New potentially bioactive alkaloids from *Crinum erubescens*

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ABSTRACT

Antimalarial bioassay-guided fractionation of the swamp lily *Crinum erubescens* led to the isolation of four compounds with potent antiplasmodial activity. Compounds **1** and **2** were determined from their spectroscopic data to be the known pesticidal compound cripowellin A and the known pesticidal and antiproliferative compound cripowellin B. 1D and 2D-NMR techniques were used to determine the identities of **3** and **4** as the new compounds cripowellin C and D. A fifth compound was identified as the known alkaloid hippadine, which was inactive against *Plasmodium falciparum*. The antiplasmodial IC₅₀ values of compounds **1** – **4** were determined to be 30 ± 2, 180 ± 20, 26 ± 2, and 260 ± 20 nM, respectively, and their antiproliferative IC₅₀ values against the A2780 human ovarian cancer cell line were 11.1 ± 0.4, 16.4 ± 0.1, 25 ± 2, and 28 ± 1 nM.

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1. Introduction

The genus *Crinum* contains around 110 accepted species with over 270 synonyms belonging to the family Amaryllidaceae.¹ Members of the genus *Crinum* possess large extravagant flowers on leafless stems and are distributed in moist sites, such as forests, river edges, seasonal pools, or salt pans, and can be found throughout, the tropics of Africa, Asia, America, Southern Africa, Madagascar, and Mascarene and the Pacific Islands.² Extracts from *Crinum* species have been used traditionally to treat a variety of ailments including fever, pain management, swelling, sores and wounds, cancer, and malaria.³ As a member of the Amaryllidaceae family, the *Crinum* genus is known to be a rich source of norbelladine type alkaloids, including lycorine, crinine, and narciclasine.⁴ Alkaloids isolated from various *Crinum* species have shown activity in a wide variety of assays including analgesic, anticancer, antibacterial, antiviral, antifungal, and antimalarial assays.³

Malaria is a tropical disease that has a disproportionate effect in poor and underdeveloped countries without access to western medicine, and with the appearance of artemisinin resistant parasites in five countries there is an urgent need for new and affordable medicines.⁵ Since plants have been a successful source for antimalarial medicines such as quinine and artemisinin, our group has been investigating plant extracts from the Natural Products Discovery Institute (NPDI) collection for new compounds or known compounds with new activity against *Plasmodium falciparum*.⁶ An extract of *Crinum erubescens* L. f. ex Aiton displayed strong antimalarial activity from the initial screening and was selected for isolation.

2. Results and Discussion

2.1. Isolation

A methanol extract of *C. erubescens* was partitioned between aqueous methanol and hexanes, the aqueous methanol was then dried and suspended in water and extracted with ethyl acetate to afford an active ethyl acetate fraction ($IC_{50} \lll 1.25 \mu\text{g/mL}$). Due to the high likelihood of the extract containing a large amount of alkaloids, an acid/base extraction was performed on the ethyl acetate fraction. The fraction was suspended in 2% sulfuric acid and extracted with ethyl ether to produce a "neutral" fraction. The sulfuric acid solution was then adjusted to a pH of 10 with a 20% ammonium hydroxide solution and then extracted with ethyl acetate to generate a "basic" fraction. The basic fraction was found to be active ($IC_{50} \lll 1.25 \mu\text{g/mL}$) and was subjected to a C_{18} solid phase extraction (SPE). The SPE column was loaded and eluted with methanol, chloroform, and finally with water to remove any salts that were carried over from the acid/base extraction. Separation of the active methanol SPE fraction ($IC_{50} \lll 1.25 \mu\text{g/mL}$) by HPLC over a C_{18} column with methanol/water afforded five active fractions ($IC_{50} \lll 1.25 \mu\text{g/mL}$) with slight impurities. Final HPLC purifications were conducted on the five active fractions over a C_{18} column using acetonitrile/water, affording compounds **1-5**.

2.2. Structure Elucidation

Compound **1** (Figure 1) was isolated as a white amorphous solid with a chemical formula of $C_{25}H_{31}NO_{12}$ as determined by HRESIMS (m/z 538.1916 $[M+H]^+$, calcd for $C_{25}H_{32}NO_{12}^+$, 538.1919). Proton NMR data (Table 1) revealed the presence of an aromatic ring moiety, a methylenedioxy bridge, a methoxy, and a glucose moiety. A literature search using the chemical formula and the key structural features confirmed the structure of **1** as the known compound cripowellin A.⁷ The structure of **2**

(Figure 1) was elucidated as the known compound cripowellin B from the HRESIMS data (m/z 524.2127 $[M+H]^+$, calcd for $C_{25}H_{34}NO_{11}^+$, 524.2126) and comparison of its ^1H NMR data (Table 1) to the published literature values.⁷ Both **1** and **2** possessed an intense fragment in their HRESIMS data at 320.1119 m/z , corresponding to the $[\text{Aglycone}+H]^+$ fragment.

Compound **3** (Figure 1) was isolated as a white amorphous solid. It had the chemical formula $C_{25}H_{31}NO_{11}$ as determined by HRESIMS (m/z 522.1968 $[M+H]^+$, calcd for $C_{25}H_{32}NO_{11}^+$, 522.1970). As with the HRESIMS data for cripowellins A and B, another high intensity fragment was observed correlating to the aglycone of the molecule (320.1119 m/z $[\text{Aglycone}+H]^+$, calcd for $C_{16}H_{18}NO_6^+$, 320.1129 m/z). Comparing the ^1H NMR of **1** to **3** it could be observed that the aglycones of the compounds were identical in structure, with the key differences being observed in the sugar moiety. The anomeric proton observed at δ_H 4.44 (d, $J = 7.9$ Hz, 1H) along with a doublet methyl at δ_H 1.36 (d, $J = 6.1$ Hz, 3H) indicated the presence of a 6-deoxypyranosyl sugar. Two-dimensional NMR spectroscopic data, including COSY, HSQC, HMBC, and NOESY were used to determine the connectivity and configuration of the sugar moiety. HSQC was used to determine all direct proton to carbon attachments while COSY correlations were used to determine the connectivity around the pyranosyl sugar from H-1' to H-6'. An HMBC correlation from the anomeric proton observed at δ_H 4.44 to δ_C 84.3 (C-15) confirmed the attachment of the sugar moiety to C-15. The more unusual protons associated with the sugar moiety were found in the 1,3,5-trioxepane ring system, which is formed by part of the sugar moiety (C-2' and C-3') and by two isolated methylene units containing four diastereotopic protons. The first methylene unit had two protons at δ_H 4.94 (d, $J = 6.0$ Hz, 1H) and δ_H 4.82 (d, $J = 6.0$ Hz, 1H) both attached to δ_C 91.5 (C-1''), while the second methylene unit had two protons at δ_H 5.01 (d, $J = 5.6$ Hz, 1H) and δ_H 4.87 (d, $J = 5.6$ Hz, 1H), both attached to δ_C 92.3 (C-2''). HMBC correlations from H-1''b to C-2' and C-2'', and from H-2''b to C-3' and C-1'' confirmed the attachment and location of the 1,3,5-trioxepane ring system. The methoxy group at δ_H 3.54 / δ_C 61.0 (s, 3H) had a single HMBC correlation to C-4', confirming its attachment to the sugar moiety. NOESY correlations from H-1' to H-3' and H-5', and from H-2' to H-4', and from H-4' to H-6' confirmed the stereochemistry of the sugar moiety as 15-([2',3']-[1,3,5]-trioxepane-4'-methoxy- β -D-quinovose). Compound **3** was assigned the name cripowellin C.

Compound **4** (Figure 1) was isolated as a white amorphous solid. It had the chemical formula $C_{25}H_{33}NO_{11}$ as determined by HRESIMS (m/z 508.2131 $[M+H]^+$, calcd for $C_{25}H_{34}NO_{10}^+$, 508.2177). As in the HRESIMS data for cripowellins A, B, and C another high intensity fragment was observed correlating to the aglycone of the molecule (m/z 320.1096 $[\text{Aglycone}+H]^+$, calcd for $C_{16}H_{18}NO_6^+$, 320.1129). Comparison of the ^1H NMR data of **4** with that of **3** indicated that the aglycones of the two compounds were identical, that the 6-dexopyranosyl sugar moiety was also present, there were two additional methoxy methyl groups, and that the 1,3,5-trioxepane-ring moiety was absent. Again, 2D NMR spectroscopic data, including COSY, HSQC, HMBC, and NOESY, were used to determine the connectivity and configuration of the sugar moiety. An HMBC correlation from the anomeric proton observed at δ_H 4.31 (d, $J = 7.9$ Hz, 1H) to δ_C 84.7 (C-15) confirmed the attachment of the sugar moiety to C-15. Once the connectivity around the ring from H-1' to H-6' had been established as before from HSQC and COSY correlations, HMBC was used to determine the locations of the methoxy methyl groups. The methoxy methyls at δ_H 3.44 / δ_C 60.6 (s, 3H), δ_H 3.58 / δ_C 60.8 (s, 3H), and δ_H 3.53 / δ_C 60.7 (s, 3H) each had a single HMBC correlation to their respective attachment locations

at C-2', C-3', and C-4' respectively. NOESY correlations from H-1' to H-15, H-3', and H-5', and from H-2' to H-4', and from H-4' to H-6' confirmed the stereochemistry of the sugar moiety as 15-(2',3',4'-methoxy- β -D-quinovose). Compound **4** was assigned the name cripowellin D.

Compound **5** (Figure 1) was isolated as an amorphous solid with the composition $C_{16}H_9NO_3$ as determined by HRESIMS ($264.0659\ m/z\ [M+H]^+$, calcd for $C_{16}H_{10}NO_3^+$, 264.0655). The 1H NMR spectrum displayed signals for five aromatic protons, two olefinic doublets, and a methylenedioxy group. Three of the five aromatic protons were determined to be in the same spin system due to the observed coupling constants from δ_H 7.94 (br d, $J = 7.7$ Hz, 1H), δ_H 7.76 (dd, $J = 7.6, 0.7$ Hz, 1H), and δ_H 7.49 (t, $J = 7.7$ Hz, 1H). The broad doublet at δ_H 7.94 is most likely the result of unresolved meta coupling. The remaining two aromatic signals are both isolated singlets at δ_H 8.00 (s, 1H), and δ_H 7.68 (s, 1H). There was also a characteristic methylenedioxy resonance at δ_H 6.17 (s, 2H). Lastly, the two olefinic protons were both coupled to one another with resonances at δ_H 6.91 (d, $J = 3.6$ Hz, 1H) and δ_H 8.05 (br d, $J = 3.6$ Hz, 1H). Putting the observed proton resonances, molecular formula, and the thirteen degrees of unsaturation together the structure of hippadine was produced. Comparison of the 1H NMR literature data for hippadine with the observed spectroscopic data confirmed the identity of **5** as the known compound hippadine.⁸

2.3. Circular Dichroism and Stereochemistry

Optical rotations of cripowellins A and B matched the published values,⁷ and the measured optical rotations for cripowellins C and D were very similar to those of cripowellins A and B. This observation combined with the NOESY data provided evidence to support the indicated stereochemistry for

Table 1. 1H and ^{13}C NMR data for **1** – **4** in $CDCl_3$

	1 Cripowellin A	2 Cripowellin B	3 Cripowellin C	4 Cripowellin D		
Position	δ_{H} , mult. (J Hz) ^a	δ_{H} , mult. (J Hz) ^a	δ_{H} , mult. (J Hz) ^a	δ_{C} ^b	δ_{H} , mult. (J Hz) ^a	δ_{C} ^b
1	3.26, brdd (4.8, 2.9)	3.27, brdd (4.4, 3.0)	3.26, t (3.9)	55.9 (CH)	3.27, t (4.0)	55.8 (CH)
2	--	--	--	130.8 (C)	--	127.8 (C)
3	6.55, s	6.68, s	6.54, s	112.1 (CH)	6.55, s	112.1 (CH)
4	--	--	--	147.5 (C)	--	147.6 (C)
6-a,b	6.00, m	6.01, s	6.00, s	101.6 (CH ₂)	6.01, m	101.6 (CH ₂)
8	--	--	--	147.6 (C)	--	147.6 (C)
9	6.66, s	6.56, s	6.65, s	107.1 (CH)	6.67, s	107.0 (CH)
10	--	--	--	127.4 (C)	--	130.8 (C)
11-a	5.28, d (17.5)	5.27, d (17.5)	5.28, d (17.8)	54.8 (CH ₂)	5.26, d (17.5)	54.9 (CH ₂)
11-b	4.49, d (17.8)	4.49, d (17.5)	4.46, d (17.8)	--	4.47, d (17.5)	--
13	--	--	--	170.7 (C)	--	170.8 (C)
14	4.66, d (7.4)	4.66, d (7.2)	4.60, d (8.1)	70.3 (CH)	4.57, dd (7.9, 5.6) ^c	70.6 (CH)
15	4.11, ddd (11.7, 7.4, 4.0)	4.11, ddd (11.4, 7.0, 4.3)	4.10, ddd (11.8, 8.3, 3.8)	84.3 (CH)	4.09, ddd (11.8, 8.0, 3.9)	84.7 (CH)
16-a	3.10, dd (14.8, 12.0)	3.07, m	3.03, dd (14.0, 12.0)	40.2 (CH ₂)	2.99, dd (14.4, 11.8)	40.2 (CH ₂)
16-b	2.23, dd (14.7, 4.0)	2.24, m	2.20, dd (14.6, 3.9)	--	2.22, dd (14.3, 4.1)	--
17	--	--	--	206.3 (C)	--	206.2 (C)
18-a	3.15, m	3.11, m	3.12, m	36.5 (CH ₂)	3.12, m	36.5 (CH ₂)
18-b	2.21, m	2.22, m	2.24, dd (9.0, 4.0)	--	2.22, m	--
19-a	4.35, m	4.34, brt, (11.6)	4.37, m	41.5 (CH ₂)	4.38, brt (11.8)	41.5 (CH ₂)
19-b	2.77, dt (13.5, 8.5)	2.76, dt (13.4, 8.5)	2.74, dt (14.0, 8.0)	--	2.73, m	--
1'	4.54, d (7.9)	4.41, d (7.7)	4.44, d (7.9)	100.1 (CH)	4.31, d (7.9)	102.4 (CH)

cripowellins C and D. To further support this claim ECD spectra were obtained on cripowellins A – D in methanol and it was observed that all four compounds had very similar spectra.

Figure 1. Structures of compounds **1** – **5**.

2.4. Bioassay Data

Compounds **1** – **5** were evaluated for their antiparasitic activity against the chloroquine/mefloquine-resistant Dd2 strain of *Plasmodium falciparum*. Compounds **1** – **4** exhibited potent antimalarial activity, with IC_{50} values of 30 ± 2 , 180 ± 20 , 26 ± 2 , and 260 ± 20 nM, respectively. Comparing the IC_{50} values of **1** – **4** indicates that replacement of the hydroxyl at 6' by methyl does not change the activity significantly, while removal of the 1,3,5-trioxapane-ring decreases the activity by about an order of

2'	3.32, brt (8.3)	3.16, brt (8.8)	3.32, brt (8.3)	79.6 (CH)	2.94, dd (9.2, 7.9)	83.7 (CH)
3'	3.58, brt (8.8)	2.96, m	3.51, m	83.7 (CH)	3.07, t (9.0)	86.4 (CH)
4'	3.23, brt (9.3)	3.06, m	2.92, brt (9.2)	82.8 (CH)	2.78, t (9.3)	85.1 (CH)
5'	3.38, ddd (9.0, 6.2, 2.6)	3.31, ddd (9.0, 7.0, 3.0)	3.36, dd (9.4, 6.2)	71.7 (CH)	3.29, dd (9.5, 6.2)	71.4 (CH)
6'-a	3.91, dd (11.7, 2.7)	3.86, brd (11.5)	--	--	--	--
6'-b	3.71, dd (11.6, 6.1)	3.65, m	--	--	--	--
6'-CH ₃	--	--	1.36, d (6.1)	17.6 (CH ₃)	1.31, d (6.2)	17.6 (CH ₃)
1''-a	4.95, d (6.1)	--	4.94, d (6.0)	91.5 (CH ₂)	--	--
1''-b	4.81, d (6.1)	--	4.82, d (6.0)	--	--	--
2''-a	5.03, d (5.8)	--	5.01, d (5.6)	92.3 (CH ₂)	--	--
2''-b	4.88, d (5.8)	--	4.87, d (5.6)	--	--	--
2'-OCH ₃	--	3.44, s	--	--	3.44, s	60.6 (CH ₃)
3'-OCH ₃	--	3.50, s	--	--	3.58, s	60.8 (CH ₃)
4'-OCH ₃	3.52, s	3.59, s	3.54, s	61.0 (CH ₃)	3.53, s	60.7 (CH ₃)

^aData (δ) measured at 600 MHz; s = singlet, d = doublet, brd = broad doublet, t = triplet, brt = broad triplet, dd = doublet of doublets, brdd = broad doublet, ddd = doublet of doublets of doublets, dt = doublet of triplets, and m = multiplet. *J* values are in Hz and are omitted if the signals overlapped as multiplets. Overlapping signals were assigned from HSQC and HMBC spectra for compounds **3** and **4**.

^bData (δ) measured at 150 MHz; CH₃, CH₂, CH, and C multiplicities were determined by HSQC spectra. All carbon assignments were made from HSQC and HMBC spectra for compounds **3** and **4**.

^cIn some cases coupling interactions can be observed between CH and OH protons, and are observed around *J* = 5 Hz.⁹

magnitude. Compound **5** was found to be inactive at 38 μ M. This loss of activity as compared with the activity of fraction containing **5** is most probably due to the small amount of **2** (cripowellin B) that was present in these fractions until the final purification of **5**.

Compounds **1** – **4** were also tested for antiproliferative activity against the A2780 human ovarian cancer cell line, and were found to have IC₅₀ values of 11.1 $\pm$ 0.4, 16.4 $\pm$ 0.1, 25 $\pm$ 2, and 28 $\pm$ 1 nM, respectively. Compound **2** has also been shown to exhibit nM antiproliferative activity against human melanoma (A375), colon (SW620), and cervical (HeLa) cell lines.¹⁰ Compound **5** was not tested in the A2780 assay since it has been previously reported to be inactive against multiple human cells lines including; lung (A549), colon (LoVo), lymphocytic leukemia (6T-CEM), and promyelocytic leukemia cells (HL-60).¹¹

3. Conclusions

The genus *Crinum* continues to be a rich source of bioactive alkaloids, but although antiplasmodial activity has been reported for some *Crinum* alkaloids,^{3, 12-13} this work adds cripowellins A – D as a new class of antiplasmodial alkaloids characterized by a nanomolar level of activity. The two new compounds **3** and **4** were isolated along with the three known compounds, **1**, **2**, and **5** from *C. erubescens*.

The unusual 1,3,5-trioxepane ring moieties of **1** and **3** might conceivably be considered to be artefacts formed from reaction of a putative diol precursor with formaldehyde (present as a trace impurity) under the acidic conditions of partition with aqueous sulfuric acid. A comparison of the ¹H NMR spectra of compounds **1** and **3** with that of the crude extract was thus conducted, and the spectrum of the crude extract was shown to contain peaks corresponding to the methylene protons of the trioxepane groups. An HPLC comparison of the crude extract with that of a fraction containing compounds **1** – **4** also indicated the presence of these compounds in the crude extract. Compounds **1** – **4** are thus all considered to be authentic natural products.

When **1** and **2** were originally isolated from *Crinum powellii* in 1997 they were found to possess insecticidal activity,¹⁴ and in 2006 **2** was reported to possess potent antiproliferative activity against a variety of human cancer cell lines.¹⁰ Although compounds **1** – **4** have now been shown to possess potent antiplasmodial activity, their antiproliferative activities against human cancer cell lines and the complex nature of the cripowellin structure regrettably combine to make the cripowellins less than ideal candidates for development as antimalarial drugs unless their antiplasmodial and antiproliferative activities can be separated by appropriate structure modifications.

4. Experimental Section

4.1. General

Optical rotations were recorded on a JASCO P-2000 polarimeter, and UV spectra were measured on a Shimadzu UV-1201 spectrophotometer. ECD analysis was performed on a JASCO J-810 spectropolarimeter with a 1 cm cell in methanol at room temperature under the following conditions: speed 100 nm/min, time constant 1 s, bandwidth 1.0 nm. ¹H and ¹³C NMR spectra were obtained either on a Bruker Avance 600 or Bruker Avance 500 spectrometer. Mass spectra were obtained on an Agilent 6220 LC-TOF-MS spectrometer. Semipreparative HPLC was performed using Shimadzu LC-10AT pumps coupled with a Shimadzu SPD-M10A diode array detector, a SCL-10A system controller, and a Phenomenex 5 μ m, 100 Å, Luna C₁₈(2) (250 x 10 mm) column (Column A) or a Cogent 4 μ m, 100 Å, Bidentate C₁₈ (250 x 10 mm) column (Column B). Solid phase extractions were conducted with Thermo Scientific HyperSep C₁₈ 500 mg, 3 mL SPE cartridges, and all reverse phase TLC was conducted with Sorbtech C₁₈-W silica TLC plates, w/UV254 on an aluminum backing with a thickness of 150 μ m.

4.2. Plant material

Specimens of above-ground parts of *Crinum erubescens* were collected by J. Francisco Morales of the Instituto Nacional de Biodiversidad, Costa Rica, along the road leading to Playa Cacao in Llano Bonito, Puntarenas. Vouchers are on deposit at INBIO under accession number FM01522.

4.3. Extraction and Isolation

Dried, powdered plant material was exhaustively extracted with MeOH to give a MeOH-soluble extract designated 13103-C4 X-2776; a total of 478 mg of this extract was made available to Virginia Tech. This extract had an IC_{50} value $<<< 3 \mu\text{g/mL}$ against *P. falciparum* Dd2 strain. The extract of *C. erubescens* (478 mg) was dissolved in 200 mL of MeOH and to which water (20 mL) was added. This aqueous methanolic solution was then extracted with 8 x 200 mL of hexanes. The aqueous MeOH was then concentrated in vacuo, and suspended in 200 mL of water. The resulting aqueous suspension was then extracted with 8 x 200 mL of EtOAc to afford an active EtOAc fraction (342 mg) with an IC_{50} value of $<<< 1.25 \mu\text{g/mL}$. The dried EtOAc fraction was suspended in 200 mL of a 2% H_2SO_4 solution and extracted with 3 x 200 mL of Et_2O to produce a “neutral” fraction, which was then dried over Na_2SO_4 . The H_2SO_4 solution was then adjusted to a pH of 10 with a 20% NH_4OH solution, and extracted with 3 x 200 mL of EtOAc to generate a “basic” fraction.¹⁵ Any insoluble material that formed was kept with the aqueous fraction throughout the acid/base extraction. The “basic” fraction (68 mg) was found to be active with an IC_{50} value of $<<< 1.25 \mu\text{g/mL}$ and was then further separated on a C_{18} solid phase extraction (SPE). The sample was dissolved in MeOH and loaded onto the SPE cartridge and eluted with 100 mL of MeOH to generate the first fraction. Then the SPE cartridge was eluted with 100 mL of chloroform to form the second fraction, and finally with 50 mL of water to remove any salts that were carried over from the acid/base extraction. The MeOH fraction (59 mg) was found to have an IC_{50} value of $<<< 1.25 \mu\text{g/mL}$ and was then separated on C_{18} HPLC. A $\text{H}_2\text{O}/\text{MeOH}$ gradient was developed using Column A, starting from 62:38 to 30:70 over 40 minutes followed by a maintained flow at 0:100 for 20 minutes. A total of thirteen fractions were collected, fractions 6 (t_R 25.90 min), 8 (t_R 29.12 min), 9 (t_R 31.33 min), 10 (t_R 32.20 min), and 12 (t_R 35.10 min) all possessed IC_{50} values of $<<< 1.25 \mu\text{g/mL}$ but still contained slight impurities. Fraction 6 was purified with Column A using an isocratic water/acetonitrile flow of 72:28 for 22 minutes, which yielded 1.2 mg (t_R 20.20 min; IC_{50} $30 \pm 2 \text{ nM}$) of **1** (cripowellin A). Fraction 8 was purified with Column B using a water/acetonitrile gradient from 45:55 to 35:65 over 20 minutes, which yielded 1.4 mg (t_R 11.14 min; IC_{50} $180 \pm 20 \text{ nM}$) of **2** (cripowellin B). Fraction 9 was purified using Column A with a water/acetonitrile gradient starting from 55:45 to 45:55 over 30 minutes, which yielded 0.4 mg (t_R 25.32 min; IC_{50} not active) of **5** (hippadine). Fraction 10 was purified using Column A with an isocratic water/acetonitrile flow of 58:42 for 15 minutes, which yielded 1.2 mg (t_R 11.94 min; IC_{50} $26 \pm 2 \text{ nM}$) of **3** (cripowellin C). Fraction 12 was purified using Column A with an isocratic water/acetonitrile flow of 52:48 for 12 minutes, which yielded 0.7 mg (t_R 9.57 min; IC_{50} $260 \pm 20 \text{ nM}$) of **4** (cripowellin D).

4.4. Antimalarial Bioassay

Each fraction and isolated compound was tested against the chloroquine/mefloquine-resistant Dd2 strain of *Plasmodium falciparum* in a 72-hour treatment using the malaria SYBR green I-based fluorescence assay as described previously.¹⁶⁻¹⁷

Artemisinin was used as the positive control with an IC_{50} of $6 \pm 1 \text{ nM}$.

4.5. Antiproliferative Bioassay

The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University, as previously reported.¹⁸⁻¹⁹ The A2780 cell line is a drug-sensitive ovarian cancer cell line.²⁰

4.6. Compound Information

Compound 1 (cripowellin A): $[\alpha]_D^{21} -28.5^\circ$ (c 5.5×10^{-6} , MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.19), 291 (2.32) nm; ECD (MeOH) $[\Delta\epsilon]_{302 \text{ nm}} +2.80$, $[\Delta\epsilon]_{282 \text{ nm}} -4.40$, $[\Delta\epsilon]_{249 \text{ nm}} +0.80$; HRESIMS $[2\text{M}+\text{Na}]^+ m/z$ 1097.3602 (calcd for $\text{C}_{50}\text{H}_{62}\text{N}_2\text{NaO}_{24}^+$ 1097.3585), $[\text{M}+\text{Na}]^+ m/z$ 560.1744 (calcd for $\text{C}_{25}\text{H}_{31}\text{NNaO}_{12}^+$ 560.1738), $[\text{M}+\text{H}]^+ m/z$ 538.1916 (calcd for $\text{C}_{25}\text{H}_{32}\text{NO}_{12}^+$ 538.1919), $[\text{Aglycone}+\text{H}]^+ m/z$ 320.1121 (calcd for $\text{C}_{16}\text{H}_{18}\text{NO}_6^+$ 320.1129). ^1H and ^{13}C NMR in CDCl_3 see Table 1.

Compound 2 (cripowellin B): $[\alpha]_D^{21} -51.8^\circ$ (c 1.1×10^{-5} , MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (3.09), 290 (2.49) nm; ECD (MeOH) $[\Delta\epsilon]_{302 \text{ nm}} +1.35$, $[\Delta\epsilon]_{281 \text{ nm}} -2.09$, $[\Delta\epsilon]_{248 \text{ nm}} +0.48$; HRESIMS $[2\text{M}+\text{Na}]^+ m/z$ 1069.4022 (calcd for $\text{C}_{50}\text{H}_{66}\text{N}_2\text{NaO}_{22}^+$ 1069.3999), $[\text{M}+\text{Na}]^+ m/z$ 546.1955 (calcd for $\text{C}_{25}\text{H}_{33}\text{NNaO}_{11}^+$ 546.1946), $[\text{M}+\text{H}]^+ m/z$ 524.2127 (calcd for $\text{C}_{25}\text{H}_{34}\text{NO}_{11}^+$ 524.2126), $[\text{Aglycone}+\text{H}]^+ m/z$ 320.1129 (calcd for $\text{C}_{16}\text{H}_{18}\text{NO}_6^+$ 320.1129). ^1H and ^{13}C NMR in CDCl_3 see Table 1.

Compound 3 (cripowellin C): $[\alpha]_D^{21} -41.1^\circ$ (c 5.9×10^{-6} , MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (3.26), 290 (2.44) nm; ECD (MeOH) $[\Delta\epsilon]_{306 \text{ nm}} +2.90$, $[\Delta\epsilon]_{270 \text{ nm}} -3.14$, $[\Delta\epsilon]_{251 \text{ nm}} +1.34$; HRESIMS $[2\text{M}+\text{Na}]^+ m/z$ 1065.3638 (calcd for $\text{C}_{50}\text{H}_{62}\text{N}_2\text{NaO}_{22}^+$ 1065.3686), $[\text{M}+\text{Na}]^+ m/z$ 544.1784 (calcd for $\text{C}_{25}\text{H}_{31}\text{NNaO}_{11}^+$ 544.1789), $[\text{M}+\text{H}]^+ m/z$ 522.1968 (calcd for $\text{C}_{25}\text{H}_{32}\text{NO}_{11}^+$ 522.1970), $[\text{Aglycone}+\text{H}]^+ m/z$ 320.1119 (calcd for $\text{C}_{16}\text{H}_{18}\text{NO}_6^+$ 320.1129). ^1H and ^{13}C NMR in CDCl_3 see Table 1.

Compound 4 (cripowellin D): $[\alpha]_D^{21} -87.1^\circ$ (c 2.9×10^{-6} , MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.43), 291 (2.50) nm; ECD (MeOH) $[\Delta\epsilon]_{305 \text{ nm}} +1.77$, $[\Delta\epsilon]_{273 \text{ nm}} -2.29$, $[\Delta\epsilon]_{250 \text{ nm}} +0.84$; HRESIMS $[2\text{M}+\text{Na}]^+ m/z$ 1037.4022 (calcd for $\text{C}_{50}\text{H}_{66}\text{N}_2\text{NaO}_{20}^+$ 1037.4101), $[\text{M}+\text{Na}]^+ m/z$ 530.1969 (calcd for $\text{C}_{25}\text{H}_{33}\text{NNaO}_{10}^+$ 530.1997), $[\text{M}+\text{H}]^+ m/z$ 508.2131 (calcd for $\text{C}_{25}\text{H}_{34}\text{NO}_{10}^+$ 508.2177), $[\text{Aglycone}+\text{H}]^+ m/z$ 320.1096 (calcd for $\text{C}_{16}\text{H}_{18}\text{NO}_6^+$ 320.1129). ^1H and ^{13}C NMR in CDCl_3 see Table 1.

Compound 5 (hippadine): HRESIMS $[\text{M}+\text{H}]^+ m/z$ 264.0659 (calc. for $\text{C}_{16}\text{H}_{10}\text{NO}_3^+$ 264.0655); ^1H NMR (500 MHz, CDCl_3) δ_{H} 8.05 (br d, $J = 3.6 \text{ Hz}$, 1H), 8.00 (s, 1H), 7.94 (br d, $J = 7.7 \text{ Hz}$, 1H), 7.76 (dd, $J = 7.6, 0.7 \text{ Hz}$, 1H), 7.68 (s, 1H), 7.49 (t, $J = 7.7 \text{ Hz}$, 1H), 6.91 (d, $J = 3.6 \text{ Hz}$, 1H), 6.17 (s, 2H); ^1H NMR (500 MHz, MeOD) δ_{H} 8.14 (br d, $J = 7.7 \text{ Hz}$, 1H), 8.04 (d, $J = 3.6 \text{ Hz}$, 1H), 7.92 (s, 1H), 7.87 (s, 1H), 7.82 (dd, $J = 7.6, 0.8 \text{ Hz}$, 1H), 7.53 (t, $J = 7.7 \text{ Hz}$, 1H), 7.02 (d, $J = 3.6 \text{ Hz}$, 1H), 6.21 (s, 2H).

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**New potentially bioactive alkaloids from
*Crinum erubescens***

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