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RESEARCH ARTICLE

Indole alkaloid ellipticine as efficient multitarget compound

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Abstract: First isolated from the tropical plant *Oschrosia elliptica*, indole alkaloid ellipticine provoked huge interest since it demonstrated antitumor activity was demonstrated along with limited toxic side effects and a complete lack of hematological toxicity. In this work, a five-step Cranwell and Saxton synthesis was used for obtaining ellipticine (Ell). Ellipticine hydrochloride salt (Ell×HCl) was also synthesized. Detailed in vitro studies of anticancer, antimalarial, and leishmanicidal activities were performed. Antiproliferation assay using DU145 cancer cell line treated with Ell showed a consistent reduction in cell proliferation and cell viability starting from 5 μM Ell concentrations. Anti-proliferation activity was more pronounced for the Ell×HCl solutions. Both the Ell and Ell×HCl revealed moderate activity in vitro against *Leishmania amazonensis* promastigotes, which is related to insufficient solubility of the drugs. IC_{50} values of Ell and Ell×HCl were determined in vitro against multidrug resistant *Plasmodium falciparum* strain K1. The Ell×HCl was shown to be almost three times more potent than the Ell in DMSO. Upon dilution with water, Ell solubility and activity drops down, while the activity and solubility of Ell×HCl is enhanced up to 10 times in 50:50 aqueous DMSO solutions.

Keywords: ellipticine; *in vitro*; anticancer; antimalarial; antileishmanial activity.

Introduction

Recently, indole alkaloid ellipticine and its derivatives have been thoroughly studied as therapeutics against different forms of cancer and as potent antimalarials [1-2]. Ellipticine (Ell) is one of the simplest naturally occurring alkaloids with a planar structure, which has been the center of attention of many research groups owing to its antitumor activity. The alkaloid was first isolated from leaves of the tropical plant *Oschrosia elliptica* (Apocynaceae) by Goodwin *et al.* [3].

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Subsequently, Ell was isolated from several other plants *of Ochrosia* genus like *O. vieillardii*, *O. acuminate*, and *O. moorei*, as well as from *Strychnos dinkagei* (Loganiaceae). Currently most commercially available ellipticine is derived from plants.

Ellipticine exhibits rather limited toxic side effects and a complete lack of hematological toxicity [4-6]. Different hypotheses on ellipticine action mechanisms have been proposed. In the last decade, evidence of different cell-cycle effects of Ell has come to light [7]. Particularly, the alkaloid is capable of interacting with p53 tumor suppressor protein, Akt- and c-Kit kinases, while its effect on other cellular proteins is still under investigation. Thus, ellipticine exhibits a multimodal cytotoxic activity that is not specified [7]. A bio-oxidation pathway was originally proposed [8] with ellipticine as a possible substrate for peroxidases *in vivo*. Stiborová's group demonstrated that ellipticine covalently binds to DNA after being enzymatically

activated with cytochromes P450 or peroxidases [9]. Pharmacological properties, such as strong DNA topoisomerase II inhibition activity, and the formation of covalent DNA adducts mediated by ellipticine oxidation could also explain the antimalarial activity of ellipticine derivatives [10].

Malaria is one of the oldest infectious parasitic diseases in the world that remains a serious public health problem worldwide [11]. The disease is caused by protozoan parasites of the genus *Plasmodium*. Six species that mainly infect humans are *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* (*P. ovale curtisi*, *P. ovale wallikeri*), *P. knowlesi*, and *P. cynomolgi* [12]. Active substances of plant origin contribute to malaria chemotherapy, either directly as antimalarials or as prototypes of synthetic drugs such as chloroquine, primaquine, mefloquine, *etc.* [13].

Traditional Amazonian medicine uses the bark of *Aspidosperma vargasii* (Apocynaceae) tree as an antimalarial remedy [14]. Alkaline ethanol extract of the bark contains ellipticine that showed a high *in vitro* activity against *P. falciparum* [15-17]. The antimalarial activity *in vitro* was further confirmed by two independent studies, as shown in Table 1. However, the IC_{50} values reported in the literature often show significant differences. This fact is due to the use of different parasite strains that have different responses to substances under investigation, which are often associated with genetic mutations that alter the response to the drug.

To the best of our knowledge, the experimental data about the leishmanicidal activity of ellipticine are practically absent in the literature. Ogungbe *et al.* [18] studied the Ell interaction with *Leishmania* proteins by molecular docking, but no obvious activity was reported. Other research was devoted to *ex vivo* splenic explant system screening for anti-leishmanial activity [19], where ellipticine was classified as an antiprotozoal compound.

A variety of positive ellipticine functions prompted a need to develop a reliable scalable synthetic pathway to produce Ell and its analogues [7]. Up to date, a very high ellipticine price of about $10/mg (*cf.* Merck, Santa Cruz Biotec, Calbiochem) is a critical point that has limited considerably the use of the compound.

Taking into account the huge interest in ellipticine, various synthetic routes have been developed, starting from R. B. Woodward's approach [20]. Generally, synthetic strategies leading to the target molecule are classified according to assembly of the last ring as B, C, D, and B + C types (Passemar *et al.*, 2011) [10] (see Figure 1). Among the different synthetic routes available [21-29], the method originally proposed by Cranwell and Saxton appears to be the most practical one [21, 30]. It consists of a five-stage ''D-type'' procedure starting from appropriately substituted indoles or carbazoles.

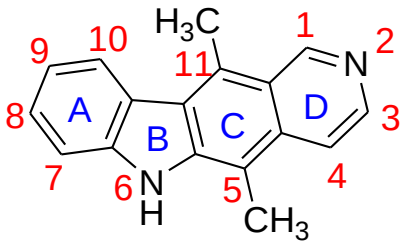

Figure 1. The structural formula of ellipticine with the IUPAC numbering of atoms.

Table 1. Antimalarial activity of ellipticine derivatives against *P.falciparum.*

Compound	strain FcM29-Cameroon [10]		strain K1 [17]		strain 3D7 [17]	
	μg/mL	μmol/mL	μg/mL	μmol/mL	μg/mL	μmol/mL
Ellipticine	0.28	1.13	0.19	0.81	0.085	0.35
Ellipticine×HCl	0.17	0.6				
3,4-Dihydroellipticine	0.25	1.01				
9-Hydroxyellipticine [a]	0.08	0.3				
9-Methoxyellipticine	0.32	1.15				
7,9-Dibromoellipticine : 9-Bromoellipticine 3:1[a]			0.30		0.20	
9-Nitroellipticine[a]			0.20	0.55		
Chloroquine		0.4	0.17	0.33	0.061	0.11

[a] Semisynthetic, obtained by modification of natural compounds.

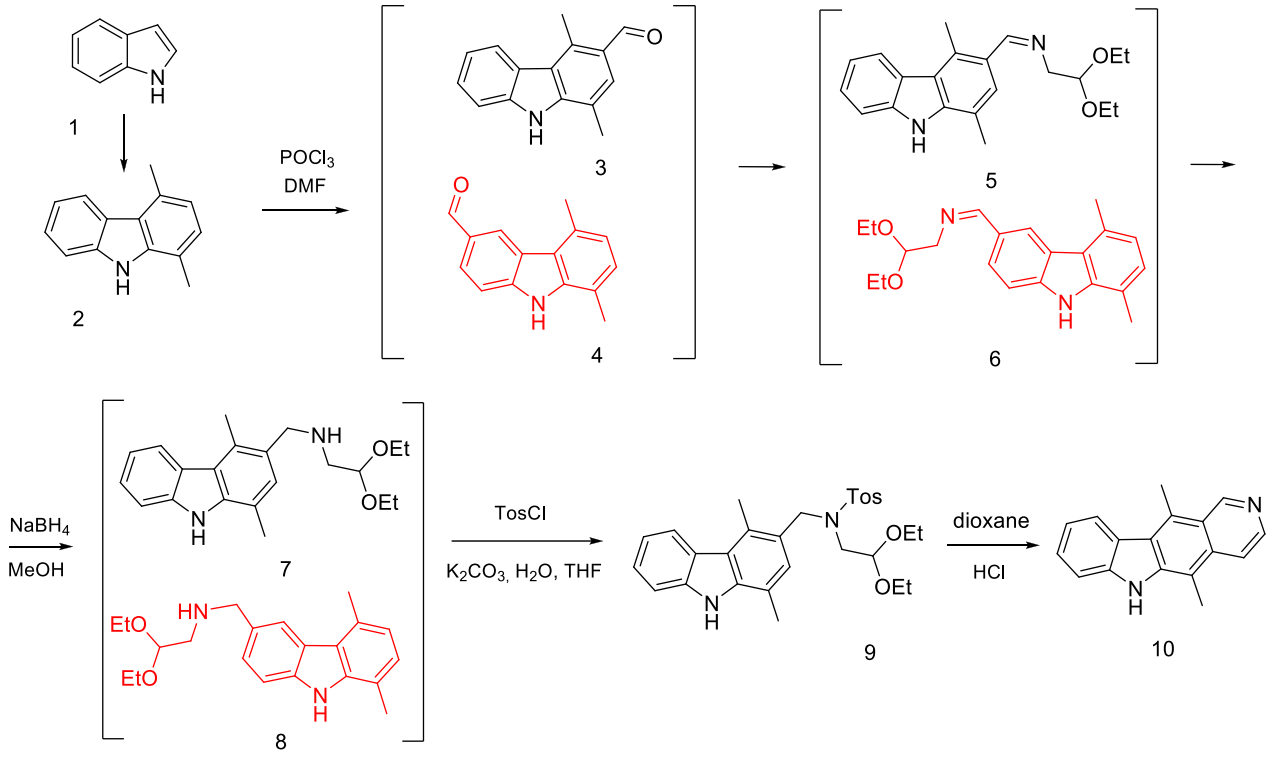

Figure 2. Synthesis of ellipticine **10** (admixture products are shown in red color).

Since then, various improvements have been proposed to make it more convenient and to increase the total yield. However, there are still some problematic synthetic steps, *e.g.* the formylation and the cyclization reactions (see Figure 2). In the former case, the difficulty stems from the fact that carbazole has three sites for an electrophilic attack, and thus a mixture of products is formed [31-32]. At the cyclization step (see Figure 2), a mixture of *N*-tosyl-1,2-dihydroellipticine and ellipticine is obtained. That mixture is difficult to separate leading to a significant decrease in the ellipticine yield.

In the present paper, we propose a significantly improved Cranwell and Saxton's procedure and performed different biological activity assays *in vitro*. Ell and Ell×HCl were used to investigate their activity against human prostate cancer DU145 cells, multidrug-resistant K1 strain of *P. falciparum*, and *Leishmania amazonensis* promastigo-tes. We hypothesize that the more water-soluble ellipticine hydrochloride would exhibit better activity compared to the more hydrophobic ellipticine.

Results and discussion

The most convenient synthetic pathway to ellipticine (5,11-dimethyl-6H-pyrido[4,3-b]carbazole, **10**) was proposed by Cranwell and Saxton [21] with later modifications [30-44] (see Figure 2). We supposed that it might be possible to further improve the overall procedure by improving the synthesis of 1,4-di-methylcarbazole **2**, the Vilsmeier-Haack formylation, and the cyclization steps. In the first case, all previously described procedures required purification of dimethylcarbazole **2** by chromatography and the use of ethanol as a solvent. We performed the reaction in toluene which has a higher boiling point and it gives the possibility of removing the evolving water with a Dean-Stark apparatus. That change led to an essential increase in yield (70%) and allowed us to substitute a chromatographic purification of the product with a much more practical crystallization one. Predominantly, the subsequent Vilsmeier-Haack formylation of 1,4-dimethylcarbazole was carried out using *N*-methylformanilide and phosphorus oxychloride in *o*-di-chlorobenzene. For that step, we adopted the procedure proposed by Deane *et al.* involving $POCl_3$ and DMF in chlorobenzene [30]. It should be noted that formylation of **2** proceeded non-regioselectively affording a mixture of isomers: 3-formyl-1,4-dimethylcarbazole **3** as a major product (about 9:1) and 6-formyl-1,4-dimethylcarbazole **4** [30]. To improve the yield, the workup of the reaction mixture after neutralization and evaporation of the volatile products was modified by performing three extractions with hot ethyl acetate. The mixture of **3** and **4** were used for further transformations without separation. The two following steps were carried out according to the literature [21, 30-31, 35-36]. Subsequently, the mixture of **7** and **8** was tosylated with *p*-toluenesulfonyl chloride at the exocyclic amino group to give *N*-tosyl-3-(2,2-diethoxy-ethylaminomethyl)-1,4-dimethylcarbazole **9**. Usually, the tosylation reaction is carried out in pyridine at room temperature for 3 days [30-

31, 35, 41]. We applied a more rapid procedure in aqueous tetrahydrofuran [43], but used potassium carbonate as a base; the reaction was complete at room temperature overnight. Pure compound **9** was separated by recrystallization.

The last step, the cyclization of *N*-tosyl-3-(2,2-diethoxyethylaminomethyl)-1,4-dimethylcarbazole **9** into ellipticine was carried out using a standard procedure by heating a dioxane solution with aqueous HCl. Here, the major problems of the reaction mixture workup arose from poor solubility of ellipticine and its contamination with an intermediate *N*-tosyl-3,4-dihydroellipticine. The mixture is usually separated by chromatography. According to the literature, a mixture of dichloromethane : methanol 9:1 is the most frequently used eluent [30]. A complete separation of the two products was achieved using consecutive flash-chromatography treatments with two different eluents: ethyl acetate and methanol. First, the *N*-tosyl-3,4-dihydro-ellipticine was eluted with ethyl acetate, then ellipticine **10** was eluted with methanol as a single pure product.

Antiproliferation activity of Ell and Ell×HCl against the DU145 cells was assessed spectrophotometrically by a MTT assay. The percentage of inhibition was determined by comparing the absorbance values of drug-treated cells with that of untreated controls. Experimental data and percentages of the cell viability are shown in Figure 3.

As shown in the figure, a decrease in cell viability with an increase in the drug concentration was observed. Such a tendency is more pronounced for Ell×HCl owing to its better solubility and hence, bioavailability. Perceptible drug activity was recorded at all concentrations except 0.625 μM. 5.0 μM was the first concentration of Ell that provoked a considerable reduction in the cancer cell viability (~62%). The viable DU145 count of only ~6% was registered for treatment with 20.0 μM of Ell. Antiproliferation activity is more pronounced for the Ell×HCl treatment. Thus, about 50% of viable cells were observed after treatment with 5.0 μM of the drug. Moreover, only ~3-5% of the viable DU145 cells were observed at 10.0 μM and 20.0 μM.

The results of antimalarial activity *in vitro* for ellipticine and ellipticine hydrochloride against *P. falciparum* strain K1 are shown in Table 2. The data demonstrate a marked advantage of the Ell×HCl over Ell. In DMSO, the Ell×HCl was almost three times more potent than Ell (*cf.* IC_{50} = 0.7 μmol/mL and 2.3 μmol/mL, respectively) and was of comparable potency to chloroquine which was used as a positive control (IC_{50} = 0.4 μmol/mL). In 50:50 aqueous DMSO solution, the ellipticine hydrochloride was more efficient than Ell by an order of magnitude (*cf* IC_{50} = 0.5 μmol/mL and 5.0 μmol/mL, respectively). Further dilution (DMSO:H_2O = 25:75) resulted in a decrease in the Ell×HCl activity. At that dilution, the ellipticine-base became insoluble and hence inactive so the measurement was not performed. Therefore, the 50:50 aqueous DMSO was found to be the best solvent for the ellipticine hydrochloride.

Leishmanicidal activity of ellipticine and ellipticine hydrochloride against *L. amazonensis* promastigotes was assessed. Ell×HCl noticeably inhibited the promastigote growth, leaving only 26.7% of viable cells after 48 h treatment at the concentration of 250 μg/mL, when compared to the negative control (Figure 4). The IC_{50} value was found to be 11.02 μg/mL.

The leishmanicidal activity found for both Ell and Ell×HCl was rather low when compared to commercial drug pentamidine isethionate (Pentacarinat®) which was used as the positive control. In the case of pentamidine,

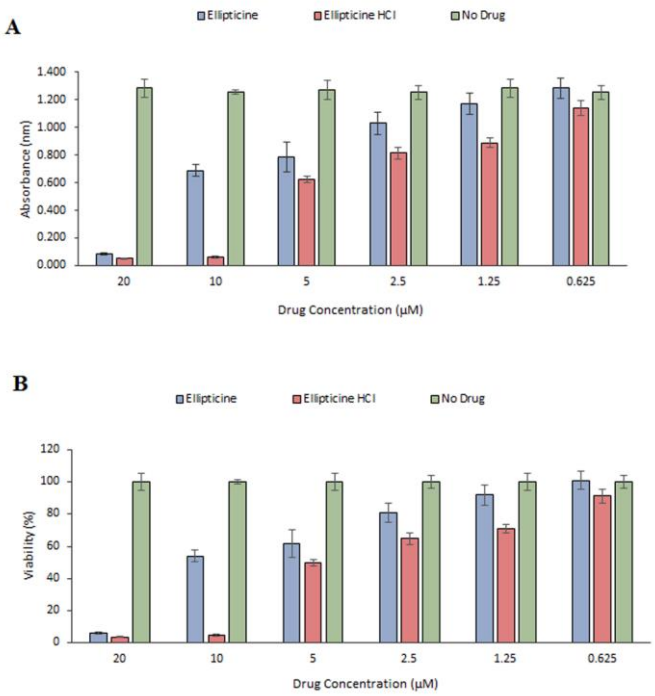

Figure 3. Proliferation assay. DU145 cells were treated with ellipticine, and ellipticine hydrochloride (0.625, 1.25, 2.5, 5, 10, and 20 μM). DMSO 0.1% treatment (no drug) was used as a control throughout the experiments. Proliferation was measured 24 hours post-treatment. Data shown are mean±SD of triplicate independent experiments. (A) Absorbance at 595 nm (B) The cell viability is shown as a percentage of the control treatment (DMSO 0.1%; No drug) set as 100%.

Table 2. IC50 of Ell and Ell×HCl *in vitro* against *P. falciparum* strain K1.

Sample	IC_{50} (μg/mL)	IC_{50} (μmol/mL)
Ellipticine in DMSO	0.55	2.3
Ellipticine in DMSO:H_2O (50:50)	1.25	5.0
Ellipticine·HCl in DMSO	0.21	0.7
Ellipticine·HCl in DMSO:H_2O (50:50)	0.14	0.5
Ellipticine·HCl in DMSO:H_2O (25:75)	0.47	1.7
Chloroquine in DMSO	0.25	0.4

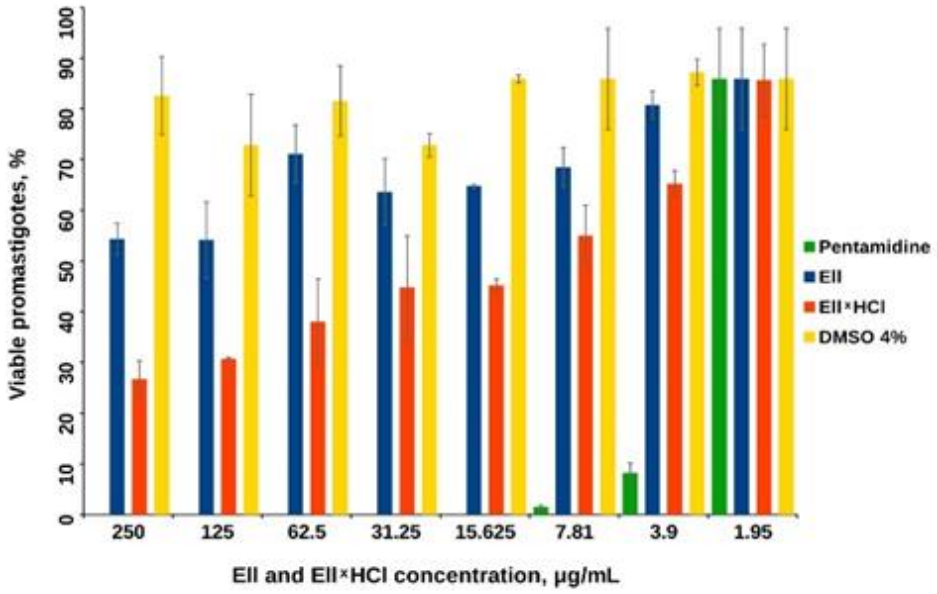

Figure 4. Leishmanicidal activity of Ell and Ell×HCl against promastigotes of *L. amazonensis* (MHO/BR/2009/IM5584): 10^6 cells mL^{-1} were incubated with different individual concentrations at 25 °C. Pentacarinat® solutions 10 µg/mL, 5 µg/mL, and 2.5 µg/mL were used as the positive control. Schneider medium containing 4% of DMSO was used as the negative control.

only 1.5% of promastigotes remained viable at the end of the experiment with the concentration of 10 µg/mL. At the same time, such an activity was never reached for all Ell and Ell×HCl concentration values. As expected, no leishmanicidal activity was detected for the negative control sample, a 4% aqueous DMSO solution. The best activity for both Ell and Ell×HCl was found at the concentration of 250 µg/mL; however, 54.4% of the parasites remained viable upon treatment with Ell, while a viability of 26.7% was observed for Ell×HCl. Our experimental data confirms previously obtained theoretical results [18]. The authors had studied molecular docking of ellipticine with *Leishmania* proteins, but no conspicuous activity was calculated. Moderate activity values for the ellipticine and its salt may also be associated with poor solubility of the active compounds in the culture medium containing insufficient DMSO quantities (4%).

Conclusions

Ellipticine and its hydrochloride salt were synthesized by an improved, reliable, and scalable procedure. Detailed study *in vitro* of their anticancer, antimalarial, and antileishmanial activity was performed. High to very high anticancer and antimalarial activities were demonstrated along with rather moderate antileishmanial activity. As low solubility of ellipticine in aqueous media is an obstacle to its efficacy, we were able to partially circumvented it by obtaining the ellipticine hydrochloride. Ell×HCl exhibited much better biological activity, owing to its better solubility and thus bioavailability.

Experimental section

All reagents were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) unless otherwise specified. Solvents were purified by standard procedures used in synthetic organic chemistry.

Synthesis

^{1}H NMR spectra were recorded on a Varian Mercury 300 instrument operating at 300 MHz.

*1,4-Dimethylcarbazole (**2**)*

A single neck round-bottom flask 250 mL was charged with indole **1** (3.2 g, 27 mmol), hexane-2,5-dione (4.1 g, 36 mmol), p-toluenesulfonic acid (0.6 g, 3 mmol), and toluene (100 mL). The flask was fitted with a Dean-Stark apparatus. First, the flask was kept at 93 °C for 1 h and then refluxed on an oil bath for 2.5 h collecting water, keeping the temperature of the bath at 150 °C. The hot reaction mixture was decanted from a tar formed. The solvent was evaporated to give a red powder. The latter was re-dissolved in boiling cyclohexane (3 x 35 mL). The combined solvent were reduced to the volume of 40 mL. The precipitated pink crystals were collected by filtration, and washed with cyclohexane (20 mL) to give the target 1,4-dimethylcarbazole **2** (3.94 g, 74%). M.p. 93 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, *J* 7.1 Hz, 1H, C5-*H*), 7.83 (br s, 1H, N*H*), 7.41-7.50 (m, 2H, C7-*H* and C8-H), 7.28 (dd, J_1 7.6 Hz, 1H, C6-*H*), 7.16 (d, *J* 7.3 Hz, 1H, C2-*H*), 6.97 (d, *J* 7.3 Hz, 1H, C3-*H*), 2.88 (s, 3H, C4-CH_3), 2.55 (s, 3H, C1-CH_3).

A mixture of *3-formyl-1,4-dimethylcarbazole (**3**) and 6-formyl-1,4-dimethylcarbazole (**4**)*

1,4-Dimethylcarbazole formylation procedure was adopted by Deane et al. [30] using $POCl_3$ and DMF in chlorobenzene. The reaction proceeded non-regioselectively affording a mixture of 3-formyl-1,4-dimethylcarbazole (**3**) as a major product and 6-formyl-1,4-dimethylcarbazole **4** (ca. 9:1) [30]. $POCl_3$ (2.5 mL, 27 mmol) was added dropwise to an ice-cold solution of 1,4-dimethylcarbazole **2** (5.15 g, 26 mmol) and dimethylformamide (DMF, 2.06 mL) in chlorobenzene (80 mL). The reaction mixture was kept at 0 °C for 15 min, and then the temperature was raised to 140 °C and maintained for 3.5 h. At that stage, the reaction mixture was cooled down and DMF (0.4 mL) and $POCl_3$ (0.5 mL) were added. The reaction mixture was then heated and kept at 140 °C for an additional 3 h. After cooling the mixture to room temperature, an aqueous solution of sodium acetate (29 mL) prepared from $CH_3COONa \times 3H_2O$ (7.3 g) was added. The reaction mixture was evaporated to dryness and the residue was extracted with hot EtOAc (3x30 mL). The EtOAc solution was cooled to room temperature, washed with water (70 mL), brine (70 mL), and dried over anhydrous Na_2SO_4. After removal of the solvent, a brown powder 5.3 g (91%) was obtained as a mixture of **3** and **4** (ca. 9:1). ^{1}H NMR (300 MHz, $CDCl_3$) (major isomer **3**) δ 10.44 (s, 1H, C*H*O), 8.54 (br s, 1H, N*H*), 8.25 (d, *J* 8.0 Hz, 1H, C5-*H*), 7.74 (s, 1H, C2-*H*), 7.47-7.53 (m, 2H, C7-*H* and C8-*H*), 7.29-7.34 (m, 1H, C6-*H*), 3.16 (s, 3H, C4-C*H3*), 2.55 (s, 3H, C1-C*H3*).

The two following steps, viz. condensation with aminoacetaldehyde diethyl acetal with a further reduction to a mixture of 3-(2,2-diethoxyethylaminomethyl)-1,4-dimethylcarbazole **7** as a major isomer and 6-(2,2-diethoxyethylaminomethyl)-1,4-dimethylcarbazole **8** (ca. 9:1) were carried out according to the literature [21, 30-31, 35-36].

A mixture of *3-(2,2-diethoxyethyliminomethyl)-1,4-dimethylcarbazole (**5**) and 6-(2,2-diethoxyethyliminomethyl)-1,4-dimethylcarbazole (**6**)*

A mixture of 3- and 6-formyl-1,4-dimethylcarbazoles (**3** and **4**) (ca. 9:1) (4.5 g, 20.1 mmol) and aminoacetaldehyde diethyl acetal (3.06 mL, 21 mmol) was heated at 110 °C with stirring for 4 h. The reaction mixture was then cooled, diluted with toluene (15 mL), and evaporated under reduced pressure to give a mixture of 3- and 6-(2,2-diethoxyethyliminomethyl)-1,4-dimethylcarbazoles (**5** and **6**) as an orange oil (6.42 g, 94%) that was used without further purification.

A mixture of *3-(2,2-diethoxyethylaminomethyl)-1,4-dimethylcarbazole (**7**) and 6-(2,2-diethoxyethylaminomethyl)-1,4-dimethylcarbazole (**8**)*

To a cooled (0 °C) solution of the mixture of 3- and 6-(2,2-diethoxyethyliminomethyl)-1,4-dimethylcarbazoles (**5** and **6**) (8.30 g, 24.6 mmol) in methanol (200 mL), 4.67 g (123 mmol) of $NaBH_4$ was added portion-wise with stirring during 20 min. The reaction mixture was kept at room temperature for 2 h with stirring. Subsequently, the reaction mixture was evaporated to dryness and 1 M aqueous HCl solution was added until pH 6. The aqueous phase was decanted from the tar formed, and neutralized by a 20% aqueous NaOH solution until the pH reached 7. The solution was extracted with toluene (3x50 mL). The organic layer was separated, washed with brine (2x30 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated to dryness to give a mixture of 3- and 6-(2,2-diethoxyethylaminomethyl)-1,4-dimethylcarbazoles (**7** and **8**) (ca. 9:1) as a brown oil (7.26 g, 87%), which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) (major isomer **7**) δ 8.24 (d, *J* 8.0 Hz, 1H, C5-*H*), 8.07 (br s, 1H, N*H*), 7.38-7.48 (m, 2H, C7-*H* and C8-*H*), 7.18-7.27 (m, 2H, C2-*H* and C6-*H*), 4.67 (t, *J* 5.6 Hz, 1H, C*H*$(OEt)_2$), 3.98 (s, 2H, ArCH_2NH), 3.65-3.75 (m, 2H, OCH_2CH$_3$), 3.49-3.59 (m, 2H, OCH_2CH$_3$), 2.87 (s, 3H, C4-CH_3), 2.85 (m, 2H, NHCH_2CH), 2.50 (s, 3H, C1-CH_3), 1.81 (br s, 1H, ArCH$_2$N*H*), 1.22 (t, *J* 7.0 Hz, 6H, 2×OCH$_2$CH_3).

*N-tosyl-3-(2,2-diethoxyethylaminomethyl)-1,4-dimethylcarbazole (**9**)*

To a mixture of 3- and 6-(2,2-diethoxyethylaminomethyl)-1,4-dimethylcarbazoles (**7** and **8**) (ca. 9:1) (5.42 g, 16 mmol) in THF (87 mL) 42 mL of water were added, followed by the addition of solid K_2CO_3 (3.34 g, 24 mmol) and p-toluenesulfonyl chloride (4.52 g, 24 mmol). The reaction mixture was stirred at room temperature for 16 h (Vehar et al., 2011). Subsequently, 50 mL of water followed by 100 mL of EtOAc were added. The organic layer was separated and washed successively with 1 M HCl (2x50 mL), water (50 mL), saturated solution of $NaHCO_3$ (50 mL), water (50 mL), brine (50 mL), and dried over the anhydrous Na_2SO_4. After the removal of the solvents, the solid residue was recrystallized from a boiling mixture of hexane : EtOAc (2:1) (90 mL). The obtained solution was left overnight at room temperature, the precipitate was separated by filtration, and washed with hexane to give 4.80 g (61%) of the target compound **9** as beige crystals. M.p. 180 °C (177-179 °C [30]). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J* 7.8 Hz, 1H, C5-*H*), 8.04 (br s, 1H, N*H*), 7.74 (d, *J* 8.2 Hz, 2H, Tos, C2'-*H* and C6'-*H*), 7.38-7.48 (m, 2H, C7-*H* and C8-*H*), 7.21-7.28 (m, 3H, Tos, C3'-*H*, C5'-*H* and C6-*H*), 4.67 (s, 2H, ArCH_2N),6.97 (s, 1H, C2-*H*), 4.43 (t, *J* 5.4 Hz, 1H, C*H*$(OEt)_2$), 3.48-3.58 (m, 2H, OCH_2CH$_3$), 3.26-3.36 (m, 2H, OCH_2CH$_3$), 3.21 (d, *J* 5.5 Hz, 2H, NHCH_2CH), 2.80 (s, 3H, C4-CH_3), 2.42 (s, 3H, Tos, C4'-CH_3), 2.39 (s, 3H, C1-CH_3), 1.07 (t, *J* 7.0 Hz, 6H, 2×OCH$_2$CH_3).

*Ellipticine (5,11-dimethyl-6H-pyrido[4,3-b]carbazole) (**10**)*

The compound **9** (1.94 g, 3.9 mmol) was dissolved in 77 mL of dioxane and 19 mL of 23% aqueous HCl solution were added. The reaction mixture was heated at 110-115 °C with stirring for 6.5 h [30-32, 35], and its color turned dark-green. The total volume was reduced on a rotary evaporator to approximately one-quarter of the initial one. Then 50 mL of water and 50 mL of 20% $NaHCO_3$ solution were added successively. The aqueous layer was extracted with methylene chloride (5x100 mL). The combined extracts were washed with brine (100 mL) and dried over an

anhydrous Na_2SO_4. The solvents were evaporated to give a green slurry (5 mL) of N-tosyl-1,2-dihydroellipticine and ellipticine **10**. The slurry was placed on top of a short silica gel column. The mixture was separated using flash chromatography. First, EtOAc was used as the eluent to collect mainly N-tosyl-1,2-dihydroellipticine (0.3 g) as brown crystals, and then the eluent was changed to MeOH to collect the ellipticine **10** as dark-yellow crystals (0.49 g, 52%). M.p. >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.41 (s, 1H, N*H*), 9.66 (s, 1H, C1-*H*), 8.39 (d, *J* 6.0 Hz, 1H, C3-*H*), 8.34 (d, *J* 8.0 Hz, 1H, C10-*H*), 7.87 (d, *J* 6.0 Hz, 1H, C4-*H*), 7.47-7.57 (m, 2H, C8-*H* and C7-*H*), 7.23 (t, *J* 8.0 Hz, 1H, C9-*H*), 3.21 (s, 3H, C11-CH_3), 2.76 (s, 3H, C5-CH_3).

*Ellipticine hydrochloride (**11**)*

2 mL of concentrated aqueous HCl (~36%) were added to the ellipticine **10** (0.11 g, 0.45 mmol) solution in 50 mL of methanol and thoroughly mixed. After the removal of the solvents, 30 mL of acetone was added to the residue. The ellipticine hydrochloride **11** precipitated as yellowish-green crystals, yield 0.12 g (95%). M.p. >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.19 (s, 1H, N*H*), 9.78 (s, 1H, C1-*H*), 8.26-8.33 (m, 3H, C4-*H*, C10-*H*, and C3-*H*), 7.57 (br s, 2H, C8-*H* and C7-*H*), 7.27-7.30 (m, 1H, C9-*H*), 3.14 (s, 3H, C11-CH_3), 2.72 (s, 3H, C5-CH_3).

Activity studies

In vitro bioassays. Cell lines and culture

Human prostate cancer cell line DU145 was purchased from ATCC (American Type Culture Collection, Rockville, MD, USA). The cells were authenticated by the cell bank using DNA profile (STR) and cytogenetic analysis. The cells were cultured in a culture media (Life Technologies, Carlsbad, CA) and supplemented with 10% of fetal bovine serum (FBS), 50 U of penicillin/mL, and 50 mg streptomycin/mL (Life Technologies, Carlsbad, CA, USA) and maintained in a 5% CO_2 humidified incubator at 37 ºC. The specific growth media for DU145 cells were DMEM and the cell line was routinely frozen within 2 passages after receipt from the cell bank and used within less than 6 months after recovery.

Proliferation Assay

Ell and Ell×HCl were dissolved in DMSO (Sigma-Aldrich) and diluted to get final concentrations of 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 µM. 0.1% DMSO was used as a negative control. The proliferation assay was performed using Cell Proliferation Kit I (MTT; Roche, Basel, Switzerland) according to the manufacturer's protocol. Ninety six well tissue culture plates (SPL Life Sciences, Korea) were used to seed 2×10^3 cells/well. Following the drug treatment, 10µl of yellow thiazolyl blue tetrazolium bromide (MTT) (5 mg/mL) dissolved in sterile 1×PBS pH 7.4 was added to the cells and incubated for 4 hours, to quantify the cell proliferation. The yellow MTT solution was converted into a dark blue/purple formazan crystals by mitochondrial dehydrogenases of live actively metabolizing cells and was solubilized following the 4-hour incubation by the addition of 100 µL of solubilization reagent (10% sodium lauryl sulfate, 0.01 M HCl in H_2O) and incubation for 16 hours. Subsequently, the absorbance at 595 nm was measured using a Multiskan^{TM} FC microplate photometer.

Culture and in vitro inhibition assay of P. falciparum

Multidrug-resistant strain K1 (MRA-159, MR4, ATCC Manassas Virginia) of *P. falciparum* used in the assay were maintained in continuous culture according to Trager and Jensen method [45]. Initial parasitemia of 1-2% and hematocrit of 2% were used for the assay. RPMI 1640 (Sigma-Aldrich) supplemented with 10% human serum (Sigma-Aldrich) and containing 25 mmol of HEPES and 2 mmol of glutamine was used as a complete medium. The substances were solubilized in DMSO at a stock concentration of 10 mg/mL and subsequently diluted in the complete medium to obtain seven test concentrations (0.006-100 µg/mL). Ellipticine and ellipticine×HCl stock solutions of 10 mg/mL were prepared both in pure DMSO and in DMSO mixtures with water in the ratios of 50:50 and 25:75 v/v. The stock solutions were subsequently diluted in the complete RPMI 1640 culture medium to obtain seven different concentrations between 0.006 and 100 µg/mL. The assay was performed as described by Andrade-Neto et al. [15]. Dilutions of the samples were applied to microplate wells containing parasitized erythrocytes. Each dilution was tested in triplicate. The plate was incubated at 37 °C for 48 h. Viable parasites were counted by a hemocytometer. Parasite growth inhibition was determined by comparison to the growth controls without the sample. The half-maximal inhibitory responses (IC_{50}) as compared to the drug-free controls were estimated by interpolation using MicroCal Origin software. The concentration values in log µg/mL were plotted against the parasite viability (ratio of the average parasitemias of test wells to the average parasitemias of the control wells) for each concentration using the sigmoidal fitting analysis function to generate a smooth curve. The IC_{50} values were obtained from the graph and correspond to viability of 0.5.

Antileishmanial assays

Leishmania (Leishmania) amazonensis strain MHO/BR/2009/IM5584 cryopreserved and kept at the Laboratory of Leishmaniasis and Chagas disease, CSAS, INPA. The promastigotes used in the bioassays were maintained and cultured in a complete Schneider Drosophila Medium (Sigma-Aldrich) supplemented with a heat-inactivated 10% Fetal Bovine Serum (FBSi, Sigma-Aldrich) and gentamycin (40 µg/mL) and kept in an oven at 25 °C, according to Morel [46]. A growth curve was performed before the bioassay realization. The strain J774 of murine macrophages was maintained in RPMI-1640 (Himedia®) in culture bottles in an oven at 37 °C. Evaluation of antileishmanial activity was performed in 96 well culture plates using promastigotes in the late log phase (10^6 cells/mL), according to Fumarola et al. [47]. The active compounds were dissolved in dimethylsulfoxide (DMSO, Vetec®, 4% v/v) and Schneider medium and then filtered

through 0.22 μm Millipore membrane in a sterile environment. The samples were evaluated at concentrations of 1.95 μg/mL to 250 μg/mL. Pentamidine (Pentacarinat®) was used as a positive control at a maximum concentration of 10 μg/mL and DMSO (4% v/v) was used as a negative control. The activity of the samples was assessed by growth inhibition and mortality of the *L. amazonensis* promastigotes in periods of 24 and 48 hours at 25 °C. The bioassays were performed in triplicates. Viable parasites were counted by a hemocytometer. The average number of live cells was used to calculate the IC_{50} values.

Notes

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The authors declare no conflict of interest.

Author contributions. **K. V. S.**: Synthesis of compounds, writing experimental section. **I. A. G.**: Formal analysis. **T. I. S.**: Synthesis of compounds. **A. N. K.**: review & editing **N. S. P.**: Investigation, **L. F. R. S.**: Investigation, **A. K.**: Investigation, **C. D. C. W.**: Investigation, **L. F. Z.**: Investigation, **W. P. T.**: Investigation, **A. M. R. F. P.**: Investigation, **M. L.**: Conceptualization, **A. V. G.**: Supervision, Writing most of the manuscript.

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Індольний алкалоїд еліптицин як ефективна багатоцільова сполука

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Резюме: Вперше виділений з тропічної рослини *Oschrosia elliptica*, індольний алкалоїд еліптицин викликав величезний інтерес, оскільки була продемонстрована протипухлинна активність разом з обмеженими токсичними побічними ефектами та повною відсутністю гематологічної токсичності. У цій роботі для отримання еліптицину (Ell) був використаний п'ятистадійний синтез по Cranwell і Saxton. Також був синтезований гідрохлорид еліптицину (Ell×HCl). Було проведено детальне дослідження *in vitro* протипухлинної, протималярійної та лейшманіцидної дії. Аналіз антиракової активності проведений з використанням лінії ракових клітин DU145, обробленої Ell, показав послідовне зниження проліферації та життєздатності клітин при обробці Ell починаючи з концентрації 5 мкмоль/л. Антипроліферацийна активність була більш вираженою для розчинів Ell×HCl. Як Ell, так і Ell×HCl виявили помірну активність *in vitro* щодо промастигот *Leishmania amazonensis*, що пов'язано з недостатньою розчинністю препаратів. Значення IC_{50} Ell та Ell×HCl визначали *in vitro* проти штаму K1 *Plasmodium falciparum*, стійкого до багатьох лікарських засобів. Було показано, що Ell×HCl майже втричі потужніший, ніж Ell в ДМСО. При розведенні водою розчинність і активність Ell падають, тоді як у випадку Ell×HCl, вони збільшуються до 10 разів у водному розчині DMSO 50:50.

Ключові слова: фтор; піролізидин; індолізидин; хінолізидинові алкалоїди; стратегія синтезу.