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Indole alkoloids from *Nauclea officinalis* with weak antimalarial activity

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Abstract

Five indole alkaloids (naucleofficines **A**–**E**) were isolated from the stems (with bark) of *Nauclea officinalis*: (*E*)-2-(1-β-D-glucopyranosyloxybut-2-en-2-yl)-3-(hydroxymethyl)-6,7-dihydro-indolo[2,3-a]quinolizin-4(12*H*)-one (**1**), (*E*)-1-propenyl-12-β-D-glucopyranosyloxy-2,7,8-trihydro-indolo[2,3-a]pyran[3,4-g]quinolizin-4,5(13*H*)-dione (**2**), (*E*)-2-(1-hydroxybut-2-en-2-yl)-11-β-D-glucopyranosyloxy-6,7-dihydro-indolo[2,3-a]quinolizin-4(12*H*)-one (**3**), (*E*)-1-propenyl-4-hydroxy-2,4a,7,8,13b,14,14a-hepthydro-(4α,4aβ,13bα,14aβ)indolo[2,3-a]pyran[3,4-g]quinolizin-5(13*H*)-one (**4**) and 1-(1-hydroxyethyl)-10-hydroxy-7,8-dihydro-indolo[2,3-a]pirydine[3,4-g]quinolizin-5(13*H*)-one (10-hydroxyangustoline) (**5**), together with two known compounds, naucleidinal (**6**) and angustoline (**7**). All of the structures of the seven compounds above were elucidated by spectroscopic methods including use of 1 D- and 2 D-NMR spectroscopic analyses. Compounds **2** and **3** are rare examples of monoterpene indole alkaloids with a glucopyranosyloxy group attached to position C-12. *In vitro* activity screening of the above seven compounds showed weak to moderate inhibitory activity against *Plasmodium falciparum*.

Keywords: *Nauclea officinalis*; Rubiaceae; Indole alkaloids; Naucleofficines **A**–**E**; *Plasmodium falciparum*

1. Introduction

There are about 35 species of plants belonging to the genus *Nauclea*, including *Nauclea orientalis*, *Nauclea latifolia* and *Nauclea diderrichhii*, which are used in folk medicine. Their extracts have been reported to exhibit antimicrobial and antiparasitic activities (Deeni and Hussain, 1991; Lamidi et al., 1996); two monoterpene indole alkaloids, naucleaorine and epimethoxynaucleaorine, also showed moderate antimalarial activity (He et al., 2005).

Nauclea officinalis Pierre ex Pitard (Rubiaceae), a traditional Chinese Herb, is widely used to cure colds, pink eye, and other ailments (Editorial Committee of Chinese Herbs, 1999). Several indole alkaloids were isolated from this species (Lin et al., 1984, 1985, 1989, Xuan et al., 2006). In the course of our continuing search for antitumor and antimalarial agents from traditional Chinese Herbs, we isolated five new indole alkaloids from this plant, naucleofficines **A–E** (**1–5**), as well as two known compounds, naucleidinal (**6**) (Hotellier et al., 1980) and angustoline (**7**) (Hotellier et al., 1975). Compounds **2** and **3** are rare examples of monoterpene indole alkaloids with a glucopyranosyloxy group attached to the position C-12. *In vitro* activity screening of above seven compounds showed weak to moderate inhibitory activity against *Plasmodium falciparum*. In this paper, we report the isolation and structural elucidation of these five new indole alkaloids, as well as their evaluation of antimalarial and cytotoxic effects.

2. Results and discussion

Compound **1** was isolated as an orange–yellow powder and a molecular formula of $C_{26}H_{30}N_2O_8$ was indicated by its quasi-molecular ion peak at *m/z* 497.1931 $[M-H]^-$ (Calc. for $C_{26}H_{29}N_2O_8$: 497.1929) in the HRFAB mass spectrum. The IR spectrum displayed the presence of hydroxyl (3399 cm^{-1}) and α,β-unsaturated carbonyl (1641 cm^{-1}) groups. In the ^1H NMR spectrum, two doublets at δ 7.59

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(1H, *d*, $J = 7.9$ Hz, H-9) and δ 7.41 (1H, *d*, $J = 7.9$ Hz, H-12), two triplets at δ 7.21 (1H, *t*, $J = 7.9$ Hz, H-11) and δ 7.07 (1H, *t*, $J = 7.9$ Hz, H-10), as well as two methylenes at δ 4.32 (2H, *m*, H-5) and δ 3.01–3.09 (2H, *m*, H-6) suggested a substitution pattern in rings A and C of a naucleamide derivative. Specifically, the spectroscopic data of 1 indicated a close relationship with naucleamide D (Shigemori et al., 2003) apart from the presence of a glucose moiety. The ^{13}C NMR spectrum clearly displayed signals of a sugar moiety, which was determined to be glucose with a β configuration by the coupling constant (7.7 Hz) of the anomeric proton, and verified also by comparison with reported values (Yu and Yang, 1999). The HMBC correlation of H-1′ to C-20 (δ 139.8) demonstrated that the glucosyl moiety was connected to the position C-21. Based on the above analyses, compound **1** was determined to be (*E*)-2-(1-β-D-glucopyranosyloxybut-2-en-2-yl)-3-(hydroxymethyl)-6,7-dihydroindolo[2,3-a]quinolizin-4(12*H*)-one (naucleamide D-21-O-β-D-glucopyranoside). The proton and carbon signals of compound **1** were assigned in detail according to analyses of the ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra.

Compound **2** was obtained as an orange–yellow powder and has a molecular formula of $C_{26}H_{26}N_2O_9$ according to its quasi-molecular ion peak at *m/z* 509.1565 $[M-H]^-$ (Calc. for $C_{26}H_{25}N_2O_9$: 509.1560) in the HRFAB mass spectrum. It also has four rings (rings A–D), which were deduced by NMR spectroscopic analysis. Specifically, a δ lactam ring (ring D) was identified by the correlations between H-5 (δ 4.51 and δ 4.06) and C-22 (δ 158.1), H-14 (δ 6.85) and C-16 (δ 108.0) in the HMBC spectrum of **2**. Furthermore, the HMBC correlations of H-14, H-18 (δ 2.10) to C-20 (δ 127.3), H-19 (δ 6.33) to C-15 (δ 150.2), and H-21 (δ 4.70) to C-17 (δ 160.7), C-15, C-20 and C-19 (δ 133.8) indicated that the ring D was connected to a lactone ring (ring E) possessing a trisubstituted olefin whose configuration (*E*) was established by NOESY spectroscopic correlations of H-18 to H-14 and H-19 to H-21. In the ^{1}H NMR spectrum, four aromatic proton signals were observed in the downfield region (δ 6.60–δ 7.30), a singlet at δ 6.85 for H-14, two doublets at δ 6.72 and δ 7.10 for H-9 and H-11, as well as a triplet at δ 7.19 for H-10. The structure of ring A, whose C-12 position was replaced, was also confirmed by the HMBC correlations of H-5, H-9 to C-7 (δ 116.9), H-1 (δ 12.09) to C-12 (δ 152.9) and the connectivities of C-9 (δ 103.5) to C-10 (δ 126.3) and C-10 to C-11 (δ 106.0). The ^{13}C NMR spectrum of compound **2** displayed the signals of a sugar moiety that was determined to be glucose with a β configuration by the coupling constant (7.3 Hz) of the anomeric proton and comparison with reported values (Yu and Yang, 1999). The sugar unit attached to the ring A at position C-12 was determined by the HMBC correlation of the anomeric proton with C-12. Based on the above analyses, compound **2** was established as (*E*)-1-propenyl-12-β-D-glucopyranosyloxy-2,7,8-trihydro-indolo[2,3-a]pyran-[3,4-g]quinolizin-4,5(13*H*)-dione.

Compound **3** was obtained as an orange–yellow powder and its molecular formula was determined as $C_{25}H_{28}N_2O_8$ from the HRFAB mass spectrum, showing a quasi-molecular ion peak at *m/z* 483.1776 $[M-H]^-$ (Calc. for $C_{25}H_{27}N_2O_8$: 483.1772). Rings A, B, C and D of **3** were very similar to those of **2**. The HMQC and ^{1}H–^{1}H COSY spectra suggested connectivities of C-5 (δ 39.8) to C-6 (δ 20.5), and C-9 (δ 103.4) to C-11 (δ 105.7). The attachment of a sugar to ring A at the C-12 position of compound **3** was confirmed from the long-range correlations from H-5 (δ 4.46 and δ 3.99) and H-9 (δ 6.69) to C-7 (δ 113.2) and from H-1 (δ 11.60) and the anomeric proton H-1′ (δ 5.00) to C-12 (δ 152.5). Similar to compounds **1** and **2**, the sugar moiety of compound **3** was identified as a β-glucopyranose from the chemical shifts (Yu and Yang, 1999) and the coupling constant (7.4 Hz) of H-1'. The HMBC correlations of H-5 to C-22 (δ 161.4) and H-14 (δ 6.56) to C-16 (δ 115.8) suggested the presence of a δ lactam ring (ring D) and the absence of C-17. The long-range correlations from H-14, H-18 (δ 1.70), H-19 (δ 5.81), H-21 (δ 4.31) and OH-21 (δ 4.97) to C-20 (δ 139.6), and from H-21 to C-15 (δ 149.5) showed that **3** had a trisubstituted olefin which connected to C-15 with an oxymethylene at C-20 and a methyl group at C-19 (δ 122.7). The configuration (*E*) of the trisubstituted olefin was determined by the NOESY correlation of H-18 to H-14. Based on the above analyses, compound **3** was determined to be (*E*)-2-(1-hydroxybut-2-en-2-yl)-11-β-D-glucopyranosyloxy-6,7-dihydro-indolo[2,3-a]quinolizin-4(12*H*)-one.

Compound **4** was obtained as a yellowish lamellar like solid and has a molecular formula of $C_{20}H_{22}N_2O_3$ according to a molecular ion peak at *m/z* 338.1634 $[M]^+$ (Calc. for $C_{20}H_{22}N_2O_3$: 338.1630) in the HREI mass spectrum. The ^{13}C NMR spectroscopic data indicated that **4** possesses one lactam carbonyl, eight aromatic carbons, one trisubstituted olefin, two oxidized carbons, three methines, three methylenes and one methyl group. The ^{13}C NMR chemical shifts and HMBC correlations of the aromatic carbons were characteristic of a tetrahydro-β-carboline ring, which were similar to the rings A, B and C of **1**. Furthermore, **4** also has two other rings (rings D and E) which are similar to those of **2**. The HMQC and ^{1}H–^{1}H COSY spectra of compound **4** established the connectivity from C-3 (δ 53.6) to C-17 (δ 91.0). The long-range correlations from H-14 (δ 2.92, δ 2.26), H-16 (δ 2.42), H-18 (δ 1.46) and H-21 (δ 4.64, δ 3.67) to C-20 (δ 134.8), and from H-18, H-19 (δ 5.40) and H-21 to C-15 (δ 28.3) showed connectivity between C-15 and C-20 and the presence of a trisubstituted olefin with a methyl group at C-19 (δ 119.5) and an oxymethylene at C-20. The HMBC correlations of H-15 (δ 2.72) to C-21 (δ 60.3) and C-17, H-21 to C-15, C-17 and C-20, H-16 to C-20 indicated that ring D was connected to a tetrahydropyran ring (ring E) with a trisubstituted olefin. The geometry of the trisubstituted olefin was *E* by the NOESY correlations of H-15 to H-18 and H-19 to H-21. The ^{1}H–^{1}H coupling constants ($J_{3,14a} = J_{3,14b}$ 5.7 Hz, $J_{14a,15} = 13.6$ Hz, $J_{15,16} = J_{16,17} = 5.0$ Hz) indicated an α-orientation of H-3 and β-orientations of H-15, H-16 and H-17 (δ 4.97). These were also confirmed by the NOESY

Table 1
^{1}H NMR spectroscopic data for compounds **1**–**5** (400 MHz, DMSO-d_6)[a,b]

No	1	2	3	4	5
1	11.84 (1H, *s*)	12.09 (1H, *s*)	11.60 (1H, *s*)	11.05 (1H, *s*)	11.84 (1H, *s*)
3				5.05 (1H, *d*, 5.7)	
5a	4.32 (2H, *m*)	4.51 (1H, *m*)	4.46 (1H, *dt*, 13.8,5.9)	4.78 (1H, *dd*, 12.4, 5.4)	4.37 (2H, *m*)
5b		4.06 (1H, *m*)	3.99 (1H, *m*)	2.98 (1H, *td*, 12.4, 4.5)	
6a	3.01–3.09 (2H, *m*)	3.51 (1H, *m*)	3.47 (1H, *m*)	2.80 (1H, *m*)	3.03 (2H, *t*, 6.7)
6b		3.20 (1H, *m*)	3.17 (1H, *m*)	2.60 (1H, *dd*, 15.0, 14.0)	
9	7.59 (1H, *d*, 7.9)	6.72 (1H, *d*, 7.9)	6.69 (1H, *d*, 7.9)	7.37 (1H, *d*, 7.9)	6.88 (1H, *d*, 2.2)
10	7.07 (1H, *t*, 7.9)	7.19 (1H, *t*, 7.9)	7.10 (1H, *t*, 7.9)	6.97 (1H, *t*, 7.9)	
11	7.21 (1H, *t*, 7.9)	7.10 (1H, *d*, 7.9)	7.03 (1H, *d*, 7.9)	7.07 (1H, *t*, 7.9)	6.80 (1H, *dd*, 8.7, 2.2)
12	7.41 (1H, *d*, 7.9)			7.33 (1H, *d*, 7.9)	7.30 (1H, *d*, 8.7)
14a	6.56 (1H, *s*)	6.85 (1H, *s*)	6.56 (1H, *s*)	2.92 (1H, *td*, 13.6, 6.5)	7.25 (1H, *s*)
14b				2.26 (1H, *d*, 12.0)	
15				2.72(1H, *m*)	
16			6.12 (1H, *s*)	2.42 (1H,*dd*, 5.0)	
17	4.11 (2H, *br s*)			5.29 (1H, *t*, 4.3)	9.22 (1H, *s*)
17-OH				6.55 (1H, *d*, 3.5)	
18	1.50 (3H, *d*, 6.8)	2.10 (3H, *d*, 7.3)	1.70 (3H, *d*, 6.8)	1.46 (3H, *d*, 6.7)	1.50 (3H, *d*, 6.4)
19	5.78 (1H, *q*, 6.8)	6.33 (1H, *q*, 7.3)	5.81 (1H, *q*, 6.8)	5.40 (1H, *q*, 6.7)	5.30 (1H, *q*, 6.4)
21a	4.59 (2H, *br s*)	4.70 (2H, *s*)	4.13 (2H, *d*, 5.5)	4.64 (1H, *d*, 12.6)	8.73 (1H, *s*)
21b				3.67 (1H, *d*, 12.6)	
21-OH			4.97 (1H, *t*, 5.5)		
1′	4.27 (1H, *d*, 7.7)	5.01 (1H, *d*, 7.3)	5.00 (1H, *d*, 7.4)		
2′	2.99 (1H, *m*)	3.23 (1H, *m*)	3.23 (1H, *m*)		
3′	3.09 (1H, *m*)	3.30 (1H, *m*)	3.31 (1H, *m*)		
4′	3.06 (1H, *m*)	3.33 (overlapped)	3.33 (overlapped)		
5′	3.17 (1H, *m*)	3.32 (overlapped)	3.34 (overlapped)		
6′	3.48 (1H, *m*)	3.49 (1H, *m*)	3.50 (1H, *m*)		
	3.67 (1H, *m*)	3.71 (1H, *m*)	3.71 (1H, *dd*, 10.8, 5.0)		

[a] Chemical shift values were in ppm and *J* values (in Hz) were presented in parentheses.
[b] The assignments were based on HMQC, HMBC and ^{1}H–^{1}H COSY experiments.

correlations of H-3 (δ 4.97) to H-14a and the mutual NOESY correlations among H-14b, H-15, H-16 and H-17, which indicated a *cis* relationship among them. Therefore, the relative stereochemistry of compound **4** was assigned as (*E*)-1-propenyl-4-hydroxy-2,4a,7,8,13b,14,14a-hepthydro-(4α,4aβ,13bα,14aβ)indolo[2,3-a]pyran[3,4-g]quinolizin-5(13*H*)-one.

Compound **5** was obtained as an orange–yellow powder. The HRFAB mass spectrum of **5** displayed a quasi-molecular ion peak at *m/z* 346.1196 $[M-H]^-$ (Calc. for $C_{20}H_{16}N_3O_3$: 346.1197), indicating a molecular formula of $C_{20}H_{17}N_3O_3$. The IR spectrum of **5** showed an absorption band at 3432 cm^{-1}, indicative of an hydroxyl group. Analyses of the ^{1}H and ^{13}C NMR spectra demonstrated that **5** were very similar to angustoline (Hotellier et al., 1975), except for an additional hydroxyl at C-10 (δ 152.0) in ring A. In the ^{1}H NMR spectrum, four aromatic proton signals were also observed in the downfield region (δ 6.70–δ 7.40). A singlet was assigned to H-14 (δ 7.25), however, two doublets at δ 7.30 ($J = 8.7$ Hz) and δ 6.88 ($J = 2.2$ Hz) were assigned to H-12 and H-9, and a doublet of doublets at δ 6.80 ($J = 8.7$ Hz, 2.2 Hz) was assigned to H-11 because of the HMBC correlations of H-5 (δ 4.37), H-9 to C-7 (δ 113.1), H-1 (δ 11.84) to C-12 (δ 103.4) and the NOESY correlation of H-6 (δ 3.03) to H-9. Therefore, compound **5** was determined to be 1-(1-hydroxyethyl)-10-hydroxy-7,8-dihy-

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**5** (100 MHz, DMSO-d_6)[a]

No	1	2	3	4	5
2	127.5	125.9	126.6	134.9	128.6
3	136.8	142.2	137.2	53.6	137.3
5	40.3	40.3	39.8	42.3	41.0
6	19.0	20.4	20.5	20.6	19.8
7	113.3	116.9	113.2	108.6	113.1
8	125.2	116.3	116.5	126.9	126.4
9	119.3	103.5	103.4	117.5	115.9
10	119.6	126.3	124.8	118.6	152.0
11	123.9	106.0	105.7	120.8	114.1
12	111.9	152.9	152.5	111.2	103.4
13	138.3	140.5	139.6	135.8	133.8
14	101.4	97.0	100.5	26.8	94.2
15	150.0	150.2	149.5	28.3	139.2
16	122.6	108.0	115.8	46.3	119.4
17	64.6	160.7	160.7	91.0	149.7
18	14.1	15.3	14.3	11.5	25.6
19	120.3	133.8	122.7	119.5	64.4
20	139.8	127.3	139.6	134.8	135.4
21	64.1	71.2	64.6	60.3	148.0
22	161.8	158.1	161.4	168.0	161.8
1′	103.2	100.4	100.6		
2′	70.0	69.7	69.7		
3′	77.0	76.7	76.8		
4′	73.5	73.4	73.5		
5′	76.7	77.1	77.1		
6′	61.0	60.7	60.7		

[a] The assignments were based on HMQC, HMBC and ^{1}H–^{1}H COSY experiments.

Table 3
Cytotoxicity and antimalarial activity of compounds 1–7 (IC_{50} μM)

Compounds	*Plasmodium falciparum*	SGC-7901	A-549	PC3	HL-60	K562
Positive control	0.018 ± 0.002	5.3 ± 1.9	18.7 ± 4.8	12.0 ± 3.1	10.1 ± 3.9	7.3 ± 3.4
1	9.7 ± 1.8	>200	>200	>200	>200	>200
2	42.1 ± 5.7	>200	>200	>200	>200	>200
3	40.5 ± 6.8	>200	>200	>200	>200	>200
4	39.8 ± 5.9	166	>200	77	181	>200
5	38.3 ± 6.2	>200	20.4 ± 4.3	44.4 ± 10.2	79	26.4 ± 7.1
6	26.8 ± 4.6	8.1 ± 2.7	22.5 ± 3.9	17.6 ± 3.8	18.6 ± 4.7	15.8 ± 4.2
7	20.5 ± 4.1	20.3 ± 4.4	>200	18.3 ± 4.1	53	29.6 ± 9.3

dro-indolo[2,3-a]pyridine[3,4-g]quinolizin-5(13*H*)-one (10-hydroxyangustoline).

Naucleaorine, a tetrahydro-β-carboline monoterpene indole alkaloid isolated from *N. orientalis*, was reported to have antimalarial activity *in vitro* (He et al., 2005). Therefore, the seven indole alkaloids isolated from *N. officinalis* were tested for *in vitro* inhibitory effects against *P. falciparum* and cytotoxic activities against five human cancer lines, including breast prostate PC3, leukemic K562, leukemic HL-60, lung A549 and gastro SGC 7901. As shown in Table 3 naucleidinal (**6**) and angustoline (**7**) demonstrated weak to moderate antimalarial activities with an IC_{50} value of 26.8 μM and 20.5 μM. Amongst the seven compounds, **1** exhibited the most potent antimalarial activity with an IC_{50} value of 9.7 μM, while no cytotoxic effect was observed. When the aromatic hydrogens of ring A (position 10 or 12) were replaced, the antimalarial activity was reduced to IC_{50} values of 42.1 μM for **2**, 40.5 μM for **3** and 38.3 μM for **5**. When ring D was aromatic, the antimalarial activity was increased; however when all the rings (ring A, B, C, D and E) were aromatic, the antimalarial activity also decreased. Compounds **6** and **7** showed good cytotoxic activities, but poor antimalarial activity. Therefore, the antimalarial activity of these compounds had no direct correlation with their cytotoxicity. These results led us to speculate that a compound having no replacement group in ring A, having an aromatic ring D and proper water-solubility might be an active compound for further screening antimalarial activity.

2.1. Concluding remarks

Although showing weak antimalarial and cytotoxic activities, compounds **2** and **3** are identified as a novel type of indole alkaloids, since the C-12 aromatic proton of ring A was replaced by glucose in both cases. To our knowledge, these are the first of this type of monoterpene indole alkaloids to be obtained from natural sources.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Bruker AVANCE 400, using TMS as an internal standard. MS and HRMS spectra were obtained on an Apex II FT-ICR MS spectrometer. IR spectra were recorded on a Perkin–Elmer 683 infrared spectrophotometer. UV spectra were obtained on a Shimadzu UV-160 spectrophotometer. Optical rotations were determined on a WZZ-2B polarimeter (Shanghai physical optics equipment factory). Melting points were measured on a XT-4 micro-melting point apparatus and are uncorrected. ODS (Jinouya Co., Beijing, People's Republic of China), Sephadex LH-20 (Pharmacia Co.), Silica gel (200-300 mesh) for column chromatography and silica gel GF_{254} for the precoated plates were obtained from Qingdao Haiyang Chemical Group Co. Ltd., Qingdao, People's Republic of China. TLC was conducted on Merck RP-18 F_{254} or on Merck Si gel 60 F_{254} on a plastic plate. Spots on the TLC plate were visualized under UV light (365 nm or 254 nm).

3.2. Plant material

The stems and bark of *N. officinalis* were collected in Hainan Province, People's Republic of China, in April 2005. The plant material was identified by Dr. Yanyan Zhao, and a voucher specimen (No. S200504) has been deposited in the Department of Pharmacognosy, School of Pharmacy, Yantai University (People's Republic of China).

3.3. Extraction and isolation

The dried powdered plant material (10 kg) was extracted by percolation with MeOH (80 l) at room temperature for three weeks. The extract was combined and evaporated to dryness *in vacuo*. The residue (750 g) was successively suspended in H_2O and partitioned with $CHCl_3$, EtOAc, and *n*-BuOH. The EtOAc soluble part (46.5 g) was subjected to silica gel (1.0 kg) column chromatography (CC), eluting with a gradient mixture of $CHCl_3$ and MeOH, giving twelve fractions (See Chart 1).

Fraction 3 ($CHCl_3$–MeOH (96:4, v/v), 6.2 g) was further fractionated by ODS (120 g) CC and eluted with MeOH–H_2O (2:8–6:4, v/v) (each fraction 200 ml), giving eluates (1–45). The eluates 32–35 (1.39 g) yielded **6** crystals (60 mg). **7** (30 mg) was obtained from the eluates 40–42 (0.51 g) by prep. TLC with $CHCl_3$ and MeOH (95:5, v/v).

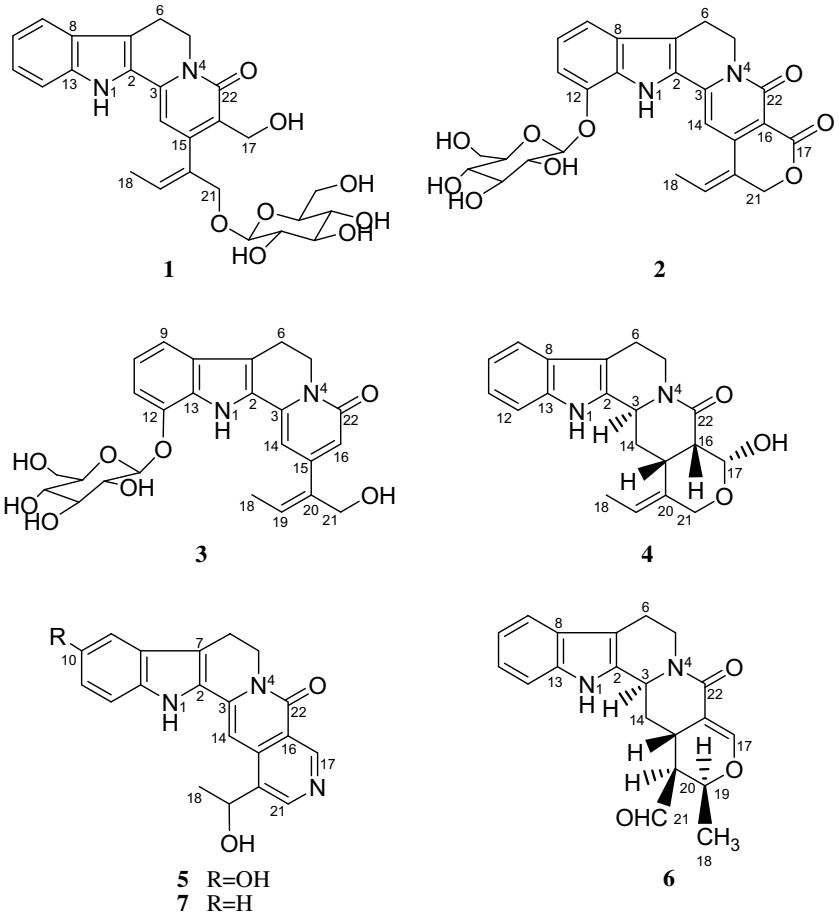

Chart 1. Structures of compounds **1**–**7**.

Fraction 5 ($CHCl_3$–MeOH (94:6, v/v), 4.8 g) was then applied to silica gel (100 g) CC and eluted with a $CHCl_3$ and MeOH gradient from 100:0 to 100:10 (v/v), giving five subfractions (A–F). Fraction B (0.9 g) was further separated by prep. TLC with $CHCl_3$ and MeOH (92:8, v/v) to give **5** (4 mg). Fraction D (1.1 g) was further fractionated by Sephadex LH-20 (70 g) CC and eluted with MeOH (each fraction 20 ml) and the resulting fraction 4–8 (0.32 g) was then separated by prep. TLC with $CHCl_3$ and MeOH (85:15, v/v) to yield **4** (8 mg).

Fraction 8 ($CHCl_3$–MeOH (87:13, v/v), 5.6 g) was fractionated by ODS (120 g) CC and eluted with MeOH–H_2O (2:8–6:4, v/v) (each fraction 200 ml) to give eluates (1–30). The eluates 7–12 (0.42 g) were then purified using Sephadex LH-20 (70 g) CC and eluted with MeOH (each fraction 20 ml) to give **2** (4 mg). The eluates 16–12 (0.50 g) and 22–26 (0.71 g) were also purified by the same method and gave **1** (25 mg) and **3** (6 mg).

*3.3.1. (E)-2-(1-β-D-Glucopyranosyloxybut-2-en-2-yl)-3-(hydroxymethyl)-6,7-dihydro-indolo[2,3-a] quinolizin-4(12H)-one (**1**)*

Orange–yellow powder. Mp 185–187°C; $[\alpha]_D^{25}$: −12.3 (MeOH; *c* 0.24). UV λ_{max}^{MeOH} nm (log ε): 220 (4.45), 282 (3.51). IR ν_{max}^{KBr} cm^{-1}: 3399, 2919, 1641, 1566, 1539, 1500, 1452, 1383, 1313, 1239, 1077, 1042, 884, 835, 744. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. HR-FABMS *m/z*: 497.1931 $[M-H]^-$ (Calc. for $C_{26}H_{29}N_2O_8$: 497.1929).

*3.3.2. (E)-1-Propenyl-12-β-D-Glucopyranosyloxy-2,7,8-trihydro-indolo[2,3-a]pyran[3,4-g]quinolizin-4,5(13H)-dione (**2**)*

Orange–yellow powder. Mp 178–179°C; $[\alpha]_D^{20}$: −11.4 (MeOH; *c* 0.21). UV λ_{max}^{MeOH} nm (log ε): 225 (4.53), 296 (3.57). IR ν_{max}^{KBr} cm^{-1}: 3429, 2915, 1706, 1619, 1588, 1513, 1430, 1383, 1250, 1158, 1108, 1070, 1029, 899, 828, 803, 778, 734. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. HR-FABMS *m/z*: 509.1565 $[M-H]^-$ (Calc. for $C_{26}H_{25}N_2O_9$: 509.1560).

*3.3.3. (E)-2-(1-hydroxybut-2-en-2-yl)-11-β-D-Glucopyranosyloxy-6,7-dihydro-indolo[2,3-a]quinolizin-4 (12H)-one (**3**)*

Orange–yellow powder. mp 175–177°C; $[\alpha]_D^{20}$: −13.1 (MeOH; *c* 0.19). UV λ_{max}^{MeOH} nm (log ε): 222 (4.49), 285 (3.50). IR ν_{max}^{KBr} cm^{-1}: 3368, 2913, 1645, 1565, 1543, 1509, 1482, 1444, 1421, 1372, 1349, 1306,1275, 1246, 1200, 1172, 1088, 1042, 990, 871, 852, 832, 778, 730. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

HR-FABMS *m/z*: 483.1776 $[M-H]^-$ (Calc. for $C_{25}H_{27}N_2O_8$: 483.1772).

3.3.4. (*E*)-1-Propenyl-4-hydroxy-2,4a,7,8,13b,14,14a-hepthydro-(4α, 4aβ,13bα,14aβ)indolo[2,3-a] pyran[3,4-g]quinolizin-5(13H)-one (**4**)

Yellowish lamellar solid. Mp 253–255°C; $[\alpha]_D^{25}$: −22.4 (MeOH; *c* 0.13). UV λ_{max}^{MeOH} nm (log ε): 221 (4.42), 277 (3.48). IR ν_{max}^{KBr} cm^{-1}: 3280, 2966, 2922, 2852, 1615, 1472, 1439, 1361, 1323, 1306, 1266, 1193, 1163, 1098, 1079, 1058, 1021, 990,910, 877, 850, 811, 790, 739.For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. HR-EIMS *m/z*: 338.1634 $[M]^+$ (Calc. for $C_{20}H_{22}N_2O_3$: 338.1630).

3.3.5. 1-(1-hydroxyethyl)-10-Hydroxy-7,8-dihydro-indolo[2,3-a]pirydine[3,4-g]quinolizin-5(13H)-one (**5**)

Orange–yellow powder. mp 279–281°C; $[\alpha]_D^{20}$: −29.3 (MeOH; *c* 0.21). UV λ_{max}^{MeOH} nm (log ε): 220 (4.31), 251 (4.02), 307 (3.90), 400 (4.67). IR ν_{max}^{KBr} cm^{-1}: 3432, 2924, 1604, 1412, 1259, 1212, 1131, 1000. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. HR-FABMS *m/z*: 346.1196 $[M-H]^-$ (Calc. for $C_{20}H_{16}N_3O_3$: 346.1197).

3.4. Biological assays

3.4.1. Antimalarial activity in vitro

P. falciparum (isolate FCC1-HN) was maintained *in vitro* by modification of the reported method (Trager and Jensen, 1976). The culture medium consisted of RPMI1640 (Gibco) supplemented with 10% rabbit serum. Cultures were maintained in type O+ human red blood cell suspensions at a hematocrit of 5%. The parasite density was maintained below 5% parasitemia under an atmosphere of a gas mixture containing 5% CO_2, 5% O_2, and 90% N_2 at 37 °C in a water-jacketed incubator. The samples of stock cultures were adjusted to final hematocrit of 5% and a parasitemia of 3%. For synchronized assays, asynchronous cultures were pretreated with sorbitol and incubated for 28–30 h to schizont stage parasites. 200 μl aliquots were distributed in 96-well microtiter plates. Test samples and chloroquine, as a positive control, dissolved in DMSO, were added at various concentrations. After incubation for 24 h, the parasitemia in each well was assessed by microscopy with a thin blood smear after Giemsa staining.

Data analyses were performed with a preprogrammed calculus sheet on Microsoft Excel 2003 that processes the parasitemia. Analysis of the counts was performed with Graphpad Prism software.

3.4.2. Cytotoxicity Assays

The cell lines of human breast prostate PC3 were cultured in DMEM medium, while leukemic K562, leukemic HL-60, lung A549, gastro SGC7901 were cultured in PRMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Each cell line was seeded in 96-well plates at a density of 2×10^4 cells per well and was incubated for 24 h. Test samples and cisplatin (PPD), as a positive control dissolved in DMSO, were added at different concentrations. Cytotoxicity was assessed using the colorimetric MTT reduction assay.

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