, 128.6, 129.2, 130.4, 131.4, 134.1, 137.4, 142.2, 146.0, 147.5, 151.7, 157.9, 162.7, 165.8, 166.5, 185.8, 189.7, 198.2. MS (FAB) *m/z* 762 ([M+H]$^+$), HRMS (FAB) calcd for $C_{43}H_{40}NO_{12}$ 762.2550 ([M+H]$^+$), found 762.2564.

4.3.4. *p*-Bromobenzoate of uroleuconaphin B_1 (5). To a suspension of **2** (14.2 mg) in CH_2Cl_2 (6 mL) with *p*-bromobenzoyl chloride (16.5 mg) was added DMAP (~10.0 mg) in small portions over a period of 3 h with stirring at ambient temperature and then the resulting mixture was quenched with water (3 mL). The mixture was separated and the organic layer was dried over Na_2SO_4. After evaporation of the solvent, the crude residue was purified by silica-gel column chromatography (2.5 g, hexane/AcOEt=2:1–1:2) to give 17.0 mg of *p*-bromobenzoate **5** as red plates (hexane/AcOEt), mp 205 °C (dec). $[\alpha]_D^{20}$ +1345.0 (*c* 0.02, $CHCl_3$). UV (CH_3CN): λ_{max} 259 (log ε 4.53), 489 (log ε 3.63) nm. IR (ATR): ν_{max} 3425 (–OH), 1744 (C=O), 1608 (C=O), 1588 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 9.6 mg/mL): δ 1.31 (3H, d, *J*=5.4 Hz), 1.35 (3H, d, *J*=6.3 Hz), 1.59 (3H, d, *J*=6.9 Hz), 1.70 (3H, d, *J*=6.9 Hz), 2.35 (1H, br s), 3.43 (1H, d, *J*=5.7 Hz), 3.72–3.91 (2H, overlapped), 4.01–4.16 (2H, overlapped), 4.43 (1H, d, *J*=7.2 Hz), 4.70 (1H, qd, *J*=6.9, 5.7 Hz), 4.85 (1H, q, *J*=6.9 Hz), 6.59 (1H, s), 6.84 (1H, d, *J*=2.3 Hz), 7.34 (1H, d, *J*=2.3 Hz), 7.61 (2H, d, *J*=6.8 Hz), 7.90 (2H, d, *J*=6.8 Hz), 8.59 (1H, s), 12.13 (1H, s), 12.91 (1H, s). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.8, 18.4, 19.3, 48.9, 64.3, 66.3, 66.8, 67.8, 68.2, 73.5, 79.6, 92.8, 105.2, 109.6, 110.0, 110.3, 110.9, 127.5, 128.0, 129.5, 131.6, 131.7, 131.9, 142.2, 145.4, 149.8, 157.5, 162.9, 163.1, 166.1, 166.7, 185.7, 191.5, 197.7. MS (FAB) *m/z* 763 ([M+H]$^+$), 765 ([M+H]$^+$), HRMS (FAB) calcd for $C_{37}H_{32}{}^{79}BrO_{13}$ 763.1027 ([M+H]$^+$), found 763.1027.

4.4. X-ray analysis of 4 and 5

The structures of **4** and **5** were solved by direct methods with the program SHELXS97 (Sheldric, 1997). The crystal data and the experimental details are summarized in Tables 2 and 3. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 275214 for **4** and 275656 for **5**. Copies of the data can be obtained, free of charge, on

Table 2. Crystal data and experimental conditions of **4**

Chemical formula/formula weight	$C_{43}H_{35}NO_{12}$/757.72
Crystal system/space group	Orthorhombic/$C222_1$
Z	16
a, *b*, *c*(Å)	22.8600(8), 39.620(2), 18.3180(6)
V/Å^3	16590.9(11)
D_x/Mgm^{-3}	1.213
Diffractometer	MXC18
Radiation	Mo Kα
λ/Å	0.71073
μ(Mo Kα)/mm^{-1}	0.11
Crystal description/crystal dimensions (mm^3)	Plate/0.35×0.3×0.2
T/K	298
θ_{max}/°	25.86
Range of *h*, *k*, and *l*	$0\le h\le 27$, $0\le k\le 48$, $0\le l\le 22$
Reflections: independent/observed	8917/4843
$R(F)(I>3\sigma(I))/wR(F^2)(I>3\sigma(I))$	0.072/0.238
S	1.434
$(\Delta/\sigma)^{max}$	0.021
Δρ/eÅ^{-3}	−0.45, 1.69

Table 3. Crystal data and experimental conditions of **5**

Chemical formula/formula weight	$C_{37}H_{31}BrO_{13}\cdot 1/3C_4H_4O_2$/791.55
Crystal system/space group	Triclinic/*P*1
Z	6
a, *b*, *c*(Å)	13.2293(14), 15.5101(16), 29.022(3)
α(°)	94.808(2)
β(°)	93.165(2)
γ(°)	107.985(2)
V/Å^3	5623.4(10)
D_x/Mgm^{-3}	1.402
Diffractometer	Bruker Smart1000 CCD
Radiation	Mo Kα
λ/Å	0.71073
μ(Mo Kα)/mm^{-1}	1.164
Crystal description/crystal dimensions (mm^3)	Plate/0.40×0.40×0.15
T/K	150
θ_{max}/°	27.54
Range of *h*, *k*, and *l*	$-16\le h\le 17$, $-20\le k\le 12$, $-33\le l\le 37$
Reflections: independent/observed	28889/16706
$R(F)(I>2\sigma(I))/wR(F^2)(I>2\sigma(I))$	0.0720/0.1986
S	0.907
Δρ/eÅ^{-3}	−0.751, 1.982

application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

4.5. Cytotoxic activities

HL-60 (human promyelocytic leukemia-60) cells were grown in suspension culture and incubated at 37 °C in RPMI-1640 medium supplemented with 10% FBS and glutamine (2 mM). The cytotoxicity of **1** and **2** in HL-60 cells was analyzed by colorimetric 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay with some modification.[38] HL-60 (1×10^4 cells) were plated on 96-well plates and allowed to adhere at 37 °C in 5% CO_2/95% air for 1 h. Then 50 μL of serial concentration of test compound **1** and **2** were added and the cells incubated for 24 h. After 24 h, 10 μL of MTT (5 mg/mL: stock solution) was added and the cells were incubated for an additional 4 h. Following this time the cells were lysed and the dark blue crystals solubilized with 100 μL of 20% sodium dodecyl sulfate in 0.01 N HCl. The optical density (OD) of each well was measured with a microplate spectrophotometer equipped with a 570 nm filter. The results of cytotoxic activity are expressed as ED_{50} (the concentration of compound that inhibited cell line replication by 50%). The ED_{50} of **1** and **2** were 45 and 20 μM, respectively.

Acknowledgements

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