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Antiplasmodial activity of synthetic ellipticine derivatives and an isolated analog

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ABSTRACT

Ellipticine has been shown previously to exhibit excellent in vitro antiplasmodial activity and in vivo antimalarial properties that are comparable to those of the control drug chloroquine in a mouse malaria model. Ellipticine derivatives and analogs exhibit antimalarial potential however only a few have been studied to date. Herein, ellipticine and a structural analog were isolated from *Aspidosperma vargasii* bark. A-ring brominated and nitrated ellipticine derivatives exhibit good in vitro inhibition of *Plasmodium falciparum* K1 and 3D7 strains. Several of the compounds were found not to be toxic to human fetal lung fibroblasts. 9-Nitroellipticine (IC_{50} = 0.55 μM) exhibits greater antiplasmodial activity than ellipticine. These results are further evidence of the antimalarial potential of ellipticine derivatives.

Despite progress made in recent years in its control, malaria continues to be the most important parasitic disease in tropical regions worldwide. Approximately 3.3 billion people, roughly half the world's population, live in regions where malaria is endemic and are thus at risk of infection. In 2010, approximately 220 million malaria infections were recorded worldwide leading to an estimated 666,000 deaths. Approximately 80% of all malaria deaths occur in Africa and most are among children less than 5 years old.[1] This death toll is related to the growing number of cases involving drug-resistant malaria parasites among other factors. The antimalarial drugs chloroquine and mefloquine (quinoline antimalarials) are synthetic mimics of the plant-derived natural product quinine. Today, chloroquine is the most used antimalarial drug worldwide and reports of chloroquine-resistance (CQR) in *Plasmodium falciparum* and *Plasmodium vivax* (together responsible for the absolute majority of all malaria infections throughout the world) are widespread. Artemisinin is a plant-derived antimalarial natural product. Its derivatives are prepared on an industrial scale from isolated artemisinin through semi-synthesis.

ACTs (artemisinin-based combined therapies) have been recommended by WHO (World Health Organization) since 2005 for the treatment of CQR malaria. ACTs comprise a synthetic quinoline antimalarial and an artemisinin derivative and highlight the importance of traditionally used antimalarial plants as sources of chemical structural classes for contemporary malaria therapy. While ACTs are clinically effective, the history of antimalarial drug treatment leads to concern that it is only a matter of time before parasites resistant to ACT therapy appear. In fact, in Cambodia, the first evidence for the resistance of malaria parasites to ACTs has been confirmed. No new class of antimalarials has been introduced into clinical practice for more than two decades. The search for new drug leads is important for the future of malaria treatment.[2] Plants are not only the origin of the antimalarials used in current clinical practice, but continue to be rich sources of natural product drug leads for other tropical diseases.[3–5]

Ellipticine (**1**), or 5,11-dimethyl-6*H*-pyrido[4,3,*b*]carbazole, is an indole alkaloid whose derivatives have important anticancer properties (Fig. 1).[6–10] Compound **1** can be isolated from the

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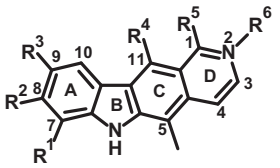


	R^1	R^2	R^3	R^4	R^5	R^6	
1	H	H	H	Me	H		
2	H	H	H	H	Me		
3	H	H	OH	Me	H		
4	H	H	OMe	Me	H		
5	H	H	H	Me	H		3,4 -dihydro
6	H	H	Br	Me	H		
7	Br	H	Br	Me	H		
8	Br	Br	Br	Me	H		
9	H	H	NO_2	Me	H		
10	NO_2	H	H	Me	H		
11	H	H	H	Me	H	Me	1,2,3,4-tetrahydro

Figure 1. Ellipticine (**1**), olivacine (**2**), ellipticine derivatives **3**–**5**[15] and **6**–**10** (synthesized herein) and isolated alkaloid **11**.

alkaline EtOH extracts of the bark of the Amazonian carapanaúba tree *Aspidosperma vargasii*[11,12] and this compound exhibits significant in vitro inhibitory activity (IC_{50} = 0.35–0.81 μM) against *Plasmodium falciparum* (Table 1).[11,13,14] Recently, significant in vivo antimalarial activity of 1 against *Plasmodium berghei* in mice has been demonstrated in the Peters 4 day suppressive test. At daily doses of 10 mg/kg/day, ellipticine suppresses parasitemia versus controls by 70–77%. At oral doses of 50 mg/kg/day, **1** exhibits total elimination of parasitemia 5–7 days after initial infection and mean survival times (MST, >40 days) that are comparable to the commercial drug standard chloroquine.[14] In that same report, the significant in vitro (Table 1) and in vivo antimalarial activity of olivacine (**2**), a structural isomer of **1** exhibiting analogous ring structure, is also demonstrated. Oral doses of 10 and 50 mg/kg/day of **2** suppress *P. berghei* by 48–68% and 90–91%, respectively, 5–7 days after infection.[14] Both **1** and **2** exhibit low toxicity to murine macrophages, significant selectivity indices (SI = 290 to >1200) and greater in vivo antimalarial activity than the much studied indole alkaloid cryptolepine and a synthetic analog of cryptolepine. Also, **1** and **2** exhibit none of the acute toxicity associated with cryptolepine and its derivatives.[14]

Recently, ellipticine derivatives **3**–**5** (Fig. 1) exhibiting in vitro activity against *P. falciparum* were described among the most active compounds in a large compound library screening.[15] In that work, significant antiplasmodial activity was described for **1** (IC_{50} = 1.13 μM), 1-HCl (IC_{50} = 0.60 μM), 9-hydroxyellipticine (**3**, IC_{50} = 0.30 μM), 9-methoxyellipticine (**4**, IC_{50} = 1.15 μM) and 3,4-dihydroellipticine (**5**, IC_{50} = 1.01 μM). To our knowledge, these are the only ellipticine derivatives whose antiplasmodial activity has been described to date.

Experience with the medicinal chemistry of synthetic quinoline alkaloids and semi-synthetic derivatives of artemisinin has shown that small structural changes can lead to improved antimalarial activity and better pharmacological properties.[16] A number of derivatives of **1** have been prepared previously.[17–22] Among the commonly prepared derivatives, the most frequently desired modifications have been hydroxylation at C-7 and C-9 positions, alkylation of the N-atoms and C-1, and substitution at the C-9 position.[22] The aim herein was to study the antiplasmodial activity of novel and heretofore unexplored ellipticine derivatives and a natural, isolated analog of ellipticine.

Despite several published and patented syntheses of ellipticine (**1**)[23] it is available from manufacturers only in tens of milligram quantities and is costly. In the present work, the natural compounds **1** and 2-methyl-1,2,3,4-tetrahydroellipticine (**11**) (Fig. 1) were isolated from *Aspidosperma vargasii* bark collected at the Ducke Forest Reserve in Manaus, Amazonas State, Brazil by adapting from previously published procedures.[11,12,24–27] Briefly, isolation involved initial extraction of powdered bark with 1% NH_4OH in EtOH, followed by evaporation of solvents and dissolution of the alkaloid-rich residue in EtOAc. Extraction of the EtOAc solution with 0.1 N HCl, basification of the aqueous phase to pH 8 with $NaHCO_3$ and extraction with $CHCl_3$ yielded $CHCl_3$ extracts that were repeatedly chromatographed on silica gel to yield **1** and **11** that were identified based on their NMR and MS data[28,29] and comparison with literature data.[12] The antiplasmodial activity (IC_{50} = 4–13 μM against *P. falciparum* K1 & 3D7)[30] and cytotoxicity to human fetal lung fibroblasts (IC_{50} >50 μg/mL)[31] of alkaloid **11** were evaluated herein (Table 1).

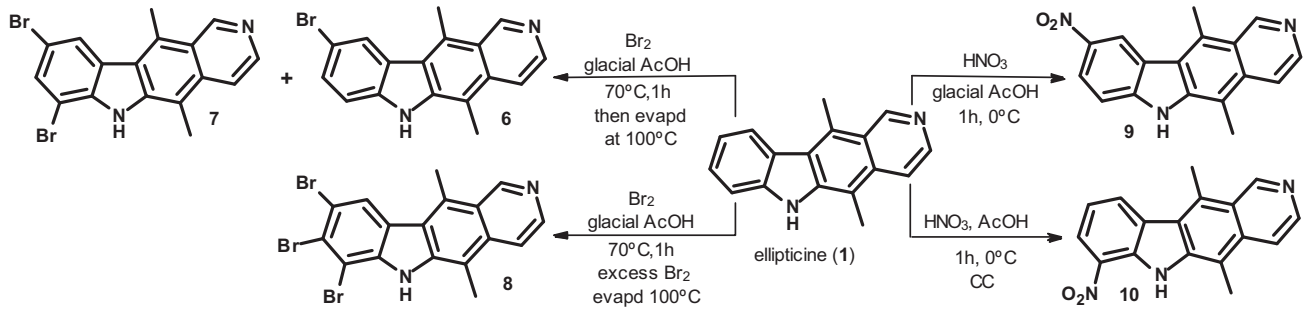
Reactions using ellipticine (**1**) as substrate were carried out on several milligram scales (Scheme 1). Treating ellipticine in glacial AcOH with excess Br_2 at 70 °C leads to the formation of a ca. 3:1 mixture of the novel 7,9-dibromoellipticine (**7**) and the known 9-bromoellipticine (**6**) based on UPLC–MS, HRMS and NMR analysis. This mixture is inseparable by standard chromatographic techniques.[32] Interestingly, it exhibits good inhibition of both *P. falciparum* K1 and 3D7 strains (IC_{50} = 0.2–0.3 μg/mL). Attempts to fully convert compound **1** to dibromo product **7** by first reacting as described above and then adding a second portion of Br_2 and heating at 100 °C yield an unstable product tentatively assigned the structure 7,8,9-tribromoellipticine (**8**) based on LC-HRMS and NMR spectra.[33] **8** could not be tested for antiplasmodial activity.

Ellipticine (**1**) dissolved in AcOH and then treated with 65% HNO_3 at 0 °C leads to a mixture of mononitration products (presumably, 6-nitro, 7-nitro and 9-nitroellipticines) and unreacted **1** according to LC–MS analysis. After flash chromatography, the novel

Table 1
In vitro inhibition of *Plasmodium falciparum* (*Pf*) strains and cytotoxicity of ellipticine derivatives and structural analogs

Compound	Classification of antiplasmodial activity[a]	*Pf*, IC_{50} (μg/mL)		*Pf*, IC_{50} (μM)		Fibroblasts, IC_{50} (μg/mL)
		K1	3D7	K1	3D7	
1	Active	0.19	0.085	0.81[b]	0.35[b]	nt[c]
2	Active	0.35	0.29	1.4[b]	1.2[b]	nt[c]
6 + **7** (ca. 1:3)	Active	0.30	0.20	—	—	>50
9-2AcOH	Active	0.20	nt	0.55	nt	nt
10	Active	3.9	4.9	13	17	>50
11	Active	1.1	3.5	4.2	13	>50
Chloroquine diphosphate	Active/Very Active	0.17	0.061	0.33	0.11	nt
Quinine sulfate	Very Active	0.040	0.052	0.12	0.15	nt

nt–not tested, — = not applicable.
[a] IC_{50} <0.1 μg/mL = very active, IC_{50} = 0.1–5 μg/mL = active, IC_{50} = 5–10 μg/mL = moderate activity, IC_{50} >10 μg/mL = inactive.[36]
[b] Data reported previously.[14]
[c] IC_{50} >1.4 × 10^2 μM against murine macrophages.[14]



Scheme 1. Synthesis of brominated and nitrated derivatives of ellipticine.

compound 7-nitroellipticine (**10**) is obtained (Scheme 1).[34] This compound exhibits the lowest antiplasmodial activity of all those tested herein (IC_{50} = 13–17 μM against *P. falciparum* K1 & 3D7). If glacial AcOH is used in the above procedure, the known 9-nitroellipticine (**9**)[20] is obtained as the sole product (no chromatography required)[35] and exhibits the highest antiplasmodial activity of all the compounds tested.

The nitration and bromination performed herein occurred by electrophilic aromatic substitution predominantly at the C-7 and/or C-9 positions of 1 due to *ortho*/*para* directed activation by the indole N-atom. Deactivation of the pyridine (D) ring to electrophilic addition, due to the electronegative sp^2 N-atom, is also presumed to be an important factor in the definition of the regiochemical outcome of these reactions.

The in vitro inhibition observed herein for 9-nitroellipticine (**9**) against the K1 strain of *P. falciparum* is greater than that of ellipticine and taken together with the result of the previous report presented in the discussion, is further evidence that A-ring modification of **1** especially in the 9-position, may be associated with improved antiplasmodial activity. More derivatives should be prepared in the future using alternative synthetic approaches that do not depend solely upon direct preparation from **1**.

In conclusion, **1** exhibits promising in vivo activity against *Plasmodium berghei* that is comparable to that of chloroquine, though at doses ca. 5× those of chloroquine. Like quinine and artemisinin, ellipticine is a potent antimalarial natural product that is isolated from a traditionally used antimalarial plant. The nitrated and brominated ellipticine derivatives prepared herein exhibit good in vitro antiplasmodial activity. Several of the compounds studied are not toxic to human fetal lung fibroblasts (MRC-5). The present cost of commercial ellipticine and its availability impose limits on the approach adopted herein (direct synthesis of derivatives from **1**). Total synthesis of **1** and its derivatives using alternative methods are beyond the scope of this work. No in vivo study on the antimalarial activity of a derivative of **1** has been reported though olivacine (**2**) exhibits in vivo activity comparable to **1**.[14] The derivatives of ellipticine prepared herein and others should be synthesized on a larger scale in future work to investigate SAR and in vivo antimalarial activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.070. These data include MOL files and InChiKeys of the most important compounds described in this article.

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28. *Ellipticine* (***1***)*:* yellow needles (56 mg), mp 311–313 °C (lit.[12]) ^{1}H NMR (250 MHz, CD_3OD): δ 2.7 (*s*, H-13) 3.2 (other CH_3 overlapped with solvent impurity peak), 7.2 (*ddd*, *J* = 7.0, 6.8 and 1.5, H-9), 7.4 (*m*, H-8 and H-7), 7.9 (*d*, *J* = 7.0 Hz, H-4), 8.3 (d, *J* = 7.0 Hz, H-10 and H-3), 9.5 (s, H-1). By UPLC–MS, peak at t_R 4.15 min exhibits adduct ion $[M+H]^+$ *m*/*z* 247 HRMS $[M+H]^+$: *m*/*z* 247.1222, for $C_{17}H_{14}N_2$ calcd. $[M+H]^+$ *m*/*z* 247.1229 (Δ = 3 ppm).

29. *2-Methyl-1,2,3,4-tetrahydroellipticine* (***11***)*:* white crystals (59 mg), 218–223 °C (lit.[12]). ^{1}H NMR (400 MHz, CD_3OD): δ 2.4 (*s*, H-13), 2.5 (*s*, H-2′), 2.6 (*s*, H-12), 2.8 (*t*, *J* = 6 Hz , H-3), 3.0 (*t*, *J* = 6 Hz, H-4), 3.7 (*s*, H-1), 7.1 (*t*, *J* = 8 Hz, H-9), 7.3 (*t*, *J* = 8 Hz, H-8), 7.4 (*d*, *J* = 8 Hz , H-7), 8.1 (*d*, *J* = 8 Hz, H-10). ^{13}C NMR (100 MHz, CD_3OD): δ 11.3 (CH_3), 14.0 (CH_3), 27.0 (CH_2), 44.8 (CH_3), 52.2 (CH_2), 56.1 (CH_2), 110.1 (CH), 114.5 (C), 118.0 (CH), 119.7 (C), 121.8 (C), 122.0 (CH), 123.9 (C), 124.1 (CH), 125.8 (C), 128.4 (C), 138.4 (C), 140.6 (C). Adduct ion $[M+H]^+$ at *m*/*z* 265, t_R 3.53 min., HRMS [M+H]+: *m*/*z* 265.1694, for $C_{18}H_{21}N_2$ calcd $[M+H]^+$ *m*/*z* 265.1705 (Δ = 4 ppm).

30. *In vitro culture of Plasmodium falciparum and assay:* The antimalarial drug-susceptible 3D7 clone of the NF54 isolate (unknown origin, MRA-102, MR4-ATCC Manassas, Virginia, USA) and the multi-drug resistant K1 (Thailand, MRA-159, MR4-ATCC) strains of *P. falciparum* were maintained in continuous culture using the Trager and Jensen method.[37] For the microtest, an initial parasitemia of 1–2% and hematocrit of 3% were used. Substances were diluted in DMSO to a stock concentration of 5 mg/mL. These stock solutions were each diluted in complete culture medium to obtain sample solutions for tests in the range 100–0.006 μg/mL. The test was performed as described previously.[11] Briefly, the diluted test samples were plated in wells containing parasitized red blood cells. Each diluted test sample was tested in triplicate. The test plate was incubated for 48 h at 37 °C. After incubation, the contents of the wells were evaluated by optical microscopy. The inhibition of the growth of parasites (IGP%) was evaluated as a percentage by comparison with controls (which did not receive sample): IGP% = 100 × [1−(parasitemia with sample/parasitemia of controls)].

31. *Cytotoxicity test using the Alamar Blue™ assay:* Human fetal lung fibroblasts (MRC-5) were grown in DMEN medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 μg/mL streptomycin and 100 U/mL penicillin, and incubated at 37 °C with 5% CO_2 atmosphere. For experiments the cells were plated in 96-well plates (10^4 cells per well) and the Alamar Blue™ assay[38] was performed as follows. After 24 h, the compounds were dissolved in DMSO (50 μg/mL) and added to each well and the cells were incubated for 48 h. Doxorubicin was used as positive control. Negative controls received 0.1% DMSO. 2 h before the end of the incubation, 10 μL of Alamar Blue™ were added to each well. The fluorescent signal was monitored with a multiplate reader using a 530–560 nm excitation wavelength and 590 nm emission wavelength.

32. *Synthesis of 9-bromoelliptcine* (***6***) *and 7,9-dibromoellipticine* (***7***)*:* **1** (3.2 mg, 13 μmol) was dissolved in glacial AcOH (60 μL, 43.1 mg). The resulting solution was placed in an ice bath. A second solution was prepared by addition of glacial AcOH (90 μL, 65 mg) to Br_2 (0.25 μL, 0.5 μmol) and chilled. Upon mixing of these two chilled solutions, a red brick-colored precipitate formed. The reaction mixture was heated in an H_2O bath at 70 °C for 1 h. The reaction was monitored by TLC using $CHCl_3$/MeOH 9:1 as eluents and UV 254 and 366 nm, I_2 chamber and *p*-anisaldehyde/heat for development of the plates. After 1 h of reaction, the solution was transferred to a watch glass and heated over a steam bath to total dryness to yield the crude product mixture (6.9 mg). CC on silica gel led to the isolation of an analytical mixture of **6** and **7** by ^{1}H NMR, UPLC–MS and HRMS analyses. *9-Bromoellipticine* (**6**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.3 (*s*, H-6), 10.1 (*s*, H-1), 8.61 (*d*, *J* = 1.8 Hz, H-10), 8.50 (overlapping peaks, H-3 and H-4), 7.82 (*dd*, *J* = 1.8, 8.6 Hz, H-8), 7.66 (*d*, *J* = 8.6 Hz, H-7), 3.17 (*s*, H-12), 2.89 (*s*, H-13). UPLC–MS exhibited a chromatographic peak at 8.63 min that had MS ions at *m*/*z* 325 and 327 $[M]^+$. HRMS $[M+H]^+$: *m*/*z* 325.0343 and 327.0443, for $C_{17}H_{13}N_2Br$ calcd $[M+H]^+$ *m*/*z* 325.0340 and 327.0321 (Δ = 0.9–37 ppm). *7,9-Dibromoellipticine* (**7**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.3 (*s*, H-6), 10.1 (*s*, H-1), 8.61 (*d*, *J* = 1.6 Hz, H-10), 8.54 (*d*, *J* = 7 Hz, H-3), 8.52, (*d*, *J* = 7 Hz, H-4), 8.10 (*d*, *J* = 1.6 Hz, H-8), 3.0 (*s*, H-12). UPLC–MS exhibited chromatographic peaks at t_R 10.25 and MS ions at *m*/*z* 403, 405 and 407 $[M+H]^+$. HRMS $[M+H]^+$: *m*/*z* 402.9480, 404.9448 and 406.9441, for $C_{17}H_{12}N_2Br_2$ calcd $[M+H]^+$ *m*/*z* 402.9445, 404.9426, 406.9408 (Δ = 5–9 ppm).

33. *Synthesis of 7,8,9-tribromoellipticine* (***8***)*:* Initially, the same procedure was used that resulted in the preparation of the above mixture of 7,9-dibromoellipticine (**7**) and 9-bromoellipticine (**6**). After 1 h, the reaction mixture was transferred to a watch glass, excess Br_2 was added and the mixture was heated over a boiling water bath until the reaction solvents had evaporated. The mixture was suspended in distilled water and extracted with $CHCl_3$/MeOH (9:1). The organic phase was dried over Na_2SO_4 and evaporated. The structure of product **8** was elucidated based on LC-HRMS, ^{1}H and ^{13}C NMR, DEPT135 and DEPT90 spectra. This compound was unstable and decomposed in DMSO-d_6 in 1 week. *7,8,9-Tribromoellipticine* (**8**): ^{1}H NMR (300 MHz, DMSO-d_6), δ 11.53 (H-6), 10.01 (H-1), 8.55–8.35 (H-3, H-4, H-10), 3.19 (CH_3), 2.90 (CH_3). ^{13}C NMR/DEPT 135 & 90 (75 MHz, DMSO-d_6) δ 144.84 (CH), 143.98 (C), 141.29 (C), 134.86 (C), 134.62 (C), 128.76 (CH), 126.65 (CH), 126.24 (C), 124.43 (C), 123.48 (C), 120.53 (C), 120.25 (CH), 115.42 (C), 112.39 (C), 107.44 (C), 15.07 (CH_3), 12.48 (CH_3). HRMS $[M+H]^+$: *m*/*z* 480.8500 (rel. int. 1), 482.8489 (rel. int. 3), 484.8467 (rel. int. 3), 486.8451 (rel. int. 1), for $C_{17}H_{11}N_2Br_3$ calcd. $[M+H]^+$ *m*/*z* 480.8551, 482.8530, 484.8510, 486.8489 (avg. Δ = 0.8 ppm).

34. *Synthesis of 7-nitroellipticine* (***10***)*:* **1** (4.5 mg, 18.2 μmol) was dissolved in AcOH (2 mL) with magnetic stirring and then chilled in an ice bath. Chilled 65% HNO_3 (0.9 mL) was added and the resulting mixture was stirred magnetically for 1 h then the solution was made pH 10 by addition of $NaCO_3$ and extracted with $CHCl_3$/MeOH 9:1 (3 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and then evaporated to dryness on a rotary evaporator to obtain a crude product mixture (39.5 mg). The mixture was separated by flash CC using a gradient of *i*-PrOH in $CHCl_3$ (0.2:9.8–0.5:9.5). Analytical normal-phase TLC was performed with $CHCl_3$/MeOH (9:1) as eluents to yield mono-nitro product **7** (R_F 0.77, yield: 1.0 mg (30%), based on recovered **1**). *7-Nitroellipticine* (**10**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.25 (*s*, H-6), 9.83 (*s*, H-1), 8.86 (*d*, *J* = 8.0 Hz, H-8), 8.55 (*d*, *J* = 6.0 Hz, H-4), 8.43 (*d*, *J* = 8.0 Hz, H-10), 8.00 (*d*, *J* = 6.0 Hz, H-3), 7.50 (*t*, *J* = 8.0 Hz, H-9), 2.95 (*s*, H-13). Ion adduct at *m*/*z* 292, HRMS $[M+H]^+$: *m*/*z* 292.1077, for $C_{17}H_{13}N_3O_2$ calcd. $[M+H]^+$ *m*/*z* 292.1086 (Δ = 3 ppm).

35. *Synthesis of 9-nitroellipticine 2AcOH* (***9***-*2AcOH*)*:* AcOH was previously fractionally distilled and dried over molecular sieves. **1** (2.0 mg) was dissolved in AcOH (ca. 0.75 mL) with stirring. Chilled 65% HNO_3 (3 drops) was added to the stirred solution of **1** in an ice bath and the resulting mixture was stirred for 1 h. Then, distilled H_2O was added and the mixture was basified to pH 11 with Na_2CO_3. The solution was extracted with $CHCl_3$/MeOH 9:1 (3 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and then evaporated to dryness on a rotary evaporator. The crude product was characterized by LC-HRMS and ^{1}H NMR and found to be the 2 AcOH salt of 9-nitroellipticine (**9**). *9-nitroellipticine* 2AcOH (**9**-2AcOH): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.72 (br s, H-1), 9.06 (*d*, *J* = 2.4 Hz, H-10), 8.47 (br s, H-3), 8.38 (*dd*, J_1 = 2.4 Hz, J_2 = 9.0 Hz, H-8), 7.93 (*d*, *J* = 6.0 Hz, H-4), 7.66 (*d*, *J* = 9.0 Hz, H-7), 3.25 (*s*, H-12), 2.80 (*s*, H-13), 1.65 (s, $2CH_3CO_2H$). HRMS $[M+H]^+$: *m*/*z* 292.1076, for $C_{17}H_{13}N_3O_2$ calcd $[M+H]^+$ *m*/*z* 292.1086 (Δ = 3 ppm).

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