Synthesis and in vitro antitumor activity of novel 2-alkyl-5-methoxycarbonyl-11-methyl-6H-pyrido[4,3-b]carbazol-2-ium and 2-alkylellipticin-2-ium chloride derivatives

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Original article

Synthesis and in vitro antitumor activity of novel 2-alkyl-5-methoxycarbonyl-11-methyl-6*H*-pyrido[4,3-*b*]carbazol-2-ium and 2-alkylellipticin-2-ium chloride derivatives

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ABSTRACT

Twenty-one types of novel ellipticine derivatives and pyridocarbazoles (5-methoxycarbonyl-11-methyl-6*H*-pyrido[4,3-*b*]carbazoles) with a nitrosourea moiety, linked by an oxydiethylene unit at the 2 position, were synthesized, and their cytotoxicity against HeLa S-3 cells was evaluated. Some of these new compounds exhibited potent antitumor activity by comparison with that of ellipticine.

1. Introduction

A great deal of interest has been generated by indole alkaloids, ellipticines [1] and β-carbolines [2] because of their intercalation, antitumor properties, and anti-HIV activities. Ellipticine was first identified in 1959 as a compound in the leaves of a small tropical evergreen *Ochrosia elliptica* Labill [1g] (see Fig. 1). Ellipticine and its derivatives cause selective inhibition of p53 protein phosphorylation in several human cancer cell lines [3], and this is correlated with their cytotoxic activity. These also uncouple mitochondrial oxidative phosphorylation [4], thereby disrupting the energy balance of cells. Further, these have been shown to react with DNA by an intercalation process [1e,f], which may account for their cytotoxicity [5], and the further inhibition of DNA topoisomerase-II activity [1a]. We have reported the interaction mode of ellipticine and its derivatives with DNA using deflection spectroscopy [6]. Ellipticine is a good intercalator and an antitumor agent like β-carbolines [7]. The unique structural features, limited toxic side effects, and complete lack of hematological toxicity of these compounds has prompted chemists to study their synthesis of a number of analogues for pharmacological evaluation [8].

The syntheses of ellipticine and its derivatives have been reported by many groups [9]. Several simple structural modifications to ellipticine derivatives have resulted in compounds with increased toxicity [10]. A low level of water solubility at physiological pH, as well as systemic toxicity, has prevented the use of ellipticine as a therapeutic agent. The introduction of a positive charge in the molecule improved water solubility and made a profound difference in the biological activity [11]. For example, the quaternization of the endocyclic nitrogen at N5 of 5-aza-ellipticine [12] and the nitrogen at N2 of ellipticine [13] with a methyl group was found to interact with DNA through intercalation, which resulted in a high affinity for binding with DNA.

This background combined with further work in this area [14] has led us to the development of a novel and efficient method for the synthesis of ellipticine via Suzuki–Miyaura coupling reaction

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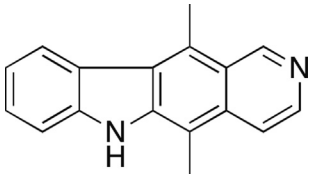


Fig. 1. Chemical structure of ellipticine.

[15]. Nitrosourea compounds generally are good DNA alkylating agents and are often used in chemotherapy to damage the DNA of cancer cells [16]. Herein, we focus on the development of new ellipticine analogues that will have greater solubility in water by quaternizing the endocyclic nitrogen atom at the 2 position with a tethered nitrosourea moiety (Fig. 2). In order to prevent a decrease in water solubility with an extension of the carbon chain, we designed a 3-oxa-pentyl tether ($X = O$ in Fig. 2). In addition, we examined the efficiency of these new molecules as potent antitumor agents with improvements in the binding ability related to cytotoxic activity, which is evaluated by MTT assay against HeLa S-3 cells.

2. Results and discussion

2.1. Synthesis of N-nitrosoureas linked to 2-alkyl-5-methoxycarbonyl-11-methyl-6H-pyrido[4,3-b]carbazol-2-ium and ellipticin-2-ium chloride derivatives

We selected a (2-aminoethoxy)ethyl (Fig. 2, $X = O$) and 5-aminopentyl groups (Fig. 2, $X = CH_2$) as linkers in the target compounds (Fig. 2a,b). Retro synthesis analysis for 2-(2-aminoethoxy) ethyl-5-methoxycarbonyl-11-methyl-6*H*-pyrido[4,3-*b*]carbazol-2-ium chloride derivatives (Fig. 2a; pyridocarbazole-5-carboxylate derivatives) is briefly shown in Fig. 3 to make the synthetic strategy understandable. We considered that the target compound was obtained from a synthetic equivalent A and a synthon B, and that the synthetic equivalent A was obtained by successive reactions of methyl 2-(3-(1-(pyridin-3-yl)vinyl)-1*H*-indol-2-yl)acetate D and an aminoalkyl linker E to give the corresponding precursor compound C (Fig. 3).

First, we synthesized various kinds of aminoalkyl linkers to accomplish the target molecules (pyridocarbazole-5-carboxylate and ellipticine derivatives), as represented in Figs. 2 and 3 (see Schemes 1 and 2). Treatment of potassium phthalimide with bis(2-chloroethyl) ether (**1**) in DMF gave the corresponding monoimide **2**. The reduction of **2** with NH_2NH_2 at 60 °C for 3 h generated the corresponding monoamine **3** (Scheme 1). The NH_2 group of **3** was protected with Ac_2O/Et_3N or 2,5-hexanedione/acetic acid to give **4a** or **4b** in 45 and 55% yields, respectively (Scheme 1; see Supporting Information). Compounds **6a**–**d** were obtained in a similar manner by protecting the NH_2 group of 2-(2-aminoethoxy)ethanol (**5**) by treatment with phthalic anhydride, Ac_2O/Et_3N, 2,5-hexanedione/acetic acid, and Boc_2O/Et_3N, respectively (Scheme 2; see Supporting Information). The obtained compounds **6a**–**d** were tosylated with TsCl in the presence of Et_3N at room temperature for 3 h of reaction time, to give the corresponding *O*-tosylates **7a**–**d** in high yields.

Next, 2-alkyl-5-methoxycarbonyl-11-methyl-6*H*-pyrido[4,3-*b*] carbazol-2-ium chloride derivatives, shown in Fig. 2, were prepared from indole (**8**), as shown in Scheme 3. First, the NH group of **8** was protected with benzenesulfonyl group to give *N*-benzenesulfonylindole (**9**) in an 85% yield, and **9** was oxalylated with dimethyl oxalate on a C-2 atom to give compound **10** in a 61% yield. The benzenesulfonyl group was easily removed upon treatment with potassium carbonate accompanied by hydrolysis of the ester group to give the corresponding carboxylic acid **11** in an 85% yield. The carbonyl group of **11** was reduced with hydrazine leading to the formation of 2-indolylacetic acid (**12**). The crude product was treated with trimethylsilyldiazomethane without further purification to give the corresponding methyl ester **13** (75%). The isolation of carboxylic acid **12** failed because of decarboxylation, which resulted in 2-methylindole. Finally, a mixture of **13** and 3-acetylpyridine was heated under reflux in methanol for 6.5 h in the presence of concentrated sulfuric acid leading to methyl 2-(3-(1-(pyridin-3-yl)vinyl)-1*H*-indol-2-yl)acetate (**14**) in 65% yield.

Furthermore, in order to construct a pyridocarbazole ring, the compound **14** was treated with **2** and **4a** in DMF, and then with sodium methoxide in the presence of 3-ethoxycarbonyl-1-methylpyridinium chloride (**17**) (Path A in Scheme 4) leading to 2-alkylpyridocarbazolium derivatives **19a**,**b** in a yield of 10% and a trace amount, respectively. When conducted in another manner, the 2-alkylpyridocarbazoliums **19a**, **19b** and **19d** were prepared from **14** in good yields (55, 60 and 52%) in two steps via treatment of **14** with **7a**, **7b** and **7d**, respectively, in the presence of NaOMe and **17** followed by the action of Amberlite IRA-900 (Path B in Scheme 4). The pyridinium chloride **17** was prepared from the corresponding pyridine carboxylate **15** and methyl iodide to give the pyridinium iodide **16** followed by treatment with Amberlite IRA-900 (chloride form). Unexpectedly, the reaction of **4b** and **7c** with **14** via paths A and B yielded complex mixtures, and the objective for **19c** was not given (Scheme 4). The stability of the quaternary salts of these molecules increases by converting them into the corresponding tosylate and chloride salts (Scheme 4). The compound **20** was obtained by the deprotection of the amino groups of **19a**, **19b** and **19d**. The compound **19b** on reflux with 9 M HCl–MeOH in 3 days and compound **19d** upon treatment with 12 M HCl in 5 h gave compound **20** in 50 and 85% yields, respectively (Scheme 4). Contrary to our expectations, **19a** did not give **20** in methanolic HCl even after reflux for 3 days.

Antitumor activity of 9-hydroxyellipticine (**23**) and its derivatives has been widely studied, and the introduction of a 9-hydroxy group may increase its antitumor activity [17]. We were interested in applying this substituent to our molecules **35a**, **38b** and **39a**,**b** tethered at the 2-position. Therefore, we planned to synthesize the compounds **35b**, **38c** and **39c** together with their 6-methylated derivatives **35c**,**d**, **38d**,**e**, and **39d**,**e** and to determine their in vitro antitumor activity. Ellipticine (**21**), 9-hydroxyellipticine (**23**), 6-methylellipticine (**27**) and 9-hydroxy-6-methylellipticine (**29**) were prepared via a modification of a previously reported method [18], as shown in Scheme 5 for **27** and **29**. Ellipticine (**21**) was heated with hexamethylenetetramine in TFA for 20 min under reflux to give the corresponding 9-formyl derivative (92% yield), and was followed by Dakin oxidation leading to the formation of 9-hydroxyellipticine (**23**) in 55% yield, according to a reported method [18]. Ellipticine (**21**), 9-hydroxyellipticine (**23**), and 9-hydroxy-6-methylellipticine (**29**) were treated with **7d** to form the corresponding ellipticinium tosylate quaternary salts **33a**–**c** (Scheme 6). In a similar manner, tosylate salt **33d** was formed by reacting **29** with **32**. The quaternary salts **33a**–**d** were then treated with Amberlite IRA-900 (chloride form) in water at room temperature, and the reaction mixture was purified by ODS column chromatography to give the corresponding chloride salts **34a**–**d** in 52, 52, 52, and 50% yields, respectively. These chloride salts (**34a**–**d**) were then refluxed for 5 h in 12 M HCl to give the 2-alkylellipticinim analogues **35a**–**d** in 86, 85, 86, and 82% yields (Scheme 6).

The obtained 2-(2-aminoethoxy)ethyl-5-methoxycarbonyl-11-methyl-6*H*-pyrido[4,3-*b*]carbazol-2-ium chloride (**20**) and its analogues **35a**–**d** were treated with *p*-nitrophenyl *N*-methylcarbamate **37a** and its *N*-nitroso derivative **37b** in the presence of $^{i}Pr_2NEt$ in

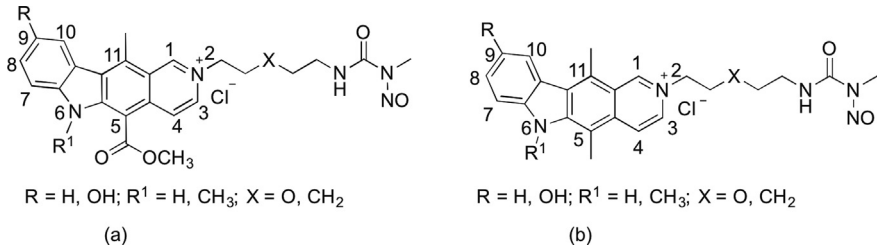


Fig. 2. 2-Alkyl-5-methoxycarbonyl-11-methyl-6*H*-pyrido[4,3-*b*]carbazol-2-ium and 2-alkylellipticin-2-ium chloride derivatives with a nitrosourea moiety.

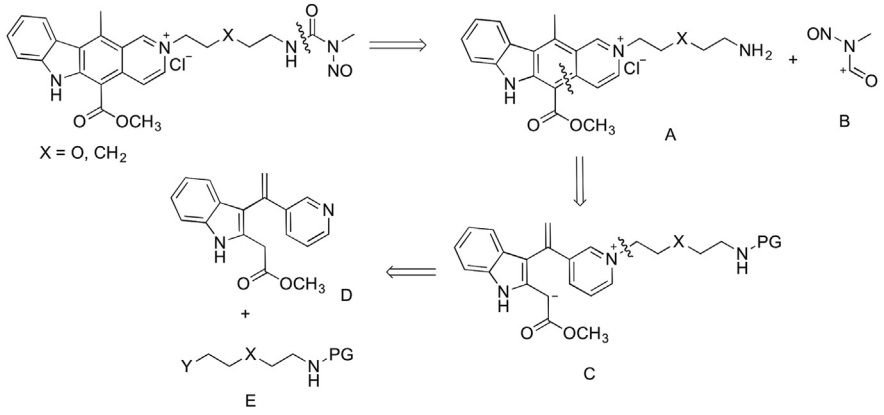
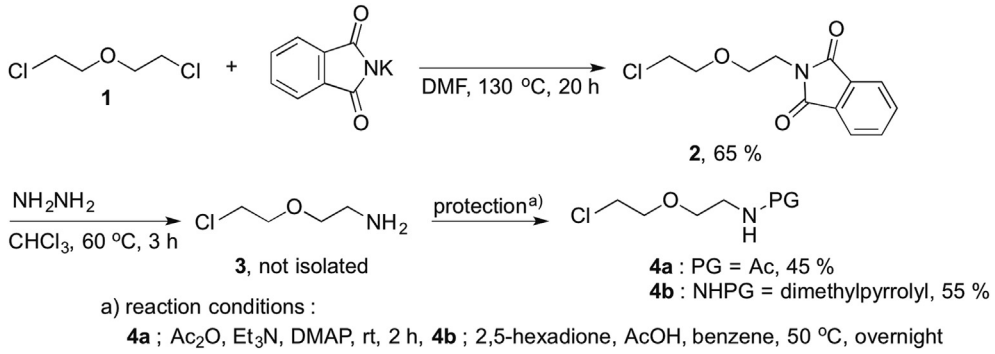
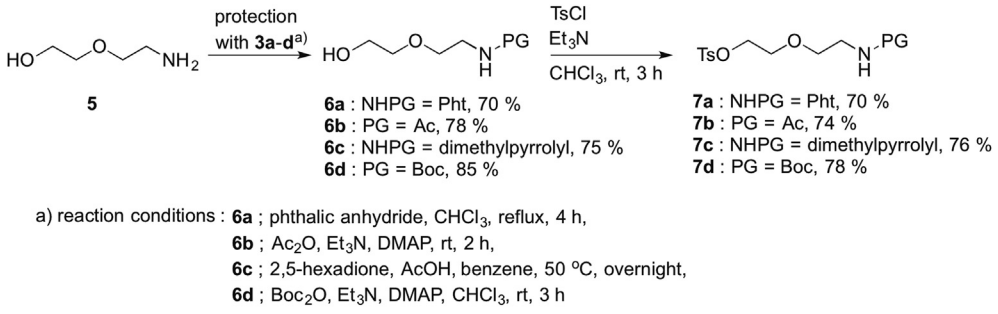


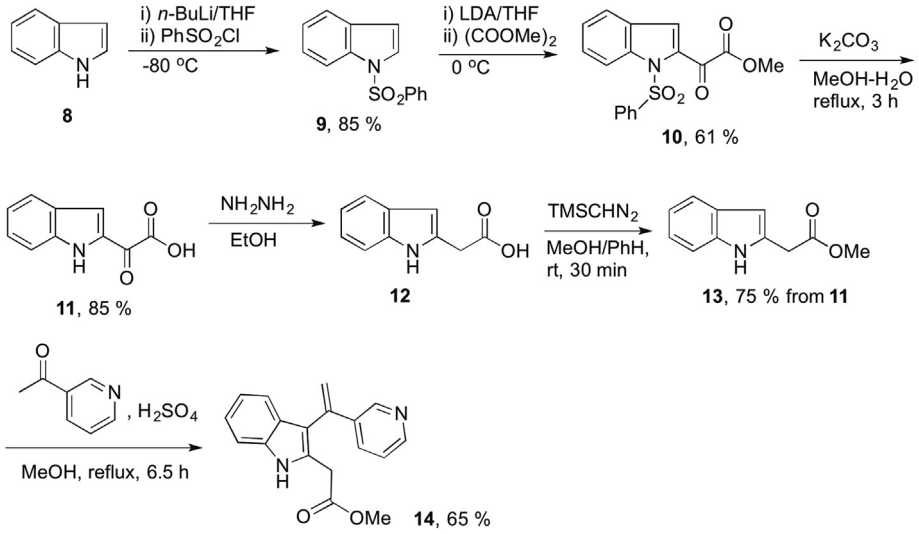
Fig. 3. Retro synthesis analysis for 2-(2-aminoethoxy)ethyl-5-methoxycarbonyl-11-methyl-6*H*-pyrido[4,3-*b*]carbazol-2-ium chloride derivatives having a methylnitrosourea moiety shown in Fig. 2a.



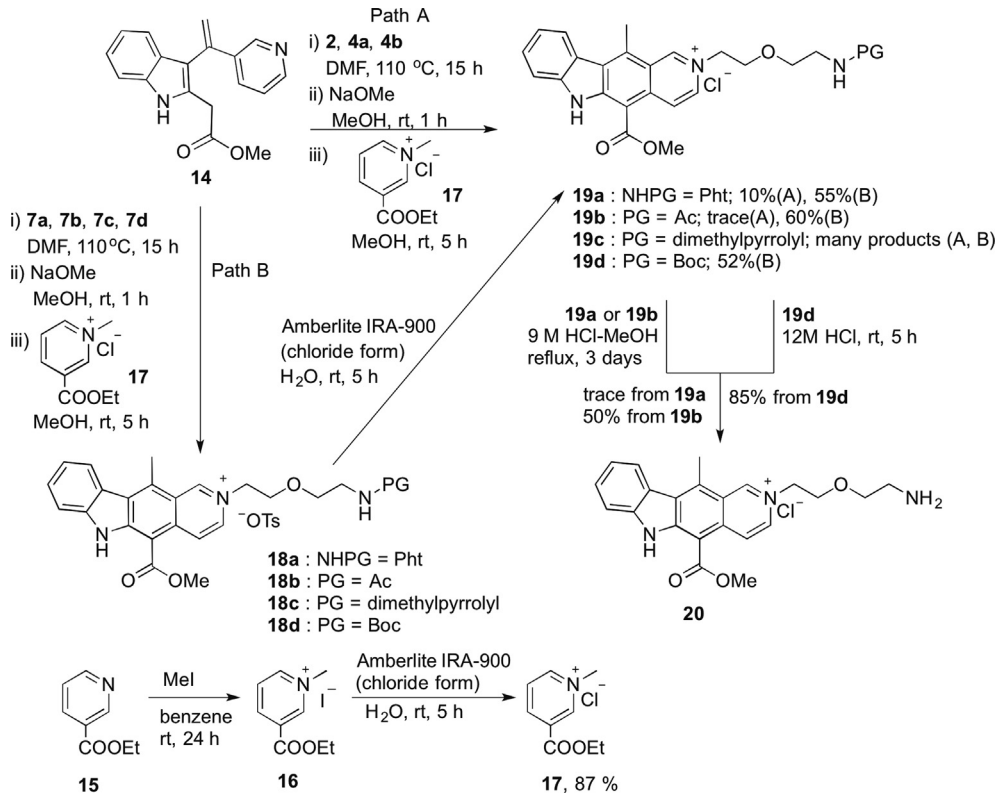
Scheme 1. Synthesis of aminoalkyl linkers having a Cl group.



Scheme 2. Synthesis of aminoalkyl linkers having a TsO group.



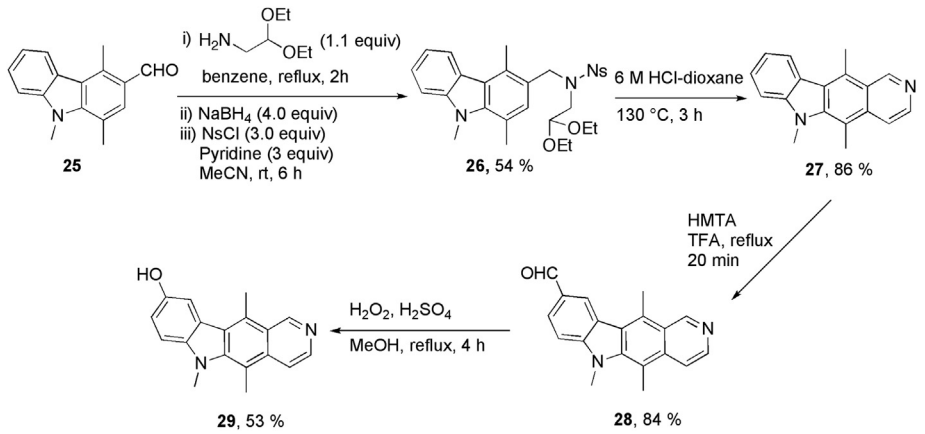
Scheme 3. Synthesis of **14.**



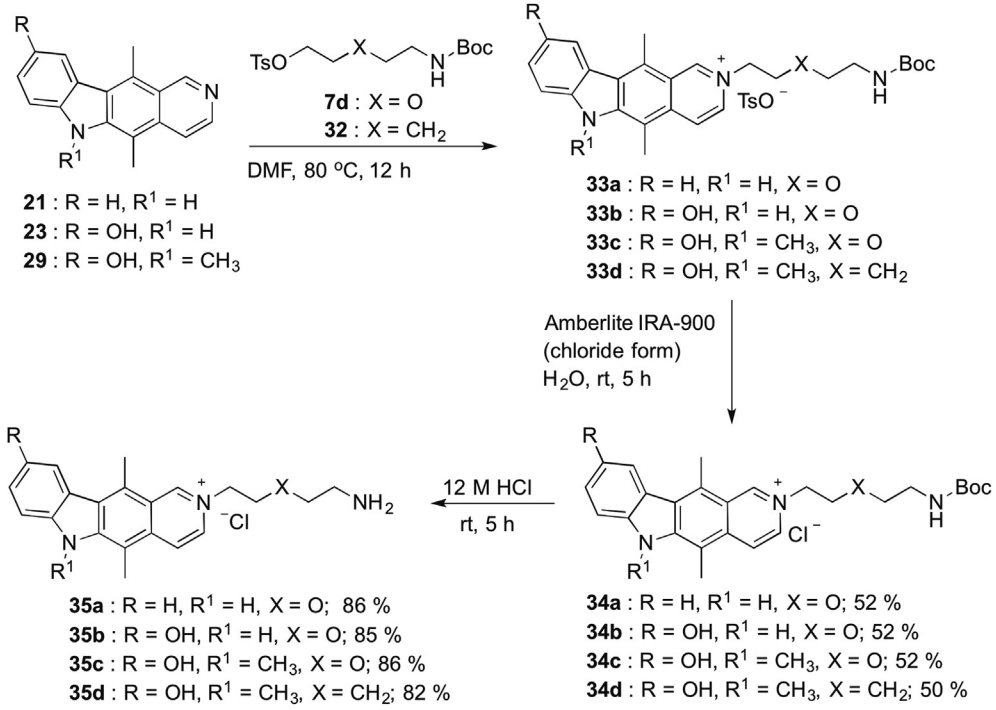
Scheme 4. Synthesis of 2-alkylellipticinium analogue **20.**

DMF to get the corresponding urea derivatives **38a**–**e** (74, 45, 43, 47, and 63% yields, respectively) and *N*-nitrosourea derivatives **39a**–**e** (64, 43, 39, 40, and 20% yields, respectively) (Scheme 7).

Finally, 2-(2-aminoethyl)- and 2-(3-aminopropyl)-pyridocarbazol-2-ium chlorides **40**–**45** were synthesized to verify the effect of linker length on antitumor activity in comparison with those of **38e** and **39e**, by a method analogous to the synthesis of **38e** and **39e** in poor to high yields (see Scheme 8). Furthermore, 2-methylellipticin-2-ium chloride (**46**) was synthesized to clarify the effect of the quaternization of the endocyclic nitrogen at the N2 of ellipticine (**21**) on antitumor activity. The compound **46** can be synthesized by a direct methylation of ellipticine (**21**) with methyl iodide, but the yield is low and the reaction time is long [30]. Therefore, we decided to synthesize **46** by the similar method for the preparation of **50** using methyl iodide instead of **49** followed by both reduction with sodium bis(2-methoxyethoxy)aluminum hydride and the ion–exchange reaction with Amberlite IRA-900 (a chloride form) in a higher yield as shown in Scheme 9.



Scheme 5. Synthesis of 6-methylellipticine (**27**) and 9-hydroxy-6-methylellipticine (**29**).

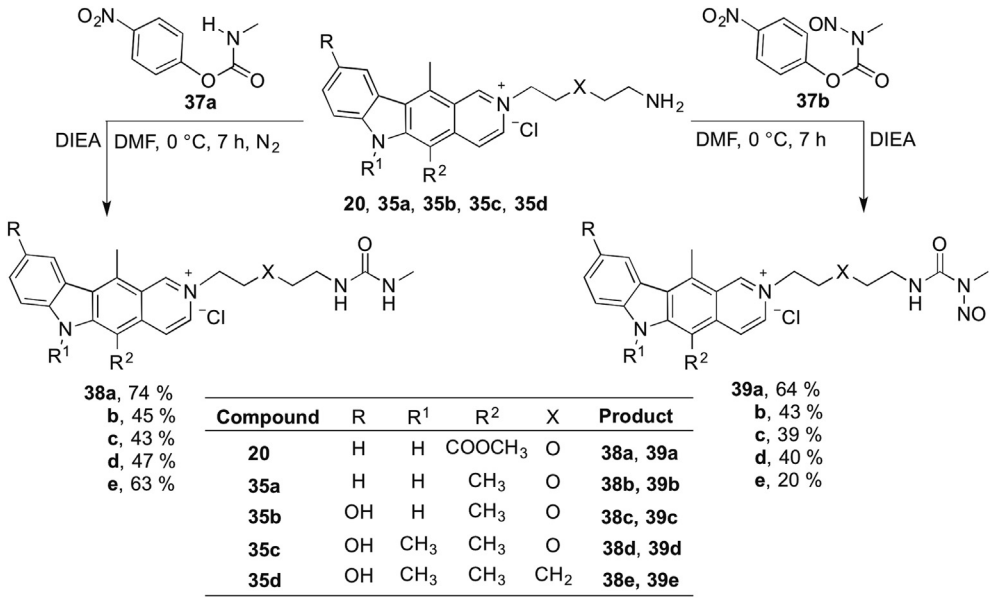


Scheme 6. Synthesis of 2-alkyl analogues of ellipticine **35a–d.**

*2.2. Antitumor activity of ellipticine and pyridocarbazole-5-carboxylate derivatives **20**, **21**, **35**, and **38–46***

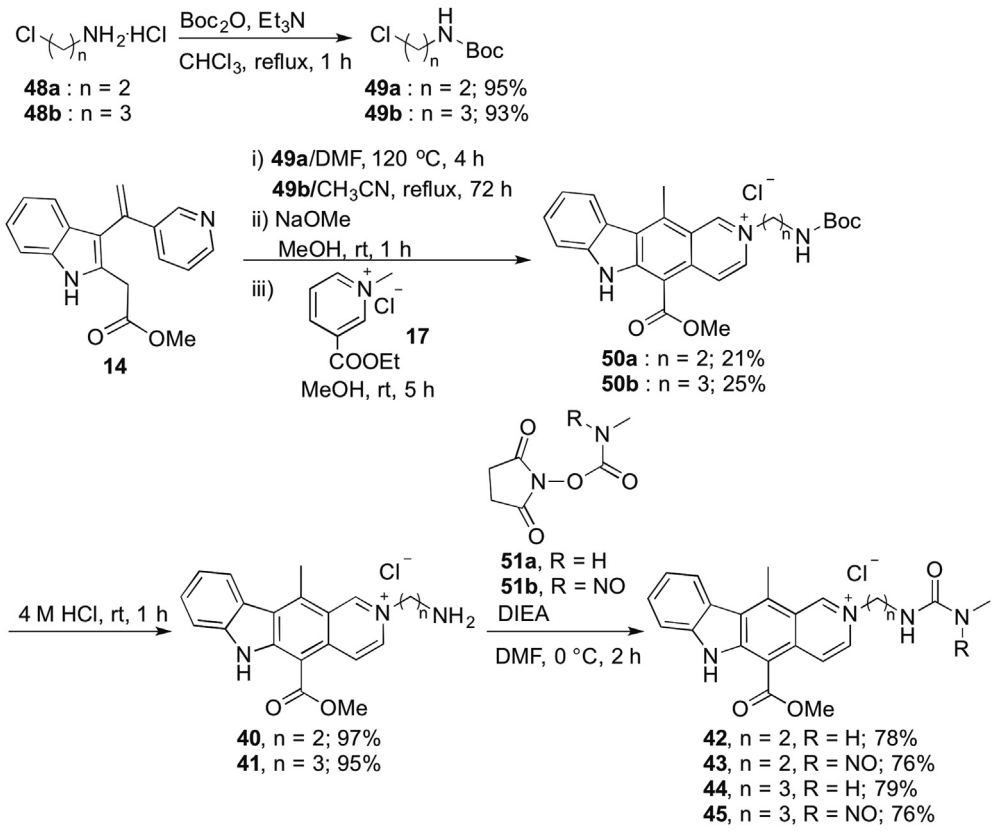
We used an MTT assay to evaluate the cytotoxicity of ellipticine and pyridocarbazole derivatives on human cervical cancer cell line HeLa S-3. The IC_{50} values in the sub-micromolar range of all compounds are given in Table 1.

First, the in vitro antitumor activity of the ellipticine series (Fig. 2(b)) is discussed. In comparison with the antitumor activity of ellipticine (**21**) itself (IC_{50} 2.1 μM), the quaternization of the endocyclic nitrogen atom at the 2-position of **21** by a methyl group did not decrease the activity (**46**, IC_{50} = 2.90 μM), as shown in Table 1. Next, the in vitro antitumor activity was compared between the derivatives with different tether lengths and a different central group X (O and CH_2). Prior to discussing the effects of the tether length on activity, it is important to examine the effects of the central X group, the terminal amino group, and the terminal urea group on antitumor activity. Although the IC_{50} value of **35c** ($X = O$; 17.9 μM) was near that of **35d** ($X = CH_2$; 19.3 μM) in the amine derivatives, the IC_{50} value of **38d** ($X = O$; 6.2 μM) was smaller than that of **38e** ($X = CH_2$; 32.5 μM) in the urea derivatives. The effect of the terminal amino group on antitumor activity was not observed in the MTT assays of either **46**, **40**, or **41** (IC_{50} = 2.90, 5.8, and 2.50 μM, respectively). However, the introduction of the terminal urea group in the tether generally decreases the antitumor activity in ellipticine derivatives; for example, the IC_{50} values of **35a** and **38b** were 27.8 and 75.3 μM; **35d** and **38e** were 19.3 and 32.5 μM; and, **41** and **44** were 2.5 and 24 μM, respectively. An increase in the activity of the 9-hydroxy-6-methylellipticinium derivatives was an exception (**35c** and **38d**: 17.9 and 6.2 μM). In comparing the IC_{50} value of **46** (Entry 2) with those of **35a** and **38b**, the compounds with the shorter tether showed the highest activity (2.9, 27.8 and



Compound	R	R^1	R^2	X	Product
20	H	H	$COOCH_3$	O	**38a**, **39a**
35a	H	H	CH_3	O	**38b**, **39b**
35b	OH	H	CH_3	O	**38c**, **39c**
35c	OH	CH_3	CH_3	O	**38d**, **39d**
35d	OH	CH_3	CH_3	CH_2	**38e**, **39e**

Scheme 7. Synthesis of urea and nitrosourea analogues of ellipticine derivatives **38a**–**e**, **39a**–**e**.

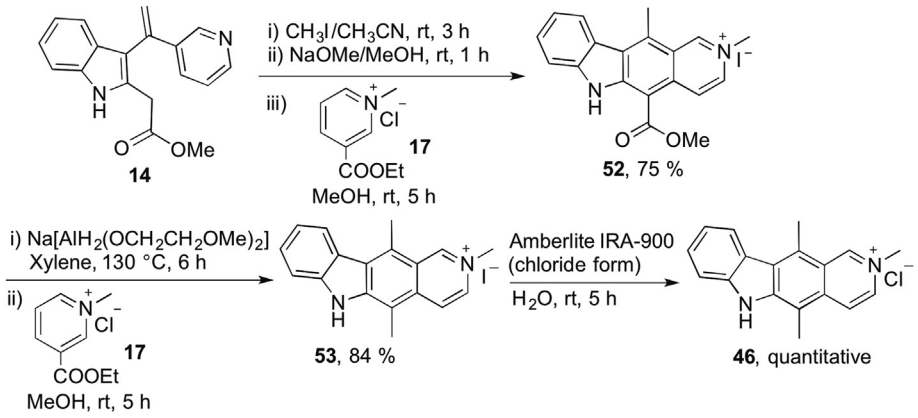


Scheme 8. Synthesis of urea and nitrosourea derivatives of pyridocarbazole-5-carboxylates **42**–**45**.

75.3 μM, respectively). As a result, it was predicted that an elongation of the tether would suppress the expression of the antitumor activity of ellipticine (**21**).

In a series of compounds with a (2-aminoethoxy)ethyl tether, replacement of the methyl group at the 5 position to the methyl ester group slightly reduced the antitumor activity (Fig. 2(a)); for example, the IC_{50} values of **35a** and **20** were 27.8 and 41.3 μM (Entries 3 and 7), respectively.

As expected, the introduction of an OH group at the 9-position increased the antitumor activity of elliptine as demonstrated by the MTT assay of pairs of compounds, **35a** and **35b** (IC_{50} = 27.8 and 17.7 μM), and **38b** and **38c** (IC_{50} = 75.3 and 29.4 μM). The reason for



Scheme 9. Synthesis of 2-methylellipticinium chloride **46**.

the increased activity by the 9-OH group is unclear. In addition, introduction of the methyl group at the 6-position also increased the activity, with the noted exception of **35b**,**c** (IC_{50} = 17.7 and 17.9 μM). For example, the IC_{50} values were 29.4 and 6.2 μM for **38c** and **38d**, and were 60 and 18.4 μM for **39c** and **39d**, respectively. Previously, we reported the synthesis of methylnitrosourea derivatives linked to a methidium chloride nucleus [19]. Despite low activity of methylnitrosourea (MNU) itself (Entry 24, $IC_{50} > 100$ μM), the introduction of a methylnitrosourea moiety in a molecule generally increases its antitumor activity. Although one of the methylnitrosourea derivatives synthesized in the present study increased the antitumor activity against HeLa S-3 cells, as shown in Table 1 (IC_{50} = 24 and 1.30 μM for **44** and **45**), the IC_{50} values of the other methylnitrosourea derivatives **39c** and **43** (60 and 15 μM, respectively), were larger than those of the corresponding methylurea derivatives **38c** and **42** (29.4 and 6.6 μM, respectively).

The quaternization of the 2-N atom of ellipticine by an alkyl group is useful for increasing solubility in water of the molecule, although some of quaternized compounds exhibited lower activity than ellipticine. The highest antitumor activity of all compounds tested was resulted from pyridocarbazole-5-carboxylate derivative **45**, and its IC_{50} value (1.30 μM) almost approximated that of ellipticine (**21**) (2.1 μM). In addition, it is remarkable that the activity of **45** (1.3 μM) is higher than that of **43** (15.0 μM) in ten times, in which only one methylene unit is added in the linker.

3. Conclusion

We developed a simple and efficient synthetic method of novel 4 ellipticine derivatives, linked by an oxydiethylene or polymethylene unit at the 2 position with a nitrosourea moiety **39b**–**e**, and that of the corresponding 8 intermediates **35a**–**d**, **38b**–**e** in good to high yields. In connection with the synthesis of these compounds, three kinds of novel pyridocarbazole-5-carboxylate derivatives, linked by an oxydiethylene or polymethylene unit at the 2 position with a nitrosourea moiety **39a**, **43**, **45**, and the corresponding 6 intermediates **20**, **38a**, **40**, **41**, **42**, **44**, were also synthesized. These compounds exhibited higher solubility in water than ellipticine (**21**) itself. In vitro antitumor activity of these compounds were evaluated by MTT assay on human cervical cancer cell line HeLa S-3. Some of these compounds exhibited potent antitumor activity (1.30–19.3 μM). Introduction of an OH group at the 9-position enhanced its antitumor activity and elongation of the tether at the 2-position had a tendency to suppress the antitumor activity of ellipticine (**21**) except in the case of **43** and **45**.

4. Experimental

4.1. Materials and methods

All solvents and chemicals were purchased from TCI and used without further purification. Reaction progress and compound purity were monitored using thin-layer chromatography (TLC) with hexane-ethyl acetate as the irrigating system and UV light at shorter wavelengths as the visualizer. Flash chromatography was performed using silica gel. Melting points were determined using a Yanaco micro-melting-point apparatus and uncorrected values were reported. ^1H and ^{13}C NMR spectra were recorded using a JNM ECP-500 (500 and 125 MHz) and a JNM ECP 300 (300 and 75 MHz) respectively. The ^1H, ^{13}C chemical shifts were referenced to TMS ($\delta = 0.00$) using the solvent residual peak as a reference and *J* values are reported in Hz. Mass spectra were recorded on a JEOL JMS-MS 700 mass spectrometer using xenon (6 kV, 10 mA) as the fast atom bombardment (FAB) gas.

4.2. Synthesis

4.2.1. 2-[2-(2-Chloroethoxy)ethyl]isoindole-1,3-dione (*2*) [20]

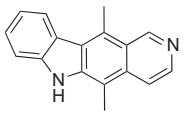
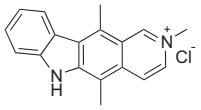
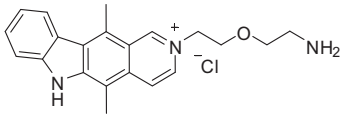
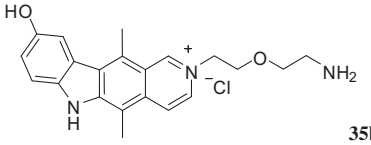
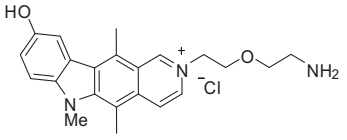
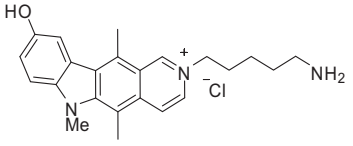
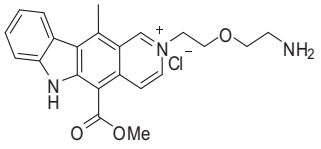
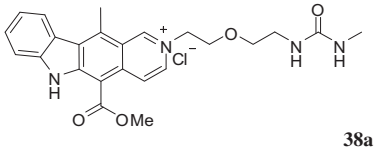
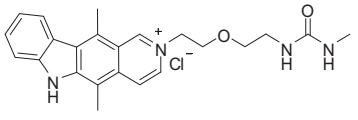
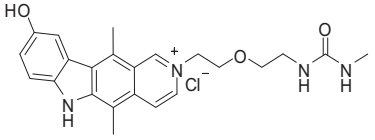
A mixture of bis(2-chloroethyl)ether (**1**, 4.6 g, 32.4 mmol) and potassium phthalimide (2.0 g, 10.8 mmol) was stirred at 130 °C for 20 h in DMF (1 mL). The residue was washed with water and extracted with $CHCl_3$ and dried ($MgSO_4$), and concentrated. The crude residue was purified by flash column chromatography ($CHCl_3$) to yield the compound **2**. 65% (lit [20]. 91%): mp 70.5–71.9 °C (lit [20]. 71.0–72.0 °C). ^1H NMR (500 MHz, $CDCl_3$): δ 7.55–7.22 (4H, m, Ar–H), 3.96 (2H, t, J = 6.0 Hz, CH_2N), 3.82 (2H, t, J = 7.5 Hz CH_2Cl), 3.68–3.60 (4H, m, 2 × CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.2, 133.9, 132.1, 123.2, 70.6, 67.9, 42.6, 37.1; MS (FAB) *m/z* 256 (M+H).

4.2.2. N-[2-(2-Chloroethoxy)ethyl]acetamide (*4a*)

A mixture of **2** (2 g, 7.9 mmol) and N_2H_4 (1.2 g, 23.7 mmol) was stirred in $CHCl_3$ for 3 h at 60 °C, and then the reaction mixture was slowly cooled to rt to get **3**. This crude material **3** was used for further reactions without purification.

To the crude **3**, Ac_2O (0.96 g, 9.4 mmol), triethylamine (9.5 g, 9.4 mmol) and DMAP (97 mg, 0.79 mmol) were added by dissolving in $CHCl_3$ (10 mL) through a dropping funnel and refluxed for 2 h. The solvent was removed under reduced pressure. The dark brown liquid remained was subjected to fractional distillation to give **4a** (45%) bp: 84–85 °C/7–8 mmHg. ^1H NMR (500 MHz, $CDCl_3$): δ 3.69–3.66 (4H, m, CH_2N, $ClCH_2$), 3.61 (2H, t, J = 5.0 Hz, CH_2O), 3.46 (2H, t, J = 5.0 Hz, OCH_2), 2.09 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.0, 66.7, 66.5, 46.5, 41.6, 21.0; MS (FAB) *m/z* 166 (M+H).

Table 1
In vitro antitumor activity (IC_{50}) of ellipticine and pyridocarbazole-5-carboxylate derivatives **20**, **21**, **35**, and **38**–**46** against HeLa S-3.

Entry	Compound	IC_{50} (μM)[a]
1	 **21**	2.1 ± 0.5
2	 **46**	2.90 ± 0.02
3	 **35a**	27.8 ± 2.5
4	 **35b**	17.7 ± 0.8
5	 **35c**	17.9 ± 2.9
6	 **35d**	19.3 ± 1.0
7	 **20**	41.3 ± 1.6
8	 **38a**	N/A[b]
9	 **38b**	75.3 ± 1.6
10	 **38c**	29.4 ± 4.3

(*continued on next page*)

Table 1 (*continued*)

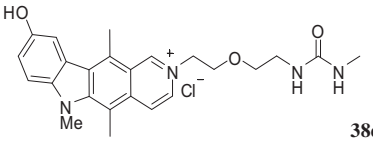
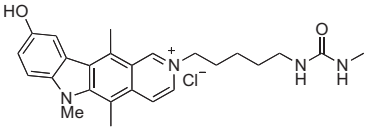
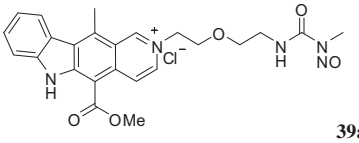
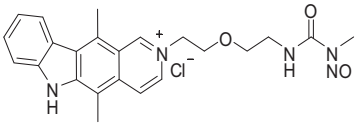
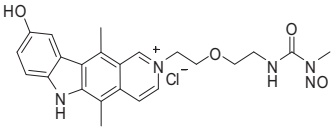
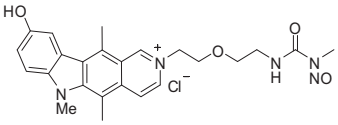
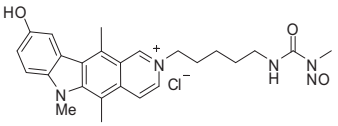
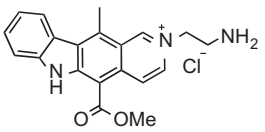
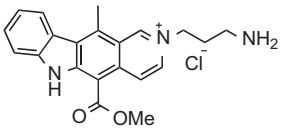
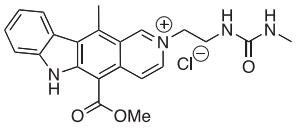
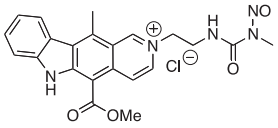
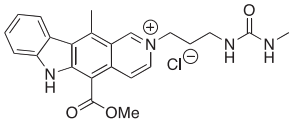
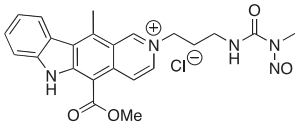
Entry	Compound	IC_{50} (μM)[a]
11	 **38d**	6.2 ± 0.2
12	 **38e**	32.5 ± 1.1
13	 **39a**	51.9 ± 2.9
14	 **39b**	N/A[b]
15	 **39c**	60 ± 6
16	 **39d**	18.4 ± 1.6
17	 **39e**	N/A[b]
18	 **40**	5.8 ± 1.8
19	 **41**	2.50 ± 0.08

Table 1 (*continued*)

Entry	Compound		IC_{50} (μM)[a]
20		**42**	6.6 ± 2.5
21		**43**	15.0 ± 3.7
22		**44**	24.0 ± 0.6
23		**45**	1.30 ± 0.03
24	Methylnitrosourea (MNU)		>100

[a] Values are reported as the means of three experiments; the standard deviations are given together.
[b] N/A: Not assayed.

*4.2.3. 1-[2-(2-Chloroethoxy)ethyl]-2,5-dimethyl-1H-pyrrole (**4b**)*

To the crude material **3** (prepared in above said lines), 2,5-hexadione (0.45 g, 7.9 mmol) and AcOH (0.19 g, 3.2 mmol) were added by dissolving in benzene (10 mL) through a dropping funnel and stirring was continued overnight at 50 °C. The solvent was removed in a rotary evaporator. The dark brown liquid remained was subjected to fractional distillation to get **4b** (55%) bp: 107–109 °C/6–7 mmHg. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.76 (2H, s, pyrrole), 3.96 (2H, t, J = 6.0 Hz, $C\underline{H}_2N$), 3.64 (4H, m, $C\underline{H}_2O$, $ClC\underline{H}_2$), 3.57 (2H, t, J = 5.5 Hz, $OC\underline{H}_2$), 2.23 (6H, s, 2 × CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 127.8, 105.3, 71.3, 70.6, 43.3, 42.7, 12.5; MS (FAB) *m/z* 202 (M+H).

*4.2.4. 2-[2-(2-Hydroxyethoxy)ethyl]isoindoline-1,3-dione (**6a**)*

2-(2-Aminoethoxy)-1-ethanol (**5**, 2.0 g, 19 mmol), phthalic anhydride (2.8 g, 19 mmol) and $CHCl_3$ (20 mL) were placed in a 100 mL reaction flask. The reaction mixture was refluxed for 4 h. After the volatile materials were removed under reduce pressure, the resulting reside was purified by flash column chromatography ($CHCl_3$) to yield **6a** (70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.86–7.85 (2H, m, Ar–H), 7.73–7.71 (2H, m, Ar–H), 3.91 (2H, t, J = 5.5 Hz, NCH_2), 3.75 (2H, t, J = 6.0 Hz, CH_2OH), 3.69 (2H, t, J = 5.5 Hz, OCH_2), 3.61 (2H, t, J = 5.5 Hz, $OC\underline{H}_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.4, 133.9, 131.9, 123.2, 72.1, 68.3, 61.6, 37.5.

*4.2.5. N-[2-(2-Hydroxyethoxy)ethyl]acetamide (**6b**)*

2-(2-Aminoethoxy)-1-ethanol (**5**, 2.0 g, 19 mmol), Ac_2O (2.1 g, 21 mmol), Et_3N (2.1 g, 21 mmol), DMAP (0.23 g, 1.9 mmol) and $CHCl_3$ (20 mL) were placed in a 100 mL reaction flask. The reaction mixture was refluxed for 2 h. After the volatile materials were removed under reduced pressure, the resulting reside was purified by flash column chromatography ($CHCl_3$) to yield **6b** (78%), ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.24 (1H, br, OH), 3.75 (2H, t, J = 4.8 Hz, $C\underline{H}_2N$), 3.60–3.55 (4H, m, $C\underline{H}_2OH$, $OC\underline{H}_2$), 3.47 (2H, t, J = 4.8 Hz, $OC\underline{H}_2$), 1.99 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6, 72.2, 69.7, 61.4, 39.2, 23.0.

*4.2.6. 2-[2-(2,5-Dimethyl-1H-pyrrol-1-yl)ethoxy]ethan-1-ol (**6c**)*

2-(2-Aminoethoxy)-1-ethanol (**5**, 2.0 g, 19 mmol), 2,5-hexadione (2.2 g, 19 mmol), AcOH (0.46 g, 7.6 mmol), and benzene (20 mL) were placed in a 100 mL reaction flask. The reaction mixture was allowed to stir overnight at 50 °C. After the volatile materials were removed under reduced pressure, the resulting reside was purified by flash column chromatography ($CHCl_3$) to get **6c** (75%), ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.77 (2H, s, pyrrole), 4.10 (2H, t, J = 6.0 Hz, CH_2N), 3.65–3.61 (4H, m, CH_2O, CH_2OH), 3.48 (2H, t, J = 4.5 Hz, OCH_2), 2.23 (6H, s, 2 × CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 127.8, 105.3, 72.5, 70.4, 61.7, 43.2, 12.5.

*4.2.7. Tert-butyl [2-(2-hydroxyethoxy)ethyl]carbamate (**6d**) [21]*

2-(2-Aminoethoxy)-1-ethanol (**5**, 2.0 g, 19 mmol), Boc_2O (4.6 g, 21 mmol), Et_3N (2.1 g, 21 mmol), DMAP (0.23 g, 1.9 mmol) and $CHCl_3$ (20 mL) were placed in a 100 mL reaction flask. The reaction mixture was allowed to stir for 3 h at rt. After the volatile materials were removed under reduced pressure, the resulting reside on flash column chromatography ($CHCl_3$) gives **6d** (85%), ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.74 (2H. t, J = 4.8 Hz, $C\underline{H}_2N$), 3.59–3.53 (4H, m, $C\underline{H}_2OH$, $OC\underline{H}_2$), 3.34 (2H, t, J = 5.1 Hz, $OC\underline{H}_2$), 1.45 (9H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.0, 79.3, 72.1, 70.2, 61.6, 40.3, 28.3.

*4.2.8. Preparation of N-protected [2-(2-hydroxyethoxy)ethyl]amine **7a–d***

A flame-dried 100 mL round-bottom flask containing a magnetic stirring bar was charged with **6a**, Et_3N (10 mmol), DMAP (0.1 mmol), and $CHCl_3$ (20 mL). The solution was cooled to 0 °C, and TsCl (10 mmol) dissolved in $CHCl_3$ was added to the solution over 10 min. The reaction mixture was stirred at rt for 3 h. After all the volatile materials were removed under reduced pressure, the crude residue was purified by flash column chromatography ($CHCl_3$) to yield **7a**. The **7b**, **7c** and **7d** were prepared in similar lines starting with **6b**, **6c** and **6d**, respectively.

7a: 70%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83–7.81 (2H, m, Pht), 7.73–7.69 (4H, m, Pht, Ts), 7.30–7.28 (2H, m, Ts); 4.08 (2H, t, J = 6 Hz, CH_2N), 3.81 (2H, t, J = 6 Hz, CH_2O), 3.65–3.63 (4H, m, OCH_2, CH_2O), 2.41 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.2, 144.7, 133.9, 132.9, 129.8, 127.9, 123.3, 69.1, 68.1, 68.0, 37.0, 21.6.

7b: 74%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (2H, d, J = 8.4 Hz, Ts), 7.33 (d, 2H, J = 8.1 Hz, Ts), 4.18 (2H, t, J = 4.5 Hz, CH_2OTs), 3.64 (2H, t, J = 4.5 Hz, CH_2N), 3.49 (2H, t, J = 4.8 Hz, OCH_2), 3.39 (2H, t, J = 4.5 Hz, OCH_2), 2.43 (3H, s, CH_3), 1.97 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2, 144.9, 133.0, 129.8, 127.7, 69.8, 69.0, 68.3, 39.0, 23.1, 21.5.

7c: 76%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (2H, d, J = 8.4 Hz, Ts), 7.34 (2H, d, J = 8.1 Hz, Ts), 5.73 (2H, s, CH), 4.10 (2H, t, J = 4.5 Hz, OCH_2), 3.87 (2H, t, J = 4.5 Hz, CH_2N), 3.56–3.52 (4H, m, OCH_2, OCH_2), 2.45 (3H, s, CH_3), 2.16 (6H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.8, 133.0, 129.8, 127.9, 127.7, 105.3, 70.7, 69.1, 68.7, 43.2, 21.6, 12.5.

7d: 78%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (2H, d, J = 8.4 Hz, Ts), 7.35 (2H, d, J = 8.1 Hz, Ts), 4.16 (2h, t, J = 4.5 Hz, CH_2OTs), 3.63 (2H, t, J = 4.5 Hz, CH_2N), 3.44 (2H, t, J = 4.8 Hz, OCH_2), 3.24 (2H, t, J = 4.5 Hz, OCH_2), 2.45 (3H, s, CH_3), 1.45 (9H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 144.9, 133.0, 129.8, 127.9, 79.3, 70.3, 69.0, 68.3, 28.4, 27.7, 21.6.

*4.2.9. 1-(Phenylsulfony1)indole (**9**) [22]*

To a solution of indole (**8**, 7.25 g, 61.8 mmol) in dry THF (75 mL) under N_2 at −78 °C, *n*-BuLi (1.58 M in hexane; 27.71 g, 64.9 mmol) was added dropwise via syringe over 15 min. The cooling bath was removed and the solution was stirred for 1 h while warming to 0 °C. The resulting indole anion precipitated as a very fine white solid in a cloudy colorless solution. After the suspension was recooled to −78 °C, neat benzenesulfonyl chloride (11.99 g, 67.9 mmol) was added via syringe over 20 min, keeping the internal temperature below −60 °C. The resulting colorless mixture was allowed to warm slowly to rt overnight, poured into 2% aq $NaHCO_3$ (500 mL), and extracted with Et_2O (4 × 200 mL). The combined extracts were washed with 2% aq $NaHCO_3$, H_2O, and brine, dried over K_2CO_3, filtered and concentrated. The crude product was washed with chilled MeOH, to get pure **9** as colorless crystals. Yield 85% (lit [22]. 91%): mp 77.2–72.9 °C. (lit [22]. 76.0–76.5 °C). ^{1}H NMR (500 MHz, $CDCl_3$); 7.92 (1H, d, J = 8.0 Hz, Ar–H), 7.79 (2H, d, J = 7.5 Hz, 2-indolyl-H, Ar–H), 7.48 (1H, d, J = 5.5 Hz, Ar–H), 7.47–7.31 (4H, m, Ar–H), 7.24 (1H, t, J = 8.5 Hz, Ar–H), 7.15 (1H, t, J = 8.5 Hz, Ar–H), 6.57 (1H, d, J = 5.5 Hz, 3-indolyl-H); ^{13}C NMR (125 MHz, $CDCl_3$): 138.2, 134.8, 133.7, 130.7, 129.2, 126.6, 126.2, 124.6, 123.3, 121.3, 113.4, 109.2; MS (FAB) *m/z* 257 ($M+H^+$).

*4.2.10. N-Benzenesulfonyl-2-methoxalylindole (**10**) [23]*

A suspension of diisopropylamine (4.44 g, 43.9 mmol) in THF (90 mL) was placed into a two-necked round-bottomed flask (1 L) equipped with a dropping funnel and an air condenser. The mixture was cooled to −78 °C and the solution containing *n*-BuLi (1.58 M in hexane; 17.47 g, 43.9 mmol) in THF was added into the flask slowly through the dropping funnel stirring the reaction mixture. The stirring was continued for 30 min under same temperature. Then, **9** (10 g, 38.9 mmol), dissolved in 10 mL of dry THF under N_2 and cooled to 0 °C, was added slowly into the reaction mixture and stirred again for 1.5 h at −78 °C then for 1 h at 15 °C. After the suspension was cooled to −50 °C, dimethyl oxalate (18.91 g, 159.5 mmol) dissolved in 50 mL of THF was added slowly over 30 min. The resulting reaction mixture was allowed to warm slowly to rt and stirred continuously for further 2 h. Then about 100 mL of ice-cold water was added slowly into the reaction mixture and stirred again for 10 min. The organic layer was separated and the aqueous layer was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brain, and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue obtained was washed with chilled MeOH to get pure **10** as colorless crystals. Yield: 61% (lit [23]. 70%). mp 108.5–109.8 °C. (lit [23]. 108.9–109.3 °C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (1H, d, J = 7.5 Hz, Ar–H), 7.71 (2H, d, J = 7.5 Hz, 3-indolyl-H, Ar–H), 7.50–7.18 (7H, m, Ar–H), 3.91 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.2, 161.4, 138.6, 136.8, 135.8, 134.1, 129.0, 128.7, 128.6, 127.1, 124.8, 123.4, 122.2, 115.3, 53.3; MS (FAB) *m/z* 344 ($M+H^+$).

*4.2.11. Synthesis of 2-(1H-indol-2-yl)-2-oxoacetic acid (**11**)*

A reaction flask was charged with **10** (3.5 g, 10.2 mmol) and K_2CO_3 (14.2 g, 0.103 mol) followed by MeOH (350 mL) and H_2O (110 mL). The reaction mixture was refluxed for 3 h and cooled to rt slowly. Then the reaction mixture was acidified using 10% aq HCl and extracted with EtOAc. The organic fraction was dried ($MgSO_4$) and the solvent was removed under reduced pressure and the residue obtained was washed with chilled MeOH to get pure **11**. Yield 85% mp 162.3–163.8 °C (lit [24]. 163–164 °C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (1H, s, NH), 7.78–7.17 (5H, m, 3-indolyl-H, Ar–H); ^{13}C NMR (125.77 MHz, CD_3OD): δ 178.6, 165.0, 140.6, 133.5, 128.8, 128.4, 124.5. 122.0, 116.3, 113.6. MS (FAB) *m/z* 189 ($M+H^+$).

*4.2.12. Synthesis of methyl 2-(1H-indol-2-yl)acetate (**13**)*

A mixture of **11** (1.2 g, 6.34 mmol) and KOH (6.7, 0.12 mol) taken in a reaction flask was dissolved in 150 mL of EtOH, to this stirred reaction mixture was slowly added 80% N_2H_4 (5 mL) at rt. Later the reaction mixture was refluxed for 5 h then the reaction mixture was heated to 150 °C and was maintained at the same temperature for 45 min. Then, the reaction mixture was cooled to 0 °C and acidified (pH = 2–3) with 5% aq HCl. The organic layer was separated and the aqueous layer was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brain, and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product **12** was dissolved in a mixture of MeOH (13 mL) and benzene (48 mL). To this solution, 4 mL of 8 mmol of $TMSCHN_2$ was added and the resulting reaction mixture was stirred for 30 min at rt. Then all the volatile materials were removed under reduced pressure, later the solid residue was dissolved in CH_2Cl_2 and washed with aq Na_2CO_3. The organic fraction was dried ($MgSO_4$) and the solvent removed under reduced pressure and the crude product was purified by flash column chromatography (EtOAc : Hexane = 3 : 2) to yield **13** (75%). mp 70.3–71.4 °C (lit [25]. 68.0–71.0 °C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.79 (1H, br-s, N–H), 7.54–7.12 (4H, m, Ar–H), 6.34 (1H, s, Ar–H), 3.82 (2H, s, CH_2), 3.74 (3H, s, CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$): δ 171.0, 136.3, 130.3, 128.1, 121.7, 120.1, 119.7, 110.8, 101.8, 52.3, 33.7. MS (FAB) *m/z* 189 (M+H).

*4.2.13. Synthesis of indolylpyridylethene derivatives (**14**) [26]*

A solution of0.709 g (3.75 mmol) of **13** and 1.73 g (14.2 mmol) of 3-acetylpyridine, and 4.25 g (43.3 mmol) concentrated H_2SO_4 in 20 mL of dry MeOH was refluxed for 6.5 h in N_2 atmosphere. The clear red solution was poured onto 500 g of ice. It was made alkaline with Na_2CO_3 and extracted with ether. The ether layer was

washed and dried ($MgSO_4$), and concentrated to dryness. The crude residue was purified by flash column chromatography to yield **14** (65%); mp 159–161 °C (lit [26]. mp 154–157 °C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.02 (1H, br, NH), 8.70 (1H, s, Ar–H), 8.54 (d, 1H, J = 4 Hz, Ar–H), 7.61 (d, 1H, J = 8.0 Hz, Ar–H), 7.30 (d, 1H, J = 8.0 Hz, Ar–H), 7.26–7.12 (m, 3H, Ar–H), 7.00 (dd, 1H, Ar–H), 5.83, 5.57 (s, s, 2H, ethenyl CH_2), 3.76 (s, 2H, CH_2), 3.72 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 148.7, 148.3, 138.9, 136.9, 135.5, 134.6, 128.5, 127.2, 123.1, 122.2, 120.0, 119.7, 117.1, 114.6, 110.8, 52.3, 32.0; MS (FAB) *m/z* 292 (M+H).

4.2.14. Synthesis of 4-nitrophenyl N-methylcarbamate (*37a*)

A flame-dried 100 mL round-bottom flask was charged with 4-nitrophenylchloroformate (**36**, 500 mg, 2.54 mmol), $MeNH_2$.HCl (170 mg, 2.54 mmol) and dioxane (30 mL). The reaction mixture was refluxed for 12 h under argon atmosphere. After the volatile materials were removed under reduced pressure the crude residue was dissolved in $CHCl_3$ and washed with aq $NaHCO_3$ and the organic phase was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (AcOEt:hexane = 1:1) to give **37a** (99%). mp 137–140 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.24 (2H, m, Ar–H), 7.30 (2H, m, Ar–H), 5.16 (1H, s, NH), 2.91 (3H, s, CH_3).

4.2.15. Synthesis of 4-nitrophenyl N-nitroso-N-methylcarbamate (*37b*)

A flame-dried 100 mL round-bottom flask was charged with sodium acetate (1.78 g, 21.7 mmol), 4-nitrophenyl *N*-methylcarbamate (**37a**, 1.78 g, 7.24 mmol), and CH_2Cl_2 (30 mL). The reaction mixture was cooled to −40 °C and was slowly added N_2O_4 (21.7 mmol) by dissolving in CH_2Cl_2. The reaction mixture was allowed to stir at the same temperature for 1 h. Later, the reaction mixture was warmed to rt and washed with aq $NaHCO_3$, brine and dried over Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography ($CHCl_3$/ether) to give **37b** (56%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (2H, d, J = 9.5 Hz, Ar–H), 7.54 (2H, q, J = 9.5 Hz, Ar–H), 3.27 (3H, s, CH_3); ^{13}C NMR (125 MHz $CDCl_3$): δ 154.8, 151.9, 145.9, 125.4, 122.2, 28.1; MS (FAB) *m/z* 226 (M^+).

4.2.16. Synthesis of 3-ethoxycarbonyl-1-methylpyridinium chloride (*17*) [27]

A reaction flask was charged with ethyl nicotinate **15** (10.87 g, 71.91 mmol), methyl iodide (30.62 g, 215.7 mmol), and benzene (40 mL). The reaction mixture was stirred at rt for 24 h. The volatile materials were removed under reduced pressure to get **16**. After drying, **16** (3.00 g, 10.7 mmol) was dissolved in 30 mL H_2O, and treated with Amberlite IRA-900 (Chloride form) 15 g with stirring for 5 h. After the water was removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield **17** (87%), mp 101.5–102.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.53 (1H, s, Ar–H), 9.18 (1H, d, J = 6.0 Hz, Ar–H), 8.96 (1H, d, J = 8.0 Hz, Ar–H), 8.26 (1H, t, J = 6.5 Hz, Ar–H), 4.46 (2H, q, J = 7.0 Hz, $C\underline{H}_2CH_3$), 4.44 (3H, s, CH_3), 1.38 (3H, t, J = 7.0 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (125.77 MHz, CD_3OD): δ 162.7, 149.7, 148.1, 146.3, 131.9, 129.4, 64.2, 14.5. MS (FAB) *m/z* 166 (M^+–Cl^-).

4.2.17. Synthesis of N-protected 2-(2-aminoethoxy)ethyl-5-metoxycarbonyl-11-methyl-6H-pyrido[4,3-b]carbazol-2-ium chloride derivatives (ellipticinium-analogues) *19a–c* using N-protected [2-(2-chloroethoxy)ethyl]amines *2*, *4a*, and *4b*; Path A

A flame-dried 100 mL round-bottom flask containing a magnetic stirring bar was charged with **14** (0.17 mmol), **2**, and DMF (1 mL). The reaction mixture was stirred at 110 °C for 15 h and cooled to rt, and then NaOMe (0.51 mmol) in MeOH was added and the reaction mixture was stirred for 1 h at rt. Later, 0.2 mmol of **17** was added by dissolving in MeOH and stirring was continued for 5 h at rt. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 7:3) to yield **19a** (10%). When **4a** was similarly used instead of **2**, a trace of **19b** was obtained. However, **4b** did not yield **19c** but a complex mixture.

4.2.18. Synthesis of N-protected 2-(2-aminoethoxy)ethyl-5-metoxycarbonyl-11-methyl-6H-pyrido[4,3-b]carbazol-2-ium chloride derivatives (ellipticinium-analogues) *19a–d* using N-protected 2-(2-aminoethoxy)ethyl tosylate *7a–d*; Path B

A flame-dried 100 mL round-bottom flask containing a magnetic stirring bar was charged with **14** (0.17 mmol), **7a**, and DMF (1 mL). The reaction mixture was stirred at 110 °C for 15 h. The reaction mixture was cool to ambient temperature, and then NaOMe (0.51 mmol) in MeOH was added and the reaction mixture was stirred for 1 h at rt. Later, 0.2 mmol of **17** was added by dissolving in MeOH and stirring was continued for 5 h at rt. After the volatile materials were removed under reduced pressure, the crude residue (crude **18a**) obtained was dissolved in 30 mL of water. To this solution, Amberlite IRA-900 (chloride form) (15 g) was added and the reaction mixture was stirred for 5 h at rt. After all the volatile material was removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 7:3) to yield **19a** (55%). Although **19b** (60%), and **19d** (52%) were obtained from **7b** and **7d**, the reaction of **7c** gave a complex mixture instead of the expected **19c**.

19a; decomp. 259 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.90 (1H, s, 1-H), 8.92 (1H, d, J = 7.0 Hz, 7-H), 8.48 (1H, d, J = 7.5 Hz, 4-H), 8.45 (1H, d, J = 7.5 Hz, 3-H), 7.91 (1H, d, J = 7.0 Hz, 10-H), 7.70 (1H, t, J = 7.0 Hz, 9-H), 7.45 (1H, t, J = 7.0 Hz, 8-H), 7.31–7.30 (4H, m, Pht-H), 4.86 (2H, t, J = 9.0 Hz, N^+CH_2), 4.12 (3H, s, CH_3), 3.97 (2H, t, J = 8.5 Hz, CH_2N), 3.69 (2H, t, J = 9.0 Hz, CH_2O), 3.63 (2H, t, J = 8.5 Hz, OCH_2), 3.26 (3H, s, CH_3); MS (FAB) *m/z* 508 (M^+–Cl^-); HRMS (FAB): Calcd for $C_{30}H_{26}N_3O_5^+$: 508.1872, found: 508.1857.

19b; decomp. 229 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.53 (1H, s, 1-H), 8.15 (1H, d, J = 7.0 Hz, 4-H), 7.69 (1H, d, J = 7.2 Hz, 3-H), 6.88 (1H, d, J = 7.5 Hz, 10-H), 6.73 (1H, t, J = 7.5 Hz, 8-H), 6.52 (1H, t, J = 7.5 Hz, 9-H), 6.35 (1H, d, J = 7.5 Hz, 7-H), 6.52 (1H, t, J = 7.5 Hz, 9-H), 6.35 (1H, d, J = 7.8 Hz, 7-H), 4.31 (2H, t, J = 4.5 Hz, N^+CH_2), 3.76 (2H, t, J = 4.8 Hz, CH_2N), 3.57 (3H, s, CH_3), 3.45 (2H, t, J = 4.5 Hz, CH_2O), 3.17 (2H, t, J = 4.8 Hz, OCH_2), 1.89 (3H, s, CH_3), 1.68 (3H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 173.2, 167.3, 147.0, 143.7, 141.4, 136.9, 135.0, 130.5, 129.7, 129.3, 125.4, 123.3, 122.7, 122.6, 122.3, 113.5, 102.6, 70.6, 70.0, 61.3, 53.1, 39.9, 22.3, 16.1; MS (FAB) *m/z* 420 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{24}H_{26}N_3O_4^+$: 420.1923, found: 420.1930.

19d; decomp. 210 °C; ^{1}H NMR (300 MHz, D_2O): δ 8.55 (1H, s, 1-H), 8.13 (1H, d, J = 7.0 Hz, 4-H), 7.69 (1H, d, J = 7.2 Hz, 3-H), 6.83 (1H, d, J = 7.5 Hz, 10-H), 6.74 (1H, t, J = 7.5 Hz, 8-H), 6.50 (1H, t, J = 7.5 Hz, 9-H), 6.36 (1H, d, J = 7.8 Hz, 7-H), 4.32 (2H, m, N^+CH_2), 3.78 (2H, m, CH_2N), 3.57 (3H, s, CH_3), 3.40 (2H, t, J = 4.5 Hz, CH_2O), 3.06 (2H, t, J = 7.5 Hz, OCH_2), 1.87 (3H, s, CH_3), 1.12 (9H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 167.2, 158.3, 146.9, 146.7, 143.4, 141.2, 136.7, 134.9, 130.5, 129.0, 125.3, 123.3, 122.7, 122.4, 13.4, 102.4, 80.0, 71.2, 70.2, 61.4, 53.2, 41.1, 28.6, 16.1; MS (FAB) *m/z* 478 (M^+–Cl^-); HRMS (FAB): Calcd for $C_{27}H_{32}N_3O_5^+$: 478.2342, found: 478.2335.

4.2.19. Deprotetion of NH_2 group in ellipticinium-analogues *19a*, *19b*, and *19d*; synthesis of *20*

Path A: A flame-dried 100 mL round-bottom flask was charged with 0.56 mmol of **19a** (or **19b**) and 9 M HCl/MeOH (15 mL). The reaction mixture was refluxed for 3 days. Purification of the crude

residue by ODS column chromatography (H_2O) gives **20** in trace from **19a** and 50% from **19b**.

Path B: A flame-dried 100 mL round-bottom flask was charged with **19d** (0.56 mmol) and 12 M aq HCl (5 mL). The reaction mixture was stirred for 5 h at rt. Purification of the crude residue by ODS column chromatography (H_2O) gives 85% of **20**.

20; decomp. 235 °C; ^{1}H NMR (300 MHz, D_2O): δ 8.71 (1H, s, 1-H), 8.31 (1H, d, J = 7.2 Hz, 4-H), 7.86 (1H, d, J = 7.2 Hz, 3-H), 7.05 (1H, d, J = 7.8 Hz, 10-H), 6.88 (1H, t, J = 7.2 Hz, 8-H), 6.68 (1H, t, J = 7.5 Hz, 9-H), 6.54 (1H, d, J = 7.2 Hz, 7-H), 4.45 (2H, t, J = 4.5 Hz, N^+CH_2), 3.81 (2H, t, J = 4.2 Hz, CH_2N), 3.65 (5H, m, CH_2O, CH_3), 3.09 (2H, t, J = 4.5 Hz, OCH_2), 2.08 (3H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 167.5, 147.4, 147.3, 143.9, 141.7, 137.2, 135.1, 130.7, 129.6, 125.6, 123.5, 123.0, 122.9, 122.6, 113.6, 102.8, 70.7, 68.1, 61.1, 53.2, 40.3, 16.4; MS (FAB) *m/z* 378 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{22}H_{24}N_3O_3^+$: 378.1818, found : 378.1830.

4.2.20. Synthesis of ellipticinium-analogue *38a* linked to a methylurea group

A reaction flask was charged with **20** (24 mg, 0.058 mmol), diisopropylethylamine (22 mg, 0.17 mmol), 4-nitrophenyl *N*-methylcarbamate (**37a**, 14 mg, 0.07 mmol), and DMF (1 mL). The reaction mixture was stirred at 0 °C for 7 h. After the volatile materials were removed under reduced pressure, the crude residue purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield **38a** (74%). Decomp. 220 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.55 (1H, s, 1-H), 8.18 (1H, d, J = 7.0 Hz, 4-H), 7.71 (1H, d, J = 7.2 Hz, 3-H), 6.91 (1H, d, J = 7.5 Hz, 10-H), 6.78 (1H, t, J = 7.5 Hz, 8-H), 6.56 (1H, t, J = 7.5 Hz, 9-H), 6.39 (1H, d, J = 7.2 Hz, 7-H), 4.32 (2H, m, N^+CH_2), 3.78 (2H, m, CH_2N), 3.60 (3H, s, CH_3), 3.42 (2H, t, J = 4.5 Hz, CH_2O), 3.10 (2H, t, J = 4.8 Hz, OCH_2), 2.33 (3H, s, CH_3), 1.91 (3H, s, CH_3); ^{13}C NMR (500 MHz, CD_3OD): δ 167.3, 161.7, 147.1, 143.6, 141.4, 136.9, 135.0, 125.4, 123.3, 122.8, 122.6, 113.5, 71.6, 70.1, 61.5, 61.3, 53.1, 40.6, 26.8, 20.8, 16.2, 14.4; MS (FAB) *m/z* 435 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{24}H_{27}N_4O_4^+$: 435.2032, found : 435.2045.

4.2.21. Synthesis of ellipticinium-analogues *39a* linked to a methylnitrosourea group

A reaction flask was charged with **20** (40 mg, 0.097 mmol), diisopropylethylamine (37 mg, 0.29 mmol), and 4-nitrophenyl-*N*-methyl-*N*-nitrosocarbamate (**37b**, 25 mg, 0.13 mmol) and DMF (1 mL). The reaction mixture was stirred at 0 °C for 7 h. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield **39a** (64%). Decomp. 215 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.67 (1H, s, 1-H), 8.24 (1H, d, J = 7.0 Hz, 4-H), 7.80 (1H, d, J = 7.2 Hz, 3-H), 7.00 (1H, d, J = 7.5 Hz, 10-H), 6.87 (1H, t, J = 7.5 Hz, 8-H), 6.65 (1H, t, J = 7.5 Hz, 9-H), 6.53 (1H, d, J = 7.8 Hz, 7-H), 4.41 (2H, m, N^+CH_2), 3.85 (2H, m, CH_2N), 3.70 (3H, s, CH_3), 3.58 (2H, t, J = 4.5 Hz, CH_2O), 3.40 (2H, t, J = 4.8 Hz, OCH_2), 2.60 (3H, s, CH_3), 2.05 (3H, s, CH_3). MS (FAB) *m/z* 464 (M^+– Cl^-); HRMS (FAB): Calcd for $C_{24}H_{26}N_5O_5^+$: 464.1934, found: 464.1937.

4.2.22. Synthesis of 2-(N-Boc-2-aminoethoxy)ethylellipticinium chloride (*34a*)

A reaction flask was charged with **21** (0.17 mmol), **7d** (0.51 mmol) and DMF (1 mL). The reaction mixture was stirred at 80 °C for 12 h. After the volatile materials were removed under reduced pressure, the crude residue (crude **33a**) was treated with 15 g of Amberlite IRA-900 (Chloride form) in water at rt for 5 h. The solvent was removed from the reaction mixture and the crude residue was purified by ODS column chromatography (H_2O/MeOH = 7:3) to afford **34a** (52%) as a white solid. Decomp. 219 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.25 (1H, s, 1-H), 7.33 (1H, m, 4-H), 6.94 (1H, m, 3-H), 6.63 (2H, m, 8/10-H), 6.34–6.30 (2H, m, 7/9-H), 4.16 (2H, m, N^+CH_2), 3.72 (2H, m, CH_2N), 3.40(2H, t, J = 5.0 Hz, CH_2O), 3.05 (2H, t, J = 5.0 Hz, OCH_2), 1.72 (3H, s, CH_3), 1.55 (3H, s, CH_3), 1.09 (9H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 158.3, 146.4, 145.0, 143.2, 133.6, 133.5, 131.7, 129.4, 126.6, 124.6, 122.8, 121.6, 120.9, 120.7, 112.0, 110.7, 80.0, 71.1, 70.3, 61.0, 41.1, 28.6, 15.1, 12.0; MS (FAB) *m/z* 434 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{26}H_{32}N_3O_3^+$: 434.2444, found: 434.2469.

4.2.23. Synthesis of 2-(2-aminoethoxy)ethylellipticinium chloride *35a*

A reaction flask was charged with **34a** (0.20 mmol) and 12 M aq HCl (5 mL). The reaction mixture was stirred at rt for 5 h. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 7:3) to afford **35a** (86%) as a white solid. Decomp. 260 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.54 (1H, s, 1-H), 7.52 (1H, m, 4-H), 7.24 (1H, m, 3-H), 7.03 (1H, m, 10-H), 6.85 (1H, m, 8-H), 6.60–6.56 (2H, m, 7/9-H), 4.33 (2H, m, N^+CH_2), 3.84 (2H, m, CH_2N), 3.64 (2H, m, CH_2O), 3.07 (2H, m, OCH_2), 2.07 (3H, s, CH_3), 1.80 (3H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 164.8, 147.6, 144.3, 134.9, 134.6, 132.1, 130.0, 125.5, 122.3, 122.0, 121.4, 112.7, 111.6, 70.9, 68.1, 60.9, 40.3, 36.9, 31.6, 15.5, 12.1; MS (FAB) *m/z* 334 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{21}H_{24}N_3O^+$: 334.1919, found: 334.1916.

4.2.24. Synthesis of ellipticinium chloride derivative *38b* linked to a methylnitrosourea group

A reaction flask was charged with **35a** (40 mg, 0.11 mmol), diisopropylethylamine (42 mg, 0.33 mmol), 4-nitrophenyl-*N*-methylcarbamate (**37a**, 25 mg, 0.13 mmol), and DMF (1 mL). The reaction mixture was stirred at 0 °C for 7 h under N_2 atmosphere. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield 45% of **38b**. Decomp. 226 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.18 (1H, s, 1-H), 7.27 (1H, d, J = 7.0 Hz, 4-H), 6.86 (1H, d, J = 6.0 Hz, 3-H), 6.57 (2H, m, 8/10-H), 6.29–6.23 (2H, m, 7/9-H), 4.11 (2H, m, N^+CH_2), 3.70 (2H, m, CH_2N), 3.39 (2H, t, J = 5.5 Hz, CH_2O), 3.07 (2H, t, J = 5.0 Hz, OCH_2), 2.35 (3H, s, $NHC\underline{H}_3$), 1.66 (3H, s, CH_3), 1.50 (3H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 161.7, 146.9, 145.6, 143.7, 134.1, 134.0, 131.8, 129.6, 127.3, 125.0, 123.3, 121.9, 121.4, 121.0, 112.3, 111.1, 71.6, 70.3, 61.1, 40.7, 26.9, 15.3, 12.0; MS (FAB) *m/z* 391 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{23}H_{27}N_4O_2^+$: 391.2134, found: 391.2131.

4.2.25. Synthesis of ellipticinium chloride derivative *39b* linked to a methylnitrosourea group

A reaction flask was charged with **35a** (50 mg, 0.14 mmol), diisopropylethylamine (52 mg, 0.4 mmol), 4-nitrophenyl-*N*-methyl-*N*-nitrosocarbamate (**37b**, 36 mg, 0.16 mmol), and DMF (1 mL). The reaction mixture was stirred at 0 °C for 7 h. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield 43% of **39b**. Decomp. 220 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.66 (1H, s, 1-H), 7.65 (1H, d, J = 7.0 Hz, 4-H), 7.45 (1H, d, J = 7.0 Hz, 3-H), 7.38 (1H, m, 10-H), 7.11 (1H, m, 8-H), 6.85 (2H, d, J = 6.0 Hz, 7/9-H), 4.39 (2H, m, N^+CH_2), 3.83 (2H, m, CH_2N), 3.54 (2H, t, J = 4.5 Hz, CH_2O), 3.28 (2H, t, J = 4.5 Hz, OCH_2), 2.29 (3H, s, CH_3), 2.12 (3H, s, CH_3), 2.08 (3H, s, CH_3); MS (FAB) *m/z* 421 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{23}H_{26}N_5O_3^+$: 420.2036, found: 420.2045.

4.2.26. Synthesis of 9-formyl-ellipticine (*22*)

A reaction flask was charged with **21** (30.0 mg, 0.0122 mmol), hexamethylenetetramine (200 mg, 1.42 mmol), and TFA (5 mL). The reaction mixture was refluxed for 20 min and cooled to rt. The resulted reaction mixture was basified with Na_2CO_3, and extracted with $CHCl_3$. The organic layer was washed with brine and dried over Na_2SO_4, and then filtered and evaporated. The crude residue

was purified by SiO_2 column chromatography ($CHCl_3$/MeOH = 9:1) to yield 92% of **22** (lit [18]. 97%). Decomp. 360 °C (lit [18] decomp. >350 °C); ^{1}H NMR (500 MHz, CD_3CD): δ 10.6 (1H, s, CHO), 9.66 (1H, s, 1-H), 8.89 (1H, s, 10-H), 8.40 (1H, d, *J* = 8.4 Hz, 3-H), 8.06 (2H, dd, N–H, 8-H), 7.65 (1H, d, *J* = 8.4 Hz, 7-H), 2.85 (3H, s, CH_3); ^{13}C NMR (125.77 MHz, DMSO-*d6*): δ 191.9, 149.8, 146.7, 141.0, 140.7, 132.9, 128.8, 128.5, 128.1, 127.2, 123.2, 122.8, 116.0, 115.7, 111.0, 109.4, 14.3, 11.9. MS (FAB) *m/z* 275 ($M+H^+$).

4.2.27. Synthesis of 9-hydroxyellipticine (*23*)

A reaction flask was charged with **22** (100 mg, 0.36 mmol), MeOH (25 mL), 31% H_2O_2 (0.2 mL), conc. H_2SO_4 (2 drops). The reaction mixture was refluxed for 4 h and cooled to rt. The reaction mixture was basified with sat. Na_2CO_3 aq, and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated. The crude residue was purified by SiO_2 column chromatography ($CHCl_3$/MeOH = 9:1) to yield **23** in 55% yield (lit [18] 91%). Decomp. 355 °C (lit [18] decomp. >350 °C); ^{1}H NMR (500 MHz, CDMSO-d_6): δ 11.04 (1H, s, NH), 9.64 (1H, s, 1-H), 9.09, (1H, s, OH), 8.37 (1H, d, *J* = 6.0 Hz, 3-H), 7.86 (1H, d, *J* = 6.0 Hz, 4-H), 7.75 (1H, s, 10-H), 7.36 (1H, d, *J* = 8.5 Hz, 7-H), 7.00 (1H, d, *J* = 8.5 Hz, 8-H), 3.19 (3H, S, 11-CH_3), 2.73 (3H, s, 5-CH_3); MS (FAB) *m/z* 263 ($M+H^+$).

4.2.28. Synthesis of 2-(N-Boc-2-aminoethoxy)ethyl-9-hydroxyellipticinium chloride (*34b*)

A reaction flask was charged with **23** (0.17 mmol), **7d** (0.51 mmol) and DMF (2 mL). The reaction mixture was stirred at 80 °C for 12 h. After the volatile materials were removed under reduced pressure, the crude residue was treated with 15 g of Amberlite IRA-900 (Chloride form) in water at rt for 5 h. The solvent was removed from the reaction mixture and the crude residue (crude **33b**) was purified by ODS column chromatography (H_2O/MeOH = 7:3) to afford **34b** (52%) as a white solid. Decomp. 250 °C; ^{1}H NMR (500 MHz, D_2O): δ 9.23 (1H, s, 1-H), 7.94 (1H, m, 4-H), 7.75 (1H, m, 3-H), 7.00–6.96 (2H, m, 8/10-H), 6.73 (1H, m, 7-H), 4.62 (2H, m, N^+CH_2), 3.87 (2H, m, CH_2N), 3.40 (2H, m, CH_2O), 3.09 (2H, m, OCH_2), 2.65 (3H, s, CH_3), 2.32 (3H, s, CH_3), 1.20 (9H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 158.3, 153.0, 146.8, 146.1, 137.4, 134.2, 133.7, 131.6, 127.3, 124.0, 121.0, 120.8, 118.2, 112.7, 110.8, 110.4, 80.0, 71.1, 70.3, 61.0, 41.1, 28.6, 15.2, 12.0; MS (FAB) *m/z* 450 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{26}H_{31}N_4O_3^+$: 449.2315, found: 434.2317.

4.2.29. Synthesis of 2-(2-aminoethoxy)ethyl-9-hydroxyellipticinium chloride (*35b*)

A reaction flask was charged with **34b** (0.22 mmol) and 12 M HCl (5 mL). The reaction mixture was stirred at 80 °C for 12 h. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 9:1) to afford **35b** (85%). Decomp. 295 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.29 (1H, s, 1-H), 7.35 (1H, m, 4-H), 6.83 (1H, m, 3-H), 5.90 (1H, m, 10-H), 5.76 (1H, m, 8-H), 5.50 (1H, m, 7-H), 4.21 (2H, m, N^+CH_2), 3.75 (2H, m, CH_2N), 3.61 (2H, m, CH_2O), 3.06 (2H, m, OCH_2), 1.48 (3H, s, CH_3), 1.36 (3H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 152.0, 146.8, 144.7, 136.0, 133.4, 132.0, 131.1, 125.8, 122.7, 119.6, 119.4, 117.3, 112.0, 109.7, 109.6, 68.9, 66.4, 58.6, 38.2, 14.9, 11.9; MS (FAB) *m/z* 350 (M^+–Cl^-); HRMS (FAB): Calcd for $C_{26}H_{31}N_4O_3^+$: 350.1869, found: 350.1866.

4.2.30. Synthesis of 9-hydroxyellipticinium chloride derivative *38c* linked to a methylurea group

A reaction flask was charged with **35b** (50 mg, 0.13 mmol), diisopropylethylamine (50 mg, 0.39 mmol), 4-nitrophenyl-*N*-methylcarbamate (**37a**, 31 mg, 0.16 mmol), and DMF (1 mL). The reaction mixture was stirred at 0 °C for 7 h under N_2 atmosphere. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield **38c** (43%). Decomp. 255 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.16 (1H, s, 1-H), 7.25 (1H, m, 4-H), 6.74 (1H, m, 3-H), 5.86 (1H, m, 10-H), 5.74 (1H, m, 8-H), 5.46 (1H, m, 7-H), 4.12 (2H, m, N^+CH_2), 3.69 (2H, m, CH_2N), 3.37 (2H, m, CH_2O), 3.08 (2H, m, OCH_2), 2.44 (3H, s, $NHCH_3$), 1.39 (3H, s, CH_3), 1.33 (3H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 161.7, 153.5, 147.4, 138.0, 135.0, 134.1, 131.7, 124.6, 121.4, 121.0, 118.6, 113.0, 111.2, 110.9, 71.6, 70.3, 61.0, 40.6, 26.8, 23.6, 15.2, 14.4, 11.9; MS (FAB) *m/z* 408 (M^+–Cl^-); HRMS (FAB): Calcd for $C_{23}H_{27}N_4O_3^+$: 407.2083, found: 407.2075.

4.2.31. Synthesis of 9-hydroxyellipticinium chloride derivative *39c* linked to a methylnitrosourea group

A reaction flask was charged with **35b** (50 mg, 0.13 mmol), diisopropylethylamine (50 mg, 0.39 mmol), 4-nitrophenyl-*N*-methyl-*N*-nitrosocarbamate (**37b**, 38 mg, 0.17 mmol), and DMF (1 mL). The reaction mixture was stirred at 0 °C for 7 h. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield **39c** (39%). Decomp. 244 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.63 (1H, s, 1-H), 7.62 (1H, m, 4-H), 7.35 (1H, m, 3-H), 6.54 (1H, m, 10-H), 6.42 (2H, m, 7/8-H), 4.40 (2H, m, N^+CH_2), 3.84 (2H, m, CH_2N), 3.54 (2H, m, CH_2O), 3.29 (2H, m, OCH_2), 2.21 (3H, s, $NHCH_3$) 2.07 (3H, s, CH_3), 1.94 (3H, s, CH_3); MS (FAB) *m/z* 437 (M^+–Cl^-); HRMS (FAB): Calcd for $C_{23}H_{26}N_5O_3^+$: 436.1985, found: 436.1966.

4.2.32. Synthesis of 1,4,9-trimethylcarbazole-3-carbaldehyde (*25*)

KOH (3.6 g, 64.1 mmol), **24** (3.6 g, 16.1 mmol), and DMSO (20 mL) were placed in a 100 mL reaction flask and the mixture was stirred at rt for 30 min. To this solution, methyl iodide (4.6 g, 32.4 mmol) was added and stirring was continued for 10 min. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc. The residue after removal of the solvent was purified by column chromatography to yield **25** (88%). Decomp. 158–160 °C (lit [28]. 157–161 °C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.39 (1H, s, CHO), 8.23 (1H, d, *J* = 7.5 Hz, 5-H), 7.64 (1H, s, 2-H), 7.51 (1H, d, *J* = 7.5 Hz, 8-H), 7.41 (1H, t, *J* = 7.5 Hz, 7-H), 7.30 (1H, t, *J* = 7.5 Hz, 6-H), 4.07 (3H, s, N–CH_3), 3.10 (3H, s, 1-CH_3), 2.81 (3H, s, 4-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.2, 142.6, 142.0, 136.4, 131.2, 126.0, 125.6, 123.6, 123.0, 122.7, 120.1, 117.9, 108.9, 32.1, 20.3, 14.9; MS (FAB) *m/z* 237 ($M+H^+$).

4.2.33. Synthesis of *26*

To a 50 mL round-bottom flask equipped with a magnetic stir bar and condenser, was added **25** (0.05 g, 0.21 mmol), aminoacetaldehyde diethyl acetal, (0.033 g, 0.246 mmol), and benzene (20 mL). This yellow solution was stirred and heated under reflux for 2 h. After completion of the reaction as indicated by TLC, the solvent was removed under reduced pressure. The residue obtained was evaporated to dryness to yield brown oil. This brown oil was dissolved in CH_3OH and treated with $NaBH_4$ (0.08 g, 2.23 mmol) and the reaction mixture was stirred at rt for 2 h. Then, the solvent was removed under reduced pressure and the obtained crude residue was dissolved in 3 mL of dry pyridine and a solution of 2-nitrobenzenesulfonyl chloride (0.15 g, 0.669 mmol) dissolved in 10 mL of dry CH_3CN was added while the solution was stirring. The resulting solution was allowed to stir at rt for 8 h. The solvent was removed under reduced pressure after completion of the reaction, and the residue obtained was extracted with CH_2Cl_2 and washed with water, dried over $MgSO_4$, filtered, and evaporated to yield orange oil, which is then dissolved in chilled CH_3OH and refrigerated overnight to get yellow solid **26** (54%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.17–6.99 (9H, m, Ar–H), 4.86 (2H, s, $ArCH_2N$), 4.55 (1H, t, *J* = 5.5 Hz, $CH(OEt)_2$), 4.05 (3H, s, N–CH_3), 3.61 (2H, d,

J = 5.5 Hz, $C\underline{H}_2CH(OEt)_2$), 3.40 (4H, q, J = 7.0 Hz, $O(C\underline{H}_2CH_3)_2$), 2.72 (3H, s, 1-$CH_3$), 2.69 (3H, s, 4-$CH_3$), 1.14 (6H, t, J = 7.0 Hz, $O(CH_2C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 147.8, 141.8, 139.3, 134.3, 132.8, 131.1, 131.0, 130.8, 130.6, 124.9, 123.8, 123.3, 123.2, 122.7, 122.4, 118.9, 117.4, 108.3, 102.1, 63.3, 50.3, 49.3, 32.2, 20.2, 16.0, 15.2; MS (FAB) m/z 540 ($M+H^+$).

*4.2.34. Synthesis of 6-methylellipticine (**27**)*

To a 100 mL round-bottom flask equipped with a magnetic stirring bar and condenser, was added **26** (100 mg, 0.19 mmol), 6 M HCl/dioxane (1:4) 20 mL. This slurry was stirred and heated to reflux with an oil bath for 3 h. After completion of the reaction as indicated by TLC, pH of the reaction mixture was adjusted to 8 with Na_2CO_3 and extracted with 3 × 50 mL of CH_2Cl_2. The combined extracts were dried over $MgSO_4$, filtered, and evaporated to yield brownish yellow oil. The residue obtained was purified by silica gel chromatography to yield **27** (86%). mp 206.4–208.1 °C (lit [29]. 207–208 °C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.56 (1H, s, 1-H), 8.45 (1H, d, J = 6.0 Hz, Ar–H), 8.21 (1H, d, J = 8.0 Hz, Ar–H), 7.89 (1H, d, J = 6.0 Hz, Ar–H), 7.51 (1H, t, J = 8.0 Hz, Ar–H), 7.25–731 (2H, m, Ar–H), 3.93 (3H, s, N–CH_3), 3.02 (3H, s, 11-CH_3), 2.86 (3H, s, 5-CH_3); ^{13}C NMR (DMSO, 500 MHz): δ 149.5, 144.9, 141.6, 140.7, 134.2, 128.6, 127.0, 124.5, 123.7, 123.3, 122.5, 119.5, 115.8, 108.4, 108.3, 33.7, 14.4, 13.7. MS (FAB) m/z 261 (M+H).

*4.2.35. Synthesis of 9-formyl-6-methylellipticine (**28**)*

To a round-bottom flask equipped with a magnetic stir bar and condenser, was added **27** (30.0 mg, 0.11 mmol), hexamethylenetetramine (200 mg, 1.42 mmol), TFA (5 mL). This slurry was stirred and heated to reflux with an oil bath for 20 min. After completion of the reaction as indicated by TLC, the reaction mixture was basified with Na_2CO_3 and extracted with 3 × 50 mL of CH_2Cl_2. The combined extracts were dried over $MgSO_4$, filtered, and evaporated to dryness. The residue obtained was purified by silica gel chromatography to yield **28** (84%) (lit [18]. 96%). mp 222–223 °C (lit [18]. 223–226 °C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 10.0 (1H, s, CHO), 8.54–8.50 (2H, m, 10/1-H), 7.99 (1H, d, J = 8.5 Hz, 3-H), 7.99 (1H, d, J = 8.5 Hz, 3-H), 7.79 (1H, m, 8-H), 7.20 (1H, d, J = 8.5 Hz, 7-H), 3.96 (3H, s, N–CH_3), 3.00 (3H, s, 11-CH_3), 2.86 (3H, s, 5-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 191.2, 149.6, 148.5, 141.5, 134.6, 129.4, 129.3, 128.8, 125.7, 123.6, 123.5, 122.8, 115.9, 109.6, 108.2, 34.0, 14.5, 13.6. MS (FAB) m/z 288 (M+H).

*4.2.36. Synthesis of 9-hydroxy-6-methylellipticine (**29**)*

To a reaction flask equipped with a magnetic stir bar and condenser, was added **28** (100.0 mg, 0.35 mmol), CH_3OH (25 mL), conc. H_2SO_4 (2 drops), and 0.2 mL of 31% H_2O_2. This slurry was stirred and heated to reflux with an oil bath for 4 h. The reaction mixture was basified with Na_2CO_3 and extracted with 3 × 150 mL of CH_2Cl_2. The combined extracts were dried over $MgSO_4$, filtered, and evaporated to dryness. The residue obtained was purified by silica gel chromatography to get **29** (53%) (lit [18]. 93%). Decomp: 345 °C (it [9]. >300 °C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.62 (1H, s, 1-H), 9.18 (1H, s, OH), 8.39 (1H, m, 3-H), 7.94 (1H, d, J = 6.0 Hz, 4-H), 7.76 (1H, s, 10-H), 7.42 (1H, d, J = 8.5 Hz, 7-H), 7.06 (1H, d, J = 8.5 Hz, 8-H), 4.05 (3H, s, N–CH_3), 3.14 (3H, s, 11-CH_3), 2.97 (3H, s, 5-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 151.0, 149.4, 141.7, 140.5, 138.5, 133.4, 128.2, 123.9, 123.1, 121.7, 115.8, 115.6, 109.5, 109.4, 108.5, 33.7, 14.1, 13.3. MS (FAB) m/z 277 (M+H).

*4.2.37. Synthesis of 2-(N-Boc-2-aminoethoxy)ethyl-9-hydroxy-6-methylellipticinium chloride (**34c**)*

A reaction flask was charged with **29** (0.17 mmol), **7d** (0.51 mmol) and DMF (2 mL). The reaction mixture was stirred at 80 °C for 12 h. After the volatile materials were removed under reduced pressure, the crude residue was treated with 15 g of Amberlite IRA-900 (Chloride form) in water at rt for 5 h. The solvent was removed from the reaction mixture and the residue (crude **33c**) was purified by ODS column chromatography (H_2O/MeOH = 7:3) to afford **34c** (52%) as a white solid. mp 195 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.45 (1H, s, 1-H), 7.64 (1H, m, 4-H), 7.33 (1H, m, 3-H), 6.04 (2H, m, 8/10-H), 5.77 (1H, m, 7-H), 4.31 (2H, m, N^+CH_2), 3.37 (2H, m, CH_2N), 3.00 (2H, m, CH_2O), 2.67 (2H, m, OCH_2), 1.70 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.15 (9H, s, $-C(CH_3)_3$); ^{13}C NMR (125 MHz, CD_3OD): δ 158.4, 153.4, 146.5, 145.5, 139.1, 134.9, 134.0, 132.0, 127.5, 123.1, 121.1, 121.0, 117.9, 111.1, 110.8, 110.3, 80.0, 71.2, 70.3, 61.0, 41.1, 34.0, 28.7, 15.2, 14.0; MS (FAB) m/z 464 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{27}H_{34}N_3O_4^+$: 464.2549, found: 464.2561.

*4.2.38. Synthesis of 2-(2-aminoethoxy)ethyl-9-hydroxy-6-methylellipticinium chloride (**35c**)*

A reaction flask was charged with **34c** (0.25 mmol), and 12 M HCl (5 mL). The reaction mixture was stirred at rt for 5 h. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 9:1) to afford **35c** (86%). Decomp. 205 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.54 (1H, s, 1-H), 7.69 (1H, m, 4-H), 7.40 (1H, m, 3-H), 6.13 (1H, m, 10-H), 6.01 (1H, m, 8-H), 5.79 (1H, m, 7-H), 4.37 (2H, m, N^+CH_2), 3.82 (2H, m, CH_2N), 3.62 (2H, m, CH_2O), 3.06 (2H, m, OCH_2), 1.79 (3H, s, N–CH_3), 1.79 (3H, s, 11-CH_3), 1.57 (3H, s, 5-CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 153.8, 147.1, 146.5, 139.9, 135.4, 134.7, 132.1, 128.4, 123.7, 121.6, 121.3, 118.3, 111.7, 111.3, 110.9, 70.8, 68.1, 60.7, 40.3, 34.3, 15.6, 14.2; MS (FAB) m/z 365 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{22}H_{26}N_3O_2^+$: 364.2025, found: 364.2021.

*4.2.39. Synthesis of 9-hydroxy-6-methylellipticine-derivative **38d** linked to a methylurea group*

A reaction flask was charged with **35c** (50 mg, 0.13 mmol), diisopropylethylamine (48 mg, 0.37 mmol) 4-nitrophenyl-*N*-methylcarbamate (**37a**, 29 mg, 0.15 mmol) and DMF (1 mL). The reaction mixture was stirred at 0 °C for 7 h under N_2 atmosphere. After the volatile materials were removed under reduced pressure, the crude residue purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield **38d** (47%). Decomp. 203 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.36 (1H, s, 1-H), 7.58 (1H, m, 4-H), 7.26 (1H, m, 3-H), 5.99 (2H, m, 8/10-H), 5.62 (1H, m, 7-H), 4.26 (2H, m, N^+CH_2), 3.72 (2H, m, CH_2N), 3.35 (2H, m, CH_2O), 3.04 (2H, m, OCH_2), 2.62 (3H, s, N–$C\underline{H}_3$), 2.42 (3H, s, NH$C\underline{H}_3$), 1.64 (3H, s, 11-CH_3), 1.35 (3H, s, 5-CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 161.8, 153.4, 146.5, 145.4, 139.0, 134.8, 133.9, 132.1, 127.4, 123.0, 121.0, 117.9, 111.0, 110.8, 110.3, 71.7, 70.3, 61.0, 40.8, 36.9, 33.9, 27.0, 15.2, 13.9; MS (FAB) m/z 422 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{24}H_{29}N_4O_3^+$: 421.2240, found: 421.2220.

*4.2.40. Synthesis of 9-hydroxy-6-methylellipticine-derivative **39d** linked to a methylnitrosourea group*

A reaction flask was charged with **35c** (50 mg, 0.13 mmol), diisopropylethylamine (48 mg, 0.38 mmol), 4-nitrophenyl-*N*-methyl-*N*-nitrosocarbamate (**37b**, 37 mg, 0.17 mmol) and DMF (1 mL). The reaction mixture was stirred at 0 °C for 7 h. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield **39d** (40%). Decomp. 200 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.52 (1H, s, 1-H), 7.68 (1H, m, 4-H), 7.38 (1H, m, 3-H), 6.16 (2H, m, 8/10-H), 5.94 (1H, m, 7-H), 4.37 (2H, m, N^+CH_2), 3.82 (2H, m, CH_2N), 3.54 (2H, m, CH_2O), 3.36 (2H, m, OCH_2), 2.85 (3H, s, N–$C\underline{H}_3$), 2.64 (3H, s, NH$C\underline{H}_3$), 1.85 (3H, s, 11-CH_3), 1.60 (3H, s, 5-CH_3); MS (FAB) m/z 451 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{24}H_{28}N_5O_4^+$: 450.2141, found: 450.2129.

*4.2.41. Synthesis of tert-butyl 5-hydroxypentylcarbamate (**31**) and the corresponding O-tosylate **32***

5-Amino-1-pentanol (**30**, 2 g, 19 mmol), Boc_2O (4.5 g, 20 mmol), Et_3N (2.1 g, 21 mmol), DMAP (0.23 g, 1.9 mmol) and $CHCl_3$ (20 mL) were placed in a 100 mL reaction flask. The reaction mixture was allowed to stir for 3 h at rt. After the volatile materials were removed under reduced pressure, the resulting reside was purified by flash column chromatography ($CHCl_3$) to yield **31**.

To a flame-dried 100 mL round-bottom flask containing a magnetic stirring bar were charged **31** (30 mmol), Et_3N (30 mmol), DMAP (3 mmol), and $CHCl_3$ (30 mL). The solution was cooled to 0 °C, and TsCl (30 mmol) dissolved in $CHCl_3$ was added to the solution over 10 min. The reaction mixture was stirred at rt for 3 h. After all the volatile materials were removed under reduced pressure, the crude residue was purified by flash column chromatography ($CHCl_3$) to get **32**.

31: 83%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.63 (2H, m, CH_2N), 3.11 (2H, m, CH_2OH), 1.61–1.39 (15H, m, 3 × CH_2, $-C(CH_3)_3$).

32: 75%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (2H, d, J = 8.5 Hz, Ts), 7.32 (2H, d, J = 8.5 Hz, Ts), 3.98 (2H, t, J = 6.0 Hz, CH_2OTs), 3.02 (2H, m, CH_2N), 1.63 (2H, m, $CH_2CH_2CH_2$), 2.45 (3H, s, Ar–CH_3), 1.40 (11H, m, $CH_2CH_2CH_2$, $-C(CH_3)_3$), 1.30 (2H, s, $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 144.6, 133.1, 129.8, 127.8, 70.3, 40.2, 29.4, 28.4, 28.3, 22.6, 21.6, 14.1.

*4.2.42. Synthesis of 2-(N-Boc-5-aminopentyl)-9-hydroxy-6-methylellipticinium chloride (**34d**)*

A reaction flask was charged with **29** (0.17 mmol), **32** (0.51 mmol) and DMF (2 mL). The reaction mixture was stirred at 80 °C for 12 h. After the volatile materials were removed under reduced pressure, the crude residue (crude **33d**) was treated with 15 g of Amberlite IRA-900 (Chloride form) in water at rt for 5 h. The solvent was removed from the reaction mixture and the residue was purified by ODS column chromatography (H_2O/MeOH = 7:3) to afford **34d** (50%) as a white solid. Decomp. 190 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.46 (1H, s, 1-H), 7.62 (1H, m, 4-H), 7.47 (1H, m, 3-H), 6.14 (1H, m, 10-H), 6.05 (1H, m, 8-H), 5.87 (1H, m, 7-H), 4.09 (2H, m, N^+CH_2), 2.96 (3H, s, NCH_3), 2.89 (2H, m, CH_2N), 1.98 (3H, s, 11-CH_3), 1.74 (2H, m, $CH_2CH_2CH_2$), 1.61 (3H, s, 5-CH_3), 1.35 (2H, m, $CH_2CH_2CH_2$), 1.19 (11H, m, $CH_2CH_2CH_2$, $C(CH_3)_3$); 13C NMR (125 MHz, CD_3OD): δ 158.6, 153.9, 146.7, 146.5, 140.1, 135.4, 134.7, 131.5, 128.7, 123.9, 121.9, 121.8, 118.4, 111.9, 111.4, 111.0, 79.9, 61.4, 40.8, 34.3, 32.0, 30.5, 28.7, 24.5, 15.4, 14.2; MS (FAB) *m/z* 462 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{28}H_{36}N_3O_3^+$: 462.2757, found: 462.2734.

*4.2.43. Synthesis of 2-(5-aminopentyl)-9-hydroxy-6-methylellipticinium chloride (**35d**)*

A reaction flask was charged with **34d** (0.25 mmol) and 12 M HCl (5 mL). The reaction mixture was stirred at rt for 5 h. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 9:1) to afford **35d** (86%). Decomp. 278 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.43 (1H, s, 1-H), 7.59 (1H, m, 4-H), 7.39 (1H, m, 3-H), 6.15 (1H, m, 10-H), 6.06 (1H, m, 8-H), 5.80 (1H, m, 7-H), 4.07 (2H, m, N^+CH_2), 2.92 (5H, m, CH_2N, N–CH_3), 1.92 (3H, s, 11-CH_3), 1.74 (2H, m, $CH_2CH_2CH_2$), 1.64 (2H, m, $CH_2CH_2CH_2$), 1.55 (3H, s, 5-CH_3), 1.36 (2H, m, $CH_2CH_2CH_2$); MS (FAB) *m/z* 363 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{23}H_{28}N_3O^+$: 362.2232, found: 362.2256.

*4.2.44. Synthesis of 9-hydroxy-6-methylellipticine-derivative **38e** linked to a methylurea group*

A reaction flask was charged with **35d** (50 mg, 0.1 mmol), diisopropylethylamine (39 mg, 0.3 mmol), 4-nitrophenyl-*N*-methylcarbamate (**37a**, 24 mg, 0.12 mmol) and DMSO (1 mL). The reaction mixture was stirred at rt for 3 h under N_2 atmosphere. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield **38e** (63%). Decomp. 213 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.34 (1H, s, 1-H), 7.57 (1H, m, 4-H), 7.38 (1H, m, 3-H), 6.04 (1H, m, 10-H), 5.97 (1H, m, 8-H), 5.71 (1H, m, 7-H), 4.02 (2H, m, N^+CH_2), 2.95 (2H, m, CH_2N), 2.85 (3H, s, N–CH_3), 2.53 (3H, s, $NHCH_3$), 1.88 (3H, s, 11-CH_3), 1.71 (2H, m, $CH_2CH_2CH_2$), 1.44–1.37 (5H, m, $CH_2CH_2CH_2$, 5-CH_3), 1.18 (2H, m, $CH_2CH_2CH_2$); 13C NMR (125 MHz, CD_3OD): δ 161.9, 153.5, 146.2, 146.0, 139.5, 135.0, 134.2, 131.5, 128.0, 123.4, 121.6, 121.5, 118.1, 111.5, 111.0, 110.6, 61.3, 40.5, 34.1, 32.0, 30.7, 27.0,24.4, 15.3, 14.1; MS (FAB) *m/z* 420 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{25}H_{31}N_4O_2^+$: 419.2447, found: 419.2420.

*4.2.45. Synthesis of 9-hydroxy-6-methylellipticine-derivative **39e** linked to a methylnitrosourea group*

A reaction flask was charged with **35d** (50 mg, 0.13 mmol), diisopropylethylamine (48 mg, 0.38 mmol) 4-nitrophenyl-*N*-methyl-*N*-nitrosocarbamate (**37b**, 37 mg, 0.17 mmol) and DMF (1 mL). The reaction mixture was stirred at 0 °C for 7 h. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 8:2) to yield **39e** (20%). Decomp. 241 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.46 (1H, s, 1-H), 7.62 (1H, m, 4-H), 7.45 (1H, m, 3-H), 6.15 (1H, m, 10-H), 6.07 (1H, m, 8-H), 5.90 (1H, m, C–H), 4.09 (2H, m, N^+CH_2), 3.25 (2H, t, J = 6.5 Hz, CH_2N), 2.96 (3H, s, N–CH_3)), 2.89 (3H, s, $NHCH_3$), 1.97 (3H, s, 11-CH_3), 1.78 (2H, m, $CH_2CH_2CH_2$), 1.67–1.52 (5H, m, $CH_2CH_2CH_2$, 5-CH_3), 1.22 (2H, m, $CH_2CH_2CH_2$); MS (FAB) *m/z* 449 (M^+-Cl^-); HRMS (FAB): Calcd for $C_{25}H_{30}N_5O_3^+$: 448.2349, found: 448.2356.

*4.2.46. Synthesis of N-Boc-2-chloroethylamine (**49a**) and N-Boc-3-chloropropylamine (**49b**)*

A reaction flask was charged with chloroethylamine hydochloride **48a** (1.00 g, 8.62 mmol), di-tert-butyl dicarbonate (1.88 g, 8.62 mmol) and $CHCl_3$ (50 mL) under argon atmosphere. To a mixture is added triethylamine (0.91 g, 9.05 mmol) slowly at 0 °C, and the mixture was heated under reflux for 1 h. After washing the reaction mixture with water, the volatile materials were removed under reduced pressure. The crude residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 8:2) to yield **49a** (95%). The **49b** was prepared in a 93% yield by a similar method starting with **48b**.

49a; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.99 (1H, brs, NH), 3.60 (2H, t, CH_2CH_2Cl), 3.45 (2H, dt, CH_2CH_2Cl), 1.46 (9H, s, $C(CH_3)_3$); MS (EI+) *m/z*: 180 (M^+).

49b; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.80 (1H, brs, NH), 3.60 (2H, t, $CH_2CH_2CH_2Cl$), 3.45 (2H, dt, CH_2CH_2Cl), 1.89 (2H, m, $CH_2CH_2CH_2Cl$), 1.36 (9H, s, $C(CH_3)_3$); MS (EI+) *m/z* 193 (M^+).

*4.2.47. Synthesis of 2-(N-Boc-2-aminoethyl)-5-metoxycarbonyl-11-methyl-6H-pyrido[4,3-b]carbazol-2-ium chloride (**50a**) and its 3-proylamino derivative (**50b**)*

A flame-dried round-bottom flask containing a magnetic stirring bar was charged with **14** (0.100 g, 0.342 mmol), **49a** (1.85 g, 10.22 mol), and DMF (5 mL). The reaction mixture was stirred at 120 °C for 4 h and concentrated after cooling to rt, and then NaOMe (0.019 g, 0.352 mmol) in MeOH (5 mL) was added and the reaction mixture was stirred for 1 h at rt. Later, 0.343 mmol of **17** was added by dissolving in MeOH and stirring was continued for 5 h at rt. After the volatile materials were removed under reduced pressure, the crude residue was purified by ODS column chromatography (H_2O/MeOH = 6:4) to yield **50a** (21%). When **49b** was similarly used instead of **49a** and acetonitirile as a solvent, **50b** was obtained in a 25% yield.

50a; decomp. ~230 °C; ^{1}H NMR (300 MHz, CD_3OD-d_4): δ 9.93 (1H, s, 1-H), 9.51 (1H, d, J = 7.7 Hz, 3-H), 8.48–8.52 (2H, m, 4, 7-H), 7.85 (1H, d, J = 7.9 Hz, 10-H), 7.74 (1H, dd, J = 7.3, 8.1 Hz, 8-H), 7.53 (1H, dd, J = 7.3, 7.9 Hz, 9-H), 4.82 (2H, t, J = 5.3 Hz, $N^+CH_2CH_2$), 3.75 (2H, t, J = 5.3 Hz, $N^+CH_2CH_2$), 4.25 (3H, s, OCH_3), 3.39 (3H, s, CH_3), 1.19 (9H, s, $C(CH_3)_3$). MS (FAB) m/z 434 ($M^+ - Cl^-$); HRMS: Calcd for $C_{25}H_{28}N_3O_4$ ($M^+ - Cl^-$): 434.2082, found: 434.2080.

50b: decomp. ~240 °C; ^{1}H NMR (300 MHz, D_2O): δ 8.54 (1H, s, 1-H), 8.29 (1H, d, J = 7.7 Hz, 3-H), 7.75 (1H, d, J = 7.7 Hz, 4-H), 7.06 (1H, d, J = 8.1 Hz, 7-H), 6.86 (1H, d, J = 7.9 Hz, 10-H), 6.65 (1H, dd, J = 7.3, 8.1 Hz, 8-H), 6.47 (1H, dd, J = 7.9, 8.1 Hz, 9-H), 4.15 (2H, m, N^+CH_2 CH_2CH_2), 3.66 (3H, s, OCH_3), 3.01 (2H, m, N^+CH_2 CH_2CH_2), 1.96 (3H, s, CH_3), 1.94 (2H, m, N^+CH_2 CH_2CH_2), 1.23 (9H, s, $C(CH_3)_3$); ^{13}C NMR (125.77 MHz, DMSO-$d6$): δ 165.5, 155.7, 146.4, 144.5, 142.2, 140.2, 134.5, 133.6, 128.9, 127.0, 124.1, 121.6, 121.0, 120.9, 120.4, 112.7, 101.0, 77.7, 57.6, 52.6, 36.8, 30.9, 28.1, 15.7. MS (FAB) m/z 448 ($M^+ - Cl^-$); HRMS: Calcd for $C_{26}H_{30}N_3O_4$ ($M^+ - Cl^-$): 448.2236, found: 448.2230.

*4.2.48. Synthesis of 2-(2-aminoethyl)-5-metoxycarbonyl-11-methyl-6H-pyrido[4,3-b]carbazol-2-ium chloride (**40**) and its 3-proylamino derivative (**41**)*

A round-bottom flask was charged with **50a** (27.6 g, 0.060 mmol) and 4 M aq HCl (10 mL). The reaction mixture was stirred for 1 h at rt. Purification of the crude residue by ODS column chromatography (H_2O/MeOH = 9:1) gives 97% of **40**. When **50b** was similarly used instead of **50a**, the compound **41** was obtained in a 95% yield.

40: decomp. ~210 °C; ^{1}H NMR (300 MHz, D_2O): δ 8.70 (1H, s, 1-H), 8.30 (1H, d, J = 7.7 Hz, 3-H), 8.30 (1H, d, J = 7.7 Hz, 4-H), 7.85 (1H, d, J = 8.1 Hz, 7-H), 7.85 (1H, d, J = 7.9 Hz, 10-H), 6.55 (1H, dd, J = 7.4, 8.1 Hz, 8-H), 6.39 (1H, dd, J = 7.4, 7.9 Hz, 9-H), 4.54 (2H, t, J = 5.2 Hz, $N^+CH_2CH_2$), 3.61 (3H, s, OCH_3), 3.50 (2H, t, J = 5.2 Hz, $N^+CH_2CH_2$), 1.91 (3H, s, CH_3); MS (FAB+) m/z 334 ($M^+ - Cl^-$); HRMS Calcd for $C_{20}H_{20}N_3O_2$: 334.1556, found 334.1546.

41: decomp. ~240 °C; ^{1}H NMR (300 MHz, D_2O): δ 8.99 (1H, s, 1-H), 8.66 (1H, d, J = 7.8 Hz, 3-H), 8.00 (1H, d, J = 7.8 Hz 4-H), 7.54 (1H, d, J = 8.1 Hz, 7-H), 7.18 (1H, d, J = 7.9 Hz, 10-H), 6.97 (1H, dd, J = 7.4, 8.1 Hz, 8-H), 6.89 (1H, dd, J = 7.4, 7.9 Hz, 9-H), 4.15 (2H, t, $N^+CH_2CH_2CH_2$), 3.66 (3H, s, OCH_3), 3.01 (2H, t, $N^+CH_2CH_2CH_2$), 1.96 (3H, s, CH_3), 1.94 (2H, m, $N^+CH_2CH_2CH_2$); MS (FAB+) m/z 348 ($M^+ - Cl^-$); HRMS Calcd for $C_{21}H_{22}N_3O_2$: 348.1712, found 348.1727.

*4.2.49. Synthesis of succinimidyl N-methylcarbamate (**51a**)*

A dried round-bottom flask was charged with *N*-hydroxysuccinimide (5.83 g, 50.7 mmol), ethyl acetate (20 mL) as a solvent and cooled at 0 °C. To the mixture were slowly added triethylamine (4.76 g) and methylisocyanate (6.39 g, 0.112 mmol) at the same temperature. The reaction temperature was slowly raised to rt, and the mixture was stirred for 24 h at rt. After the volatile materials were removed under reduced pressure the crude residue was recrystallized from ethyl acetate/diethyl ether to give **51a** (86%). Colorless needles, mp 148.0–149.0 °C (lit [19]. 148.0–152.0 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (1H, br, NH), 2.76 (4H, s, CH_2CH^2), 2.67 (3H, s-like, CH_3); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 170.66, 151.98, 27.98, 25.47; MS (FAB+) m/z 173 (M+H).

*4.2.50. Synthesis of succinimidyl N-nitroso-N-methylcarbamate (**51b**) [19]*

A dried round-bottom flask was charged with sodium acetate (409 mg, 4.99 mmol), **51a** (2.0 g, 8.0 mmol), and dichloromethane (25 mL) as a solvent, and a suspension of the mixture was stirred at −40 °C. To the mixture, N_2O_4 (5.08 g, 55.2 mmol) in dichloromethane (10 mL) was added drop-by-drop with stirring. The mixture was stirred at −40 °C for 1 h, and then the temperature was slowly raised to the room temperature followed by an additional 1 h stirring. The reaction mixture was poured into iced water, and an aqueous layer was extracted with dichloromethane. The combined organic layer was successively washed with 10% $NaHCO_3$ and a brine, and dried over Na_2SO_4 over-night in a refrigerator. To the concentrated filtrate (5 mL) was added petroleum ether to precipitate a crude product, which was recrystallized from chloroform/ether to give **51b** (73%). light yellow needles, mp not determined (lit [31]. 148–152 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.22 (4H, s, CH_2CH_2), 2.95 (3H, s, CH_3); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 168.53, 151.09, 28.44, 25.57.

*4.2.51. Synthesis of ellipticinium-analogues **42** linked to a methylurea group*

A reaction flask was charged with **40** (20.5 mg, 0.055 mmol), diisopropylethylamine (9.30 mg, 0.072 mmol), and DMF (10 mL), and cooled to 0 °C. To the mixture, succinimidyl *N*-methylcarbamate (**51a**, 10.6 mg, 0.062 mmol) in DMF (3 mL) was slowly added, and the reaction mixture was stirred at 0 °C for 2 h. After the volatile materials were removed under reduced pressure, the crude residue, precipitated from methanol solution with ethyl acetate, was purified by ODS column chromatography (H_2O/MeOH = 6:4) to yield **42** (78%). Decomp. ~250 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.53 (1H, s, 1-H), 8.19 (1H, d, J = 7.7 Hz, 3-H), 7.72 (1H, d, J = 7.7 Hz, 4-H), 6.88 (1H, d, J = 8.1 Hz, 7-H), 6.79 (1H, d, J = 7.9 Hz, 10-H), 6.55 (1H, dd, J = 7.5, 8.1 Hz, 8-H), 6.38 (1H, dd, J = 7.5, 7.9 Hz, 9-H), 4.02 (2H, t, J = 5.3 Hz, $N^+CH_2CH_2$), 3.57 (3H, s, OCH_3), 3.48 (2H, t, J = 5.3 Hz, $N^+CH_2CH_2$), 2.47 (3H, s, CH_3), 1.83 (3H, s, CH_3). MS (FAB) m/z 391 ($M^+ - Cl^-$); HRMS (FAB): Calcd for $C_{22}H_{23}N_4O_3^+$: 391.1770, found: 391.1762.

*4.2.52. Synthesis of ellipticinium-analogues **43** linked to a methylnitrosourea group*

A reaction flask was charged with **40** (24.0 mg, 0.064 mmol), diisopropylethylamine (9.29 mg, 0.072 mmol), and DMF (10 mL), and cooled to 0 °C. To the mixture, succinimidyl *N*-methyl-*N*-nitrosocarbamate (**51b**, 13.0 mg, 0.060 mmol) in DMF (3 mL) was slowly added, and the reaction mixture was stirred at 0 °C for 2 h. After the volatile materials were removed under reduced pressure, the crude residue, precipitated from methanol solution with ethyl acetate, was purified by ODS column chromatography (H_2O/MeOH = 6:4) to yield **43** (76%). Decomp. ~250 °C; ^{1}H NMR (500 MHz, CD_3OD-D_4): δ 10.00 (1H, s, 1-H), 9.49 (1H, d, J = 7.7 Hz, 3-H), 8.53 (1H, d, J = 7.7 Hz, 4-H), 8.46 (1H, d, J = 8.1 Hz, 7-H), 7.81 (1H, d, J = 7.9 Hz, 10-H), 7.69 (1H, dd, J = 7.5, 8.1 Hz, 8-H), 6.38 (1H, dd, J = 7.5, 7.9 Hz, 9-H), 4.96 (2H, t, J = 5.3 Hz, $N^+CH_2CH_2$), 4.19 (3H, s, OCH_3), 4.10 (2H, t, J = 5.3 Hz, $N^+CH_2CH_2$), 3.48 (3H, s, CH_3), 3.00 (3H, s, CH_3). MS (FAB) m/z 420 ($M^+ - Cl^-$); HRMS (FAB): Calcd for $C_{22}H_{22}N_5O_4^+$: 420.1677, found: 420.1671.

*4.2.53. Synthesis of ellipticinium-analogues **44** linked to a methylurea group*

A reaction flask was charged with**41** (22.5 mg, 0.066 mmol), diisopropylethylamine (10.23 mg, 0.079 mmol), and DMF (10 mL), and cooled to 0 °C. To the mixture, succinimidyl *N*-methylcarbamate (**51a**, 11.7 mg, 0.066 mmol) in DMF (3 mL) was slowly added, and the reaction mixture was stirred at 0 °C for 2 h. After the volatile materials were removed under reduced pressure, the crude residue, precipitated from methanol solution with ethyl acetate, was purified by ODS column chromatography (H_2O/MeOH = 6:4) to yield **44** (79%). Decomp. ~250 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.53 (1H, s, 1-H), 8.19 (1H, d, J = 7.7 Hz, 3-H), 7.72 (1H, d, J = 7.7 Hz, 4-H), 6.88 (1H, d, J = 8.1 Hz, 7-H), 6.79 (1H, d, J = 7.9 Hz, 10-H), 6.55 (1H, dd, J = 7.5, 8.1 Hz, 8-H), 6.38 (1H, dd, J = 7.5, 7.9 Hz, 9-H), 4.15 (2H, t, $N^+CH_2CH_2CH_2$), 3.66 (3H, s, OCH_3), 3.22 (2H, t, $N^+CH_2CH_2CH_2$), 1.96

(3H, s, CH_3), 2.47 (3H, s, CH_3), 1.88 (2H, m, $N^+CH_2\underline{CH_2}CH_2$). MS (FAB) *m/z* 405 ($M^+ - Cl^-$); HRMS (FAB): Calcd for $C_{23}H_{25}N_4O_3^+$: 405.1937, found: 405.1941.

4.2.54. Synthesis of ellipticinium-analogues **45** linked to a methylnitrosourea group

A reaction flask was charged with **41** (18.0 mg, 0.051 mmol), diisopropylethylamine (8.01 mg, 0.062 mmol), and DMF (10 mL), and cooled to 0 °C. To the mixture, succinimidyl *N*-methyl-*N*-nitrosocarbamate (**51b**, 9.6 mg, 0.056 mmol) in DMF (3 mL) was slowly added, and the reaction mixture was stirred at 0 °C for 2 h. After the volatile materials were removed under reduced pressure, the crude residue, precipitated from methanol solution with ethyl acetate, was purified by ODS column chromatography (H_2O/MeOH = 6:4) to yield **45** (76%). Decomp. ~240 °C; ^{1}H NMR (500 MHz, D_2O): δ 8.43 (1H, s, 1-H), 8.19 (1H, d, J = 7.7 Hz, 3-H), 7.64 (1H, d, J = 7.7 Hz, 4-H), 6.99 (1H, d, J = 8.1 Hz, 7-H), 6.89 (1H, d, J = 7.9 Hz, 10-H), 6.55 (1H, dd, J = 7.5, 8.1 Hz, 8-H), 6.38 (1H, dd, J = 7.5, 7.9 Hz, 9-H), 4.35 (2H, t, $N^+\underline{CH_2}CH_2CH_2$), 3.46 (3H, s, OCH_3), 3.22 (2H, t, $N^+CH_2CH_2\underline{CH_2}$), 2.49 (3H, s, CH_3), 1.86 (3H, s, CH_3), 1.68 (2H, m, $N^+CH_2\underline{CH_2}CH_2$). MS (FAB) *m/z* 434 ($M^+ - Cl^-$); HRMS (FAB): Calcd for $C_{23}H_{24}N_5O_4^+$: 434.1828, found: 434.1819.

4.2.55. Synthesis of 2-methyl-5-metoxycarbonyl-11-methyl-6H-pyrido[4,3-b]carbazol-2-ium iodide (**52**)

A flame-dried round-bottom flask containing a magnetic stirring bar was charged with **14** (0.4 g, 1.36 mmol), acetonitirile (15 mL), and methyl iodide (0.85 mL, 13.6 mmol). The reaction mixture was stirred at rt for 3 h and dissolved in MeOH (2.5 mL) after concentration, and then NaOMe (74 mg, 1.93 mmol) in MeOH (2.5 mL) was added drop-by-drop and the reaction mixture was stirred for 1 h at rt. Later, 365 mg (1.42 mmol) of **17** was added by dissolving in MeOH (4 mL) and stirring was continued for 5 h at rt. The precipitated crystals were collected by filtration and washed with MeOH to give **52** (75%; lit [32]. 78%) as light yellow crystals. Decomp. ~310 °C (lit [32]. 317–321 °C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.98 (1H, s, 6-H), 10.05 (1H, s, 12-H), 9.20 (1H, d, J = 7.4 Hz, 3-H), 8.57 (1H, d, J = 7.4 Hz, 4-H), 8.43 (1H, d, J = 8 Hz, 7-H), 7.84 (1H, d, J = 8 Hz, 10-H), 7.76 and 7.46 (1H, dd, J = 8, 7.4 Hz, dd, J = 8, 7.4 Hz, 8/9-H), 4.47 (3H, s, N^+CH_2), 4.12 (3H, s, OCH_3), 3.30 (3H, s, CH_3). MS (FAB+) *m/z* 305 ($M^+ - I^-$).

4.2.56. Synthesis of 2-methyl-ellipticin-2-ium iodide (**53**) and the corresponding chloride **46**

A reaction flask was charged with **52** (74 mg, 0.0171 mmol), Na[$Al(OCH_2CH_2OMe)_2$] (65% solution in toluene, 86 mg, 0.0685 mmol), and xylene (13 mL), and stirred at 130 °C for 6 h. After the reaction mixture was cooled down to rt, the reaction was quenched with water, and the mixture was condensed to dryness under reduced pressure. The residue was dissolved in 20 mL of MeOH, and treated with **17** (50 mg, 0.0171 mmol) at rt for 5 h. After the volatile materials were removed under reduced pressure, the crude residue was de-salted with Amberlite-XAD2, and purified by silica gel column chromatography (MeOH including 1% acethyl chloride) to yield **53** (84%; lit [32]. 85%). The compound **53** (100 mg, 0.26 mmol) and Amberlite IRA-900 (chloride form; 2.5 g) were stirred at rt for 5 h in water (30 mL), and the mixture was passed through a column of Amberlite IRA-900 (3 g) to give **46** in quantitative yield.

53: decomp. 355 °C (lit [1g]. decomp. 360 °C); ^{1}H NMR (300 MHz, CD_3OD): δ 9.56 (1H, s, 1-H), 8.34 (1H, d, J = 6.9 Hz, 3-H), 8.31 (1H, d, J = 6.9 Hz, 4-H), 7.96 (1H, d, J = 6.3 Hz, 10-H), 7.50–7.53 (2H, m, 7/8-H), 7.26–7.29 (1H, m, 9-H), 4.34 (3H, s, N^+-CH_3), 3.23 (3H, s, 11-CH_3), 2.77 (3H, s, 5-CH_3). MS (FAB+) *m/z* 261 ($M^+ - I^-$).

46: decomp. 355 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 9.90 (1H, s, 1-H), 8.47 (1H, d, J = 7.5 Hz, 3-H), 8.46 (1H, d, J = 7.5 Hz, 4-H), 8.29 (1H, d, J = 7.2 Hz, 10-H), 7.67 (2H, m, 7/8-H), 7.40–7.46 (1H, m, 9-H), 4.49 (3H, s, N^+–CH_3), 3.37 (3H, s, 11-CH_3), 2.91 (3H, s, 5-CH_3). MS (FAB+) *m/z* 261 ($M^+ - Cl^-$).

4.3. Antitumor activity

4.3.1. Cell lines and culture

HeLa S-3 cells were provided by Dr. Okada (Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology, Japan). The cells were maintained in MEM (Nissui) supplemented with 10% FBS (GIBCO), 2% HEPES, 3% NEAA and 2 mM L-glutamine in a water-saturated atmosphere of 5% CO_2 at 37 °C.

4.3.2. Cell viability assay

Cell viability was determined by a 3-[4,5-dimethylthiazol-2-y]-2,5-diphenyltetrazolium bromide (MTT) assay, which is a method for determining cell viability by measuring the mitochondrial dehydrogenase action. Cells were seeded in a 96-well cell culture cluster (Becton Dickinson) at a density of 2×10^4 cells/mL and cultured 3 h prior to drug treatment. Cells were exposed at 37 °C for 24–72 h to ellipticine analogues. The MTT reagent (Nacalai tesuque) was prepared at a concentration of 2 mg/ml in Dulbecco's PBS without calcium and magnesium, and stored at 4 °C. After treatment for indicated times, cells were incubated with MTT reagent for 4 h at 37 °C. The plate was centrifuged at 3000 rpm for 10 min, and the medium was removed. To solubilize the resultant MTT-formazan, 200 μL/well of dimethyl sulfoxide (DMSO) was added to each well followed by thorough mixing with a mechanical plate mixer. Absorbance at 540 nm was measured on a microplate reader (MTP-500, CORONA), and the percentage of cell viability was taken as the percentage absorbance at 540 nm of ellipticine-treated cells against control cells.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.032.

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