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Original article

# Synthesis and cytotoxic activity of the heptaphylline and 7-methoxyheptaphylline series

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ABSTRACT

Nineteen carbazole alkaloids modified from heptaphylline (**I**) and 7-methoxyheptaphylline (**II**) isolated from *Clausena harmandiana* were synthesized. Among these derivatives, **Ih** and **IIi** showed cytotoxicity against the NCI-H187 cell line with $IC_{50}$ values of 0.02 and 0.66 μM, respectively, which are about 138 and 4 fold stronger than the ellipticine standard. In addition, oxime **Ih** displayed cytotoxicity against KB cells with an $IC_{50}$ value of 0.17 μM which is about 10 times stronger than the ellipticine. This compound demonstrated weak cytotoxicity against Vero cells ($IC_{50}$ = 66.01 μM). The results show convincingly that **Ih** may be a promising lead for the development of cytotoxic agents.



## 1. Introduction

Carbazole alkaloids are a major component of the *Clausena* genus, especially *Clausena excavata* [1], *Clausena anisata* [2] and *Clausena harmandiana* [3,4]. It has been reported that carbazole alkaloids exhibit a broad pharmacological profile, including anti-platelet aggregation and vasorelaxing [5], antimycobacterial [6], antimycobial [7], antiplasmodial [3], anti-inflamatory [8], anti HIV-1 [9], and antitumor promoting activities [2], cytotoxicity against the leukemia cell line [10], as a topoisomerase II inhibitor [11] and also as having antidiabetes activity [12]. Many carbazole derivatives have been synthesized and evaluated for biological activities [13–16].

*C. harmandiana* is a health-promoting herb and is used for the treatment of illness, stomachache and headache [17]. It has been found that the roots of this plant contain a large amount of carbazole alkaloids together with coumarins [18]. In our previous study, the major components in *C. harmandiana* were heptaphylline and 7-methoxyheptaphylline which exhibited cytotoxicity against NCI-H187 and KB cell lines [4]. In order to take advantage of these major constituents and in our continuing search for new anticancer substances with high efficacy, low toxicity and minimum side effects from natural products [19–22], we therefore planned to modify the chemical structures of heptaphylline and 7-methoxyheptaphylline and also screen for cytotoxicity of all derivatives. We anticipate the discovery of new cancer chemo-preventive agents with novel structures. We report herein simple methods of synthesis and the active cytotoxic structure of modified carbazoles.

## 2. Results and discussion

### 2.1. Chemistry

Nineteen carbazole derivatives were successfully synthesized from heptaphylline (**I**) and 7-methoxyheptaphylline (**II**) which were isolated from *C. harmandiana*. Methylation of **I** using NaH/$CH_3I$ yielded monomethylated products **Ia** (15%) and **Ib** (11%) as well as dimethylated product **Ic** (65%), while methylation of **II** yielded **IIa** (15%) and **IIc** (74%) (Scheme 1). Treatment of **I** and **II** with conc. $H_2SO_4$ at room temperature afforded pyrans **Id** and **IId** in yields of 98% and 95%, respectively (Scheme 2). The $^1H$ NMR spectrum of **Id** showed two triplet signals at $\delta$ 2.82 (H-1) and $\delta$ 1.94 (H-2), and in addition, the disappearance of the phenolic proton signal at $\delta$ 11.65 was seen. The $^1H$ NMR and $^{13}C$ NMR of **IId** showed the same pattern as **Id**. Epoxidation of the isolated double bond in the prenyl moiety of **I** using *m*CPBA provided **Ie** (40%) along with cyclization products pyran **If** (21%) and furan **Ig** (31%) (Scheme 3). In

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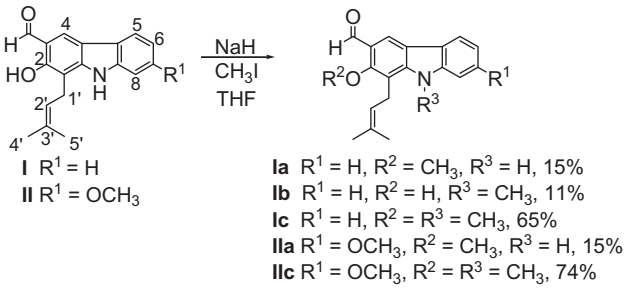



**Scheme 1.** Methylated products.

the same conditions, **Iie**–**IIg** were obtained in the yields of 37%, 23% and 32%, respectively. The reaction of **I** and **II** with $NH_2OH \cdot HCl$ under basic conditions at room temperature furnished **Ih** and **IIh** in 82% and 73% yields, respectively (Scheme 4). The $^1H$ NMR spectrum of **Ih** showed the presence of two hydroxyl protons at $\delta$ 10.22 (phenolic proton) and $\delta$ 7.15 (oxime proton). The aldehydic proton showed upfield shift from $\delta$ 9.91 (compound **I**) to $\delta$ 8.39 (compound **Ih**) and from $\delta$ 9.86 (compound **II**) to $\delta$ 8.26 (compound **IIh**). Bromination of **I** using NBS in the presence of aqueous acetonitrile at room temperature yielded **Ii** and **Ij** in 64% and 35% yield, respectively, while the reaction of **II** provided **IIi** and **IIj** in 30% and 60% yield, respectively (Scheme 5).

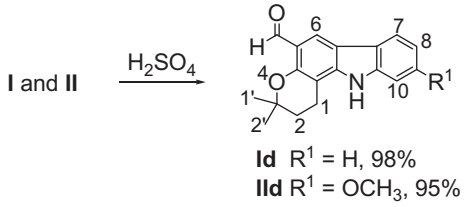
### 2.2. Biological activity

Due to the discovery of the cytotoxicity of ellipticine in 1960s (Fig. 1), many carbazole derivatives have been tested for cytotoxic activity. Many cancer cell lines such as human adrenocortical carcinoma cells (NCI-H295) [23], adenocarcinoma cells (HT29) [24], breast cancer cells (MCF-7) [14], leukemia cells (HL-60) [25], murine leukemia (L1210), human colon carcinoma (HT29 and HCT116) [26] and non-small lung cancer (NCI-H1299) [27] have been evaluated for cytotoxicity. In the present study, heptaphylline, 7-methoxyphylline and their derivatives were evaluated for cytotoxicity against cancer cells, NCI-H187 (human small cell lung cancer) and KB (human epidermoid carcinoma of the oral cavity) cell lines, together with normal cells (Vero cells; African green monkey kidney) and the results are shown in Table 1. Compounds **I** and **II** showed cytotoxicity against NCI-H187 and KB cell lines with $IC_{50}$ values ranging from 1.3 to 2.7 μM. Fortunately, these compounds showed weak and no activity against normal cells. Methylated products **Ia** and **IIc** showed cytotoxic activity against the NCI-H187 cells ($IC_{50}$ around 4–5 μM) while the other methylated products showed weaker cytotoxicity against this cell line. All methylated products (**Ia**–**Ic**, **IIa** and **IIc**) showed weak cytotoxicity against KB and Vero cell lines, except **Ia** and **IIa** which showed moderate cytotoxicity with $IC_{50}$ of around 11–13 μM. The cytotoxicity results of **Ia** and **IIa** against KB cells show convincingly that the appearance of a methoxy group at the C-2 position and an NH group are essential for cytotoxicity to this cell line. The results showed **Ia** and **IIc** were selectively active on the NCI-H187 cell line. The cytotoxicity results of pyrans **Id** and **IId** showed weak activity against all cell lines. Epoxide **Ie** and furan **Ig** showed cytotoxicity against the NCI-H187 cell line with $IC_{50}$ values of 5.31 and 3.05 μM, respectively, while they showed weak and no activity on the other two cell lines. In contrast, hydroxypyran **If** exhibited cytotoxic activity against two cancer cell lines with $IC_{50}$ values around 6 μM which were stronger than the corresponding pyran **Id** ($IC_{50}$ = 65.19 and 26.38 μM, respectively). These results showed convincingly that the polarity of compound may play an important role in cytotoxicity. It should be noted that **If** might be useful for cytotoxic agents. Among compounds **Iie**–**IIg**, hydroxypyran **IIf** shows stronger activity ($IC_{50}$ = 3.04 μM) against NCI-H187 cell line than the corresponding pyran **IId** ($IC_{50}$ = inactive) which may be due to the more polarity of this compound. From the results of **Ie**–**Ig** and **Iie**–**IIg**, it seems that these derivatives exhibit selectively cytotoxicity against the NCI-H187 cell line. In case of **IIe**, it showed weaker cytotoxicity against NCI-H187 ($IC_{50}$ = 20.27 μM) than that of the KB cell line ($IC_{50}$ = 9.22 μM) which is opposite to the activity of **Ie**, **Ig**, **IIf** and **IIg**. Comparing the structure of epoxide derivatives **Ie** and **IIe**, the reverse activity may be due to the methoxy group at the C-7 position being favorable to KB cells. In contrast, the cytotoxicity of **IIf** against KB cell ($IC_{50}$ = 120.48 μM) is lower than **If** ($IC_{50}$ = 5.99 μM). This may be explained that the methoxy group of pyrano alcohol appears to be detrimental to the activity against KB cells.

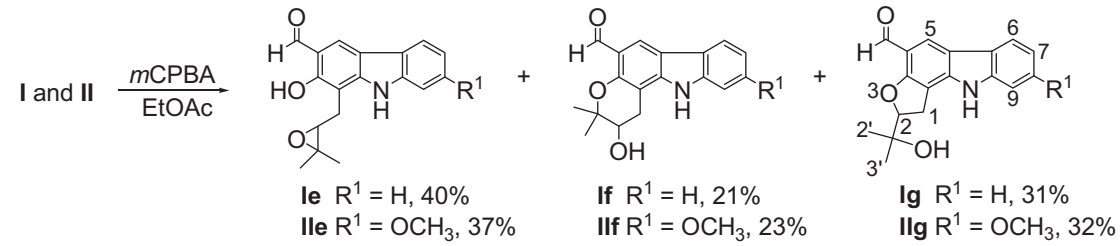
It has been reported that amidine carbazole derivatives are potent anticancer agents [28]. Then oxime derivatives which are structurally close to amidine groups were prepared and expected to have good results on cytotoxicity testing. Fortunately, oxime derivative **Ih** showed a dramatic improvement in cytotoxicity against NCI-H187 and KB cell lines with $IC_{50}$ values of 0.02 and 0.17 μM, respectively, which are about 66 and 9 fold higher than the parent **I**. In addition, **Ih** showed weak cytotoxicity against Vero cells with an $IC_{50}$ value of 66.01 μM. Hence, it is suggested that the oxime group gives an additional favorable effect on cytotoxicity. In a previous report, pyrimidocarbazoledione (ER37326; Fig. 1) was evaluated for cytotoxicity against the KB cell line and showed activity with an $IC_{50}$ value of 0.35 μM [29]. Fortunately, oxime **Ih** exhibited stronger activity than ER37326. It has been reported that 8-substituents such as amino and acetyl groups in pyrimidocarbazoles are more favorable for cytotoxicity. It should be noted that **Ih** contains an oxime group at a similar position. In the case of oxime **IIh**, it shows weaker cytotoxicity than **Ih** against NCI-H187 and KB cell lines with $IC_{50}$ values of 3.67 and 18.16 μM, respectively, and shows no activity against Vero cells. It seems that the presence of a methoxy group at the C-7 position leads to a decrease in the cytotoxicity.

It has been reported that chlorocarbazole derivatives are antitumor agents [30] and so we tried to synthesized halo derivatives. In the present study, bromo carbazoles were produced and evaluated for cytotoxicity. Among bromide derivatives, **IIi** was the most active compound with an $IC_{50}$ value of 0.66 μM against NCI-H187 cells is about 2.5 fold higher than the parent **II**. However, this compound showed moderate cytotoxicity against Vero cells with an $IC_{50}$ value of 12.53 μM. In addition, **Ij** displayed selective cytotoxicity against KB cells ($IC_{50}$ = 6 μM).

The anticancer agent used as a standard in our cytotoxic assay is ellipticine with $IC_{50}$ values ranging from 1.7 to 3.8 μM. It is interesting to note that compound **Ih** demonstrated stronger cytotoxic activity against NCI-H187 and KB cell lines by about 138 and 10 times, respectively, than the ellipticine standard while **IIi** showed stronger cytotoxicity against NCI-H187 at about 4 times than the ellipticine. The results show convincingly that **Ih** is likely to be useful as a lead compound for the development of cytotoxic agents,




**Scheme 2.** Reaction with conc. $H_2SO_4$.




**Scheme 3.** Reaction with *m*CPBA.

because it exhibits strong cytotoxicity against NCI-H187 and KB cell lines but has very weak cytotoxicity against Vero cells.

## 3. Conclusion

In summary, we have prepared a series of modified carbazoles using simple reactions and evaluated their cytotoxicity against NCI-H187, KB and Vero cell lines. The reaction of **I** and **II** with hydroxylamine hydrochloride yielded the corresponding oximes **Ih** (82%) and **IIh** (73%), respectively. It was found that oxime **Ih** demonstrated stronger cytotoxicity against NCI-H187 and KB cell lines with $IC_{50}$ values of 0.02 and 0.17 μM, respectively. Fortunately, this compound displayed weak cytotoxicity to Vero cells, which suggests a potential lead compound for the development of anticancer agents. It can be concluded that oxime derivative of heptaphylline (**Ih**) displayed significant activity against both NCI-H187 and KB cell lines. In addition, bromide **IIi** exhibited significant cytotoxicity to NCI-H187. Moreover, epoxide, pyran and furan derivatives were selectively active on the NCI-H187 cell line.

## 4. Experimental

### 4.1. General

NMR spectra were recorded on a Varian Mercury plus spectrometer operating at 400 MHz ($^1$H) and at 100 MHz ($^{13}$C). IR spectra were recorded as KBr disks or thin films, using Perkin Elmer Spectrum One FT-IR spectrophotometer. Mass spectra were determined on Micromass Q-TOF 2 hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer with a Z-spray ES source (Micromass, Manchester, UK). Melting points were determined on a SANYO Gallenkamp melting point apparatus and were uncorrected. Thin layer chromatography (TLC) was carried out on MERCK silica gel 60 $F_{254}$ TLC aluminum sheet. Column chromatography was done with silica gel 0.063–0.200 mm or less than 0.063 mm. Preparative layer chromatography (PLC) was carried out on glass supported silica gel plates using silica gel 60 $PF_{254}$ for preparative layer chromatography. All solvents were routinely distilled prior to use.

### 4.2. Physical properties and spectroscopic data of I and II

#### 4.2.1. Heptaphylline (*I*) [4]

Yellow crystals; mp 168–170 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3292, 2926, 1614, 1474, 1449, 1329, 1237, 1183, 739; $^1$H NMR ($CDCl_3$) $\delta$ 11.65 (1H, s, OH), 9.91 (1H, s, CHO), 8.23 (1H, br s, NH), 8.04 (1H, s, H-4), 7.97 (1H, d, $J$ = 7.8 Hz, H-5), 7.37–7.41 (2H, m, H-7 and H-8), 7.24–7.28 (1H, m, H-6), 5.31 (1H, t, $J$ = 6.9 Hz, H-2′), 3.64 (2H, d, $J$ = 6.9 Hz, H-1′), 1.90 (3H, s, H-5′), 1.77 (3H, s, H-4′); $^{13}$C NMR ($CDCl_3$) $\delta$ 195.4 (CHO), 157.8 (C-2), 145.0 (C-1a), 140.1 (C-8a), 134.2 (C-3′), 125.9 (CH-4), 125.3 (CH-7), 123.6 (C-5a), 121.2 (CH-2′), 120.8 (CH-6), 119.8 (CH-5), 117.3 (C-4a), 115.4 (C-3), 110.9 (CH-8), 109.0 (C-1), 25.7 ($CH_3$-4′), 22.8 ($CH_2$-1′), 18.1 ($CH_3$-5′); HRMS $m/z$ 280.1338 $[M + H]^+$ (calcd. for $C_{18}H_{17}NO_2$ + H, 280.1337).

#### 4.2.2. 7-Methoxyheptaphylline (*II*) [4]

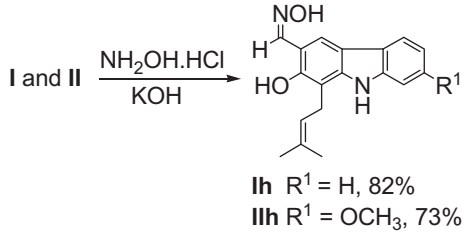
Yellow solid; mp 150–152 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3326, 2958, 1612, 1465, 1324, 1188, 1161, 1028; $^1$H NMR ($CDCl_3$) $\delta$ 11.62 (1H, s, OH), 9.86 (1H, s, CHO), 8.16 (1H, br s, NH), 7.88 (1H, s, H-4), 7.81 (1H, d, $J$ = 8.5 Hz, H-5), 6.89 (1H, br d, $J$ = 1.6 Hz, H-8), 6.87 (1H, dd, $J$ = 8.5, 1.8 Hz, H-6), 5.30 (1H, t, $J$ = 6.8 Hz, H-2′), 3.90 (3H, s, $OCH_3$), 3.60 (2H, d, $J$ = 6.8 Hz, H-1′), 1.88 (3H, s, H-5′), 1.76 (3H, s, H-4′); $^{13}$C NMR ($CDCl_3$) $\delta$ 195.3 (CHO), 159.0 (C-7), 157.3 (C-2), 145.2 (C-1a), 141.5 (C-8a), 134.1 (C-3′), 124.0 (CH-4), 121.3 (CH-2′), 120.5 (CH-5), 117.5 (C-4a), 117.2 (C-5a), 115.3 (C-3), 109.0 (C-1), 108.9 (CH-6), 95.6 (CH-8), 55.7 ($OCH_3$-7), 25.7 ($CH_3$-4′), 22.8 ($CH_2$-1′), 18.1 ($CH_3$-5′); HRMS $m/z$ 310.1440 $[M + H]^+$ (calcd. for $C_{19}H_{19}NO_3$ + H, 310.1443).

### 4.3. Preparation of carbazole derivatives

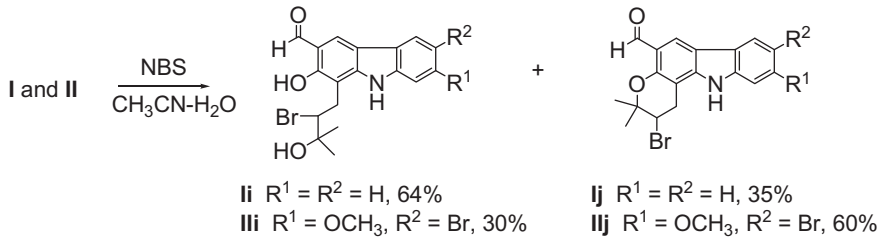
#### 4.3.1. Methylation reaction

To a solution of NaH (60% in oil, 45.2 mg, 1.13 mmol) in THF (5 mL) was added dropwise compound **I** (50 mg, 0.18 mmol) in THF (1 mL) at 0 °C under nitrogen atmosphere and added dropwise $CH_3I$ (excess, 3 mL) at 0 °C. The reaction mixture was stirred at 30 °C for 10 min. The entire reaction mixture was poured into cold water and extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with water, saturated NaCl, dried over anhydrous $Na_2SO_4$ and evaporated. After purification by PLC, compounds **Ia**, **Ib** and **Ic** were obtained. The reaction of **II** was examined with the same procedure as described above and compounds **IIa** and **IIc** were obtained.

*4.3.1.1. 2-Methoxyheptaphylline (**Ia**) [31].* 7.9 mg, 15% yield, yellow solid; mp 130–131 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3170, 2989, 1662, 1621, 1596, 1457, 1334, 1230, 1062, 735; $^1$H NMR ($CDCl_3$) $\delta$ 10.40 (1H, s, CHO), 8.50 (1H, s, H-4), 8.26 (1H, br s, NH), 8.05 (1H, d, $J$ = 7.7 Hz, H-5), 7.40–7.45 (2H, m, H-7 and H-8), 7.26–7.29 (1H, m, H-6), 5.31 (1H, t, $J$ = 6.4 Hz, H-2′), 3.94 (3H, s, $OCH_3$), 3.70 (2H, d, $J$ = 6.4 Hz, H-1′), 1.93 (3H, s, H-5′), 1.79 (3H, s, H-4′); $^{13}$C NMR ($CDCl_3$) $\delta$ 190.1 (CHO), 159.3 (C-2), 144.1 (C-1a), 140.2 (C-8a), 134.4 (C-3′), 126.3 (CH-7), 123.7 (C-5a), 122.4 (C-3), 121.4 (CH-2′), 120.8 (CH-4), 120.6 (CH-6), 120.2 (C-1), 120.0 (CH-5), 116.3 (C-4a), 110.9 (CH-8), 64.8 ($OCH_3$), 25.7 ($CH_3$-4′), 23.9 ($CH_2$-1′), 18.2 ($CH_3$-5′); HRMS $m/z$ 294.1493 $[M + H]^+$ (calcd. for $C_{19}H_{19}NO_2$ + H, 294.1494).




**Scheme 4.** Oxime derivatives.




**Scheme 5.** Reaction with NBS.

*4.3.1.2. 9-Methylheptaphylline (**Ib**).* 5.8 mg, 11% yield, yellow solid; mp 89–90 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3414, 2926, 1620, 1483, 1355, 1322, 1235, 1192, 755; $^1$H NMR ($CDCl_3$) $\delta$ 11.73 (1H, s, OH), 9.88 (1H, s, CHO), 8.04 (1H, s, H-4), 7.97 (1H, d, $J$ = 7.6 Hz, H-5), 7.45 (1H, t, $J$ = 7.4 Hz, H-7), 7.34 (1H, d, $J$ = 8.1 Hz, H-8), 7.25–7.29 (1H, m, H-6), 5.24 (1H, br t, $J$ = 5.5 Hz, H-2′), 4.03 (3H, s, N–$CH_3$), 3.84 (2H, d, $J$ = 5.5 Hz, H-1′), 1.84 (3H, s, H-5′), 1.74 (3H, s, H-4′); $^{13}$C NMR ($CDCl_3$) $\delta$ 194.9 (CHO), 158.9 (C-2), 145.3 (C-1a), 142.8 (C-8a), 132.5 (C-3′), 125.7 (CH-7), 124.8 (CH-4), 123.7 (CH-2′), 123.2 (C-5a), 120.6 (CH-6), 119.2 (CH-5), 118.0 (C-4a), 114.7 (C-3), 110.3 (C-1), 108.9 (CH-8), 32.1 ($NCH_3$), 25.7 ($CH_3$-4′), 22.8 ($CH_2$-1′), 18.2 ($CH_3$-5′); HRMS $m/z$ 294.1488 $[M + H]^+$ (calcd. for $C_{19}H_{19}NO_2$ + H, 294.1494).

*4.3.1.3. 2-Methoxy-9-methylheptaphylline (**Ic**).* 36 mg, 65% yield, yellow solid; mp 75–78 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 2924, 2853, 1666, 1622, 1592, 1450, 1429, 1321, 1242, 1196, 1049, 743; $^1$H NMR ($CDCl_3$) $\delta$ 10.38 (1H, s, CHO), 8.51 (1H, s, H-4), 8.06 (1H, d, $J$ = 7.7 Hz, H-5), 7.48 (1H, t, $J$ = 8.0 Hz, H-7), 7.37 (1H, d, $J$ = 8.2 Hz, H-8), 7.28 (1H, t, $J$ = 7.6 Hz, H-6), 5.27 (1H, t, $J$ = 5.5 Hz, H-2′), 4.01 (3H, s, N–$CH_3$), 3.93 (3H, s, $OCH_3$), 3.89 (2H, d, $J$ = 5.5 Hz, H-1′), 1.86 (3H, s, H-5′), 1.74 (3H, s, H-4′); $^{13}$C NMR ($CDCl_3$) $\delta$ 190.0 (CHO), 160.3 (C-2), 144.2 (C-1a), 142.8 (C-8a), 132.8 (C-3′), 126.2 (CH-7), 124.2 (CH-2′), 123.2 (C-5a), 121.7 (C-4a), 120.9 (C-1), 120.5 (CH-4), 120.1 (CH-6), 119.8 (CH-5), 117.8 (C-3), 109.0 (CH-8), 65.0 ($OCH_3$), 32.0 (N-$CH_3$), 25.6 ($CH_3$-4′), 23.6 ($CH_2$-1′), 18.3 ($CH_3$-5′); HRMS $m/z$ 308.1645 $[M + H]^+$ (calcd. for $C_{20}H_{21}NO_2$ + H, 308.1645).

*4.3.1.4. 2,7-Dimethoxyheptaphylline (**IIa**).* 8.7 mg, 15% yield, yellow solid; mp 148–150 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3247, 2921, 1662, 1599, 1451, 1424, 1322, 1229, 1200, 1161, 1061; $^1$H NMR ($CDCl_3$) $\delta$ 10.39 (1H, s, CHO), 8.38 (1H, s, H-4), 8.14 (1H, br s, NH), 7.90 (1H, d, $J$ = 8.5 Hz, H-5), 6.92 (1H, br d, $J$ = 2.1 Hz, H-8), 6.88 (1H, dd, $J$ = 8.5, 2.1 Hz, H-6), 5.30 (1H, t, $J$ = 6.6 Hz, H-2′), 3.93 (3H, s, $OCH_3$-2), 3.90 (3H, s, $OCH_3$-7), 3.68 (2H, d, $J$ = 6.6 Hz, H-1′), 1.93 (3H, s, H-5′), 1.79 (3H, s, H-4′); $^{13}$C NMR ($CDCl_3$) $\delta$ 190.1 (CHO), 159.4 (C-2), 158.7 (C-7), 144.2 (C-1a), 141.5 (C-8a), 134.4 (C-3′), 122.4 (C-3), 121.5 (CH-5), 121.3 (CH-2′), 120.4 (C-4a), 118.8 (CH-4), 117.4 (C-1), 116.1 (C-5a), 109.2 (CH-6), 95.4 (CH-8), 64.8 ($OCH_3$-2), 55.7 ($OCH_3$-7), 25.7 ($CH_3$-4′), 23.9 ($CH_2$-1′), 18.2 ($CH_3$-5′); HRMS $m/z$ 324.1594 $[M + H]^+$ (calcd. for $C_{20}H_{21}NO_3$ + H, 324.1594).

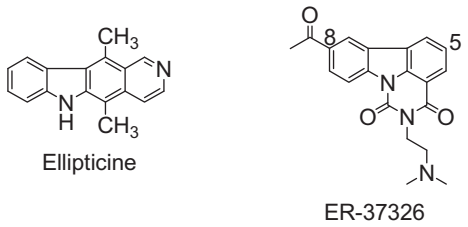



**Fig. 1.** The structures of ellipticine and ER37326.

*4.3.1.5. 2,7-Dimethoxy-9-methylheptaphylline (**IIc**).* 45 mg, 74% yield, yellow solid; mp 144–146 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 2927, 2849, 1672, 1591, 1442, 1357, 1243, 1228, 1180, 1132, 1054, 1041, 834, 804; $^1$H NMR ($CDCl_3$) $\delta$ 10.36 (1H, s, CHO), 8.37 (1H, s, H-4), 7.90 (1H, d, $J$ = 8.5 Hz, H-5), 6.88 (1H, dd, $J$ = 8.5, 1.4 Hz, H-6), 6.81 (1H, br d, $J$ = 1.4 Hz, H-8), 5.26 (1H, t, $J$ = 4.9 Hz, H-2′), 3.97 (3H, s, N–$CH_3$), 3.92 (6H, s, $OCH_3$-2 and $OCH_3$-7), 3.85 (2H, d, $J$ = 4.9 Hz, H-1′), 1.85 (3H, s, H-5′), 1.74 (3H, s, H-4′); $^{13}$C NMR ($CDCl_3$) $\delta$ 190.0 (CHO), 159.6 (C-2), 159.4 (C-7), 144.4 (C-1a), 144.2 (C-8a), 132.7 (C-3′), 124.2 (CH-2′), 121.7 (C-3), 121.1 (C-4a), 120.8 (CH-5), 118.5 (CH-4), 117.7 (C-1), 116.9 (C-5a), 108.4 (CH-6), 93.9 (CH-8), 65.0 ($OCH_3$-2), 55.7 ($OCH_3$-7), 32.0 ($NCH_3$), 25.6 ($CH_3$-4′), 23.6 ($CH_2$-1′), 18.3 ($CH_3$-5′); HRMS $m/z$ 338.1748 $[M + H]^+$ (calcd. for $C_{21}H_{23}NO_3$ + H, 338.1750).

*4.3.2. Cyclization with concentrated sulfuric acid*

The solid of carbazole **I** (0.07 mmol) was added dropwise conc. $H_2SO_4$ (0.5 mL). The mixture was ground with a pestle for 5 min and kept at room temperature for 1 h. The crude reaction was added dropwise saturated $NaHCO_3$ (0.5 mL) and EtOAc (2 mL). The reaction mixture was poured into cold water and extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with water, saturated NaCl, dried over anhydrous $Na_2SO_4$ and evaporated to give a white solid. After purification by PLC, carbazole derivative **Id** was obtained. The reaction of **II** with conc. $H_2SO_4$ was examined in the same procedure as described above and then **IId** was obtained.

**Table 1**
Cytotoxicity of derived carbazoles.

| Compound | Cytotoxicity ($IC_{50}$ μM) | | |
|---|---|---|---|
| | NCI-H187 | KB | Vero cells |
| **I** | 1.32 | 1.61 | 92.47 |
| **II** | 1.68 | 2.75 | Inactive[a] |
| **Ia** | 4.49 | 12.81 | 169.86 |
| **Ib** | 44.21 | 166.34 | 34.56 |
| **Ic** | 62.52 | 147.4 | 148.74 |
| **IIa** | 19.51 | 11.04 | 61.07 |
| **IIc** | 4.83 | 69.77 | 45.94 |
| **Id** | 65.19 | 26.38 | Inactive[a] |
| **IId** | Inactive[a] | Inactive[a] | Inactive[a] |
| **Ie** | 5.31 | 54.82 | 74.46 |
| **If** | 6.06 | 5.99 | Inactive[a] |
| **Ig** | 3.05 | 155.29 | Inactive[a] |
| **IIe** | 20.07 | 9.22 | 75.33 |
| **IIf** | 3.04 | 120.48 | Inactive[a] |
| **IIg** | 12.94 | 71.21 | Inactive[a] |
| **Ih** | 0.02 | 0.17 | 66.01 |
| **IIh** | 3.67 | 18.16 | Inactive[a] |
| **Ii** | 30.19 | 17.38 | Inactive[a] |
| **Ij** | 77.44 | 6.00 | Inactive[a] |
| **IIi** | 0.66 | 36.34 | 12.53 |
| **IIj** | Inactive[a] | Inactive[a] | Inactive[a] |
| Ellipticine | 2.77 | 1.78 | 3.82 |

*Data shown are from triplicate experiments.
[a] Inactive at >200 μM.

*4.3.2.1. 5-Formyl-3,3-dimethylpyrano[3,2-a]carbazole (**Id**) [32].* 19.2 mg, 98% yield, white solid; mp 228–230 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3263, 2972, 1661, 1603, 1456, 1331, 1238, 1167; $^1$H NMR ($CDCl_3$) $\delta$ 10.43 (1H, s, CHO), 8.38 (1H, s, H-6), 8.01 (1H, br s, NH), 7.91 (1H, d, $J$ = 7.6 Hz, H-7), 7.27–7.34 (2H, m, H-9 and H-10), 7.15–7.18 (1H, m, H-8), 2.82 (2H, t, $J$ = 6.8 Hz, H-1), 1.94 (2H, t, $J$ = 6.8 Hz, H-2), 1.39 (6H, s, H-1′ and H-2′); $^{13}$C NMR ($CDCl_3$) $\delta$ 189.9 (CHO), 155.6 (C-4a), 143.4 (C-11a), 139.9 (C-10a), 125.5 (CH-9), 124.4 (C-7a), 120.8 (CH-8), 120.2 (CH-7), 119.2 (CH-6), 119.0 (C-5), 116.4 (C-6a), 110.6 (CH-10), 102.4 (C-1a), 75.2 (C-3), 31.6 ($CH_2$-2), 26.7 ($CH_3$-1′ and 2′), 18.0 ($CH_2$-1); HRMS $m/z$ 302.1157 $[M + Na]^+$ (calcd. for $C_{18}H_{17}NO_2$ + Na, 302.1157).

*4.3.2.2. 5-Formyl-3,3-dimethyl-7-methoxypyrano[3,2-a]carbazole (**IId**).* 20.6 mg, 95% yield, white solid; mp 210–211 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3165, 2928, 1595, 1444, 1322, 1241, 1200, 1160; $^1$H NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 10.33 (1H, s, CHO), 8.47 (1H, s, H-6), 7.77 (1H, d, $J$ = 8.5 Hz, H-7), 6.89 (1H, br d, $J$ = 1.7 Hz, H-10), 6.76 (1H, dd, $J$ = 8.5, 1.7 Hz, H-8), 3.83 (3H, s, $OCH_3$), 2.84 (2H, t, $J$ = 6.8 Hz, H-1), 1.93 (2H, t, $J$ = 6.8 Hz, H-2), 1.38 (6H, s, H-1′ and H-2′); $^{13}$C NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 194.7 (CHO), 162.5 (C-9), 159.0 (C-4a), 148.6 (C-11a), 146.0 (C-10a), 133.3 (C-7a), 124.4 (CH-7), 121.78 (CH-6), 121.76 (C-5), 120.6 (C-6a), 112.0 (CH-8), 106.7 (C-1a), 99.7 (CH-10), 79.1 (C-3), 59.4 ($OCH_3$), 35.5 ($CH_2$-2), 30.4 ($CH_3$-1′ and 2′), 21.7 ($CH_2$-1); HRMS $m/z$ 310.1436 $[M + H]^+$ (calcd. for $C_{19}H_{19}NO_3$ + H, 310.1443).

*4.3.3. Epoxidation reaction*

To a solution of **I** (0.07 mmol) in EtOAc (1 mL) was added *m*CPBA (0.08 mmol) and the reaction mixture was stirred at room temperature for 20 h. The entire reaction mixture was poured into cold water and extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with water, saturated NaCl, dried over anhydrous $Na_2SO_4$ and evaporated to give solid products. After purification by PLC, carbazole derivatives **Ie**–**Ig** were obtained. Epoxidation of **II** was examined in the same procedure as described above and compounds **IIe**–**IIg** were obtained.

*4.3.3.1. 1-(3′-Methylbutyl-2′,3′-oxirane)mukonal (**Ie**).* 8.27 mg, 40% yield, yellow solid; mp 241–242 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3330, 2968, 1637, 1455, 1432, 1379, 1344, 1231, 895, 783, 747; $^1$H NMR ($CDCl_3$) $\delta$ 11.78 (1H, s, OH), 9.92 (1H, s, CHO), 9.45 (1H, br s, NH), 8.10 (1H, s, H-4), 7.98 (1H, d, $J$ = 7.7 Hz, H-5), 7.46 (1H, d, $J$ = 8.0 Hz, H-8), 7.39 (1H, t, $J$ = 8.0 Hz, H-7), 7.25 (1H, m, H-6), 3.96 (1H, dd, $J$ = 15.4, 1.1 Hz, H-1′a or b), 2.99 (1H, dd, $J$ = 9.6, 1.1 Hz, H-2′), 2.46 (1H, dd, $J$ = 15.4, 9.6 Hz, H-1′b or a), 1.58 (3H, s, H-5′), 1.41 (3H, s, H-4′); $^{13}$C NMR ($CDCl_3$) $\delta$ 195.3 (CHO), 157.9 (C-2), 146.0 (C-1a), 140.6 (C-8a), 126.1 (CH-4), 125.9 (CH-7), 123.6 (C-5a), 120.7 (CH-6), 119.7 (CH-5), 117.6 (C-4a), 115.2 (C-3), 111.4 (CH-8), 106.6 (C-1), 64.4 (CH-2′), 60.3 (C-3′), 24.8 ($CH_3$-4′), 24.1 ($CH_2$-1′), 19.0 ($CH_3$-5′); HRMS $m/z$ 318.1107 $[M + Na]^+$ (calcd. for $C_{18}H_{17}NO_3$ + Na, 318.1106).

*4.3.3.2. 5-Formyl-2-hydroxy-3,3-dimethylpyrano[3,2-a]carbazole (**If**).* 4.34 mg, 21% yield, pale yellow amorphous solid; mp 283–284 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3435, 2925, 1592, 1452, 1326, 1228; $^1$H NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 10.35 (1H, s, CHO), 8.35 (1H, s, H-6), 7.92 (1H, d, $J$ = 7.7 Hz, H-7), 7.39 (1H, d, $J$ = 7.1 Hz, H-10), 7.31 (1H, t, $J$ = 7.1 Hz, H-9), 7.16 (1H, t, $J$ = 7.7 Hz, H-8), 3.93 (1H, t, $J$ = 5.6 Hz, H-2), 3.12 (1H, dd, $J$ = 16.3, 5.6 Hz, H-1a or b), 2.80 (1H, dd, $J$ = 16.4, 5.6 Hz, H-1b or a), 1.44 (3H, s, H-1′ or H-2′), 1.35 (3H, s, H-2′ or H-1′); $^{13}$C NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 190.6 (CHO), 154.5 (C-4a), 144.6 (C-11a), 140.8 (C-10a), 125.4 (CH-9), 123.8 (C-7a), 120.1 (CH-8), 119.7 (CH-7), 119.1 (CH-6) 117.8 (C-5), 117.0 (C-6a), 110.9 (CH-10), 101.9 (C-1a), 78.1 (C-3), 68.4 (CH-2), 26.7 ($CH_2$-1), 25.0 ($CH_3$-1′ or 2′), 20.1 ($CH_3$-2′ or 1′); HRMS $m/z$ 318.1106 $[M + Na]^+$ (calcd. for $C_{18}H_{17}NO_3$ + Na, 318.1106).

*4.3.3.3. 4-Formyl-2(1′-hydroxy-1′-methylethyl)furano[3,2-a]carbazole (**Ig**).* 6.4 mg, 31% yield, yellow solid; mp 214 °C (dec.), IR (KBr) $\nu_{max}$ cm$^{-1}$: 3392, 1660, 1629, 1596, 1456, 1378, 1230, 1141, 955; $^1$H NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 10.04 (1H, s, CHO), 8.21 (1H, s, H-5), 7.92 (1H, d, $J$ = 7.6 Hz, H-6), 7.32–7.36 (2H, m, H-8 and 9), 7.17 (1H, t, $J$ = 6.8 Hz, H-7), 4.86 (1H, t, $J$ = 9.2 Hz, H-2), 3.25 (2H, d, $J$ = 9.2 Hz, H-1), 1.31 (3H, s, H-2′ or 3′), 1.23 (3H, s, H-3′ or 2′); $^{13}$C NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 189.85 (CHO), 160.9 (C-3a), 141.6 (C-10a), 141.1 (C-9a), 125.7 (CH-8), 123.7 (C-6a), 121.9 (CH-5), 120.3 (CH-7), 119.7 (CH-6), 118.99 (C-5a), 113.4 (C-4), 110.98 (CH-9), 107.6 (C-1a), 91.8 (CH-2), 71.54 (C-1′), 27.8 ($CH_2$-1), 24.7 ($CH_3$-2′ or 3′), 23.8 ($CH_3$-3′ or 2′); HRMS $m/z$ 296.1282 $[M + H]^+$ (calcd. for $C_{18}H_{17}NO_3$ + H, 296.1286).

*4.3.3.4. 1-(3′-Methylbutyl-2′,3′-oxirane)-7-methoxymukonal (**IIe**).* 8.43 mg, 37% yield, dark yellow amorphous solid; mp 145–147 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3304, 2967, 1618, 1430, 1330, 1238, 1160; $^1$H NMR ($CDCl_3$) $\delta$ 11.73 (1H, s, OH), 9.87 (1H, s, CHO), 9.37 (1H, br s, NH), 7.93 (1H, s, H-4), 7.80 (1H, d, $J$ = 8.5 Hz, H-5), 6.95 (1H, s, H-8), 6.85 (1H, dd, $J$ = 8.5, 2.0 Hz, H-6), 3.92 (1H, d, $J$ = 15.5 Hz, H-1′a or b), 3.88 (3H, s, $OCH_3$), 2.99 (1H, d, $J$ = 9.5 Hz, H-2′), 2.42 (1H, dd, $J$ = 15.5, 9.7 Hz, H-1′b or a), 1.56 (3H, s, H-5′), 1.40 (3H, s, H-4′); $^{13}$C NMR ($CDCl_3$) $\delta$ 195.3 (CHO), 159.0 (C-7), 157.3 (C-2), 146.1 (C-1a), 141.9 (C-8a), 124.7 (CH-4), 120.4 (CH-5), 117.7 (C-4a), 117.0 (C-5a), 115.0 (C-3), 109.2 (CH-6), 106.5 (C-1), 95.7 (CH-8), 64.5 (CH-2′), 60.4 (C-3′), 55.6 ($OCH_3$), 24.8 ($CH_3$-4′), 24.0 ($CH_2$-1′), 18.9 ($CH_3$-5′); HRMS $m/z$ 348.1207 $[M + Na]^+$ (calcd. for $C_{19}H_{19}NO_4$ + Na, 348.1212).

*4.3.3.5. 5-Formyl-2-hydroxy-3,3-dimethyl-9-methoxypyrano[3,2-a] carbazole (**IIf**).* 5.24 mg, 23% yield, yellow solid; mp 142–143 °C, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3388, 2929, 1596, 1323, 1233, 1199, 1159; $^1$H NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 10.33 (1H, s, CHO), 8.22 (1H, s, H-6), 7.79 (1H, d, $J$ = 8.6 Hz, H-7), 6.93 (1H, s, H-10), 6.78 (1H, d, $J$ = 8.6 Hz, H-8), 3.92 (1H, m, H-2), 3.85 (3H, s, $OCH_3$), 3.12 (1H, dd, $J$ = 16.2, 5.4 Hz, H-1a or b), 2.80 (1H, dd, $J$ = 16.2, 7.2 Hz, H-1b or a), 1.44 (3H, s, H-1′ or H-2′), 1.35 (3H, s, H-2′ or H-1′); $^{13}$C NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 190.6 (CHO), 158.8 (C-9), 154.0 (C-4a), 144.9 (C-11a), 142.3 (C-10a), 120.3 (CH-7), 117.8 (CH-6), 117.59 (C-7a), 117.56 (C-5), 117.1 (C-6a), 108.3 (CH-8), 102.0 (C-1a), 95.5 (CH-10), 78.0 (C-3), 68.4 (CH-2), 55.2 ($OCH_3$), 26.7 ($CH_2$-1), 24.9 ($CH_3$-1′ or 2′), 20.0 ($CH_3$-2′ or 1′); HRMS $m/z$ 348.1217 $[M + Na]^+$ (calcd. for $C_{19}H_{19}NO_4$ + Na, 348.1212).

*4.3.3.6. 4-Formyl-2(1′-hydroxy-1′-methylethyl)-8-methoxyfurano [3,2-a]carbazole (**IIg**).* 7.3 mg, 32% yield, pale yellow solid; mp 208–210 °C (dec.), IR (KBr) $\nu_{max}$ cm$^{-1}$: 3392, 1660, 1629, 1596, 1456, 1378, 1230, 1141, 955; $^1$H NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 10.01 (1H, s, CHO), 8.06 (1H, s, H-5), 7.76 (1H, d, $J$ = 8.5 Hz, H-6), 6.86 (1H, d, $J$ = 2.1 Hz, H-9), 6.76 (1H, dd, $J$ = 8.5, 2.1 Hz, H-7), 4.83 (1H, t, $J$ = 9.0 Hz, H-2), 3.25 (1H, dd, $J$ = 13.0, 9.0 Hz, H-1a or b), 3.17 (1H, dd, $J$ = 13.0, 9.0 Hz, H-1b or a), 1.30 (3H, s, H-2′ or 3′), 1.22 (3H, s, H-3′ or 2′); $^{13}$C NMR ($CDCl_3$) $\delta$ 189.8 (CHO), 160.4 (C-3a), 158.9 (C-8), 142.4 (C-9a), 141.8 (C-10a), 120.5 (C-6a), 120.4 (CH-5), 119.1 (CH-6) 117.5 (C-5a), 113.3 (C-4), 108.2 (CH-7), 107.6 (C-1a), 95.8 (CH-9), 91.6 (CH-2), 71.5 (C-1′), 55.5 ($OCH_3$), 27.8 ($CH_2$-1), 24.8 ($CH_3$-2′ or 3′), 23.8 ($CH_3$-3′ or 2′); HRMS $m/z$ 326.1387 $[M + H]^+$ (calcd. for $C_{19}H_{19}NO_4$ + H, 326.1392).

*4.3.4. Condensation with hydroxylamine hydrochloride*

To a solution of **I** (0.07 mmol) in EtOAc (2 mL) was added $NH_2OH$·HCl (0.36 mmol) at room temperature. To the resulting mixture was added a solution of KOH (excess) in EtOH (1 mL) and stirred at room temperature for 12 h. The entire reaction mixture was poured into cold water and extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with water, saturated

NaCl, dried over anhydrous $Na_2SO_4$ and evaporated to give solid products. After purification by PLC, carbazole derivative **Ih** was obtained. The reaction of **II** with $NH_2OH{\cdot}HCl$ was examined in the same procedure as described above and then **IIh** was obtained.

*4.3.4.1. Heptaphylline oxime (**Ih**).* 16.9 mg, 82% yield, dark brown amorphous solid; mp 167–169 °C, IR (KBr) $\nu_{max}$ $cm^{-1}$: 3443, 2927, 1612, 1438, 1363, 1328, 1305, 1236, 1179, 998, 741; $^1H$ NMR ($CDCl_3$) $\delta$ 10.22 (1H, br s, OH), 8.39 (1H, s, CH-oxime), 8.03 (1H, br s, NH), 7.93 (1H, d, $J$ = 7.7 Hz, H-5), 7.71 (1H, s, H-4), 7.32–7.40 (2H, m, H-7 and H-8), 7.21 (1H, t, $J$ = 7.7 Hz, H-6), 7.15 (1H, br s, N-OH), 5.35 (1H, t, $J$ = 6.7 Hz, H-2′), 3.68 (2H, d, $J$ = 6.8 Hz, H-1′), 1.91 (3H, s, H-5′), 1.77 (3H, s, H-4′); $^{13}C$ NMR ($CDCl_3$) $\delta$ 154.2 (CH-oxime), 153.6 (C-2), 141.6 (C-1a), 139.8 (C-8a), 133.6 (C-3′), 125.0 (CH-7), 123.7 (C-5a), 121.8 (CH-2′), 121.0 (CH-4), 120.0 (CH-6), 119.5 (CH-5), 116.7 (C-4a), 110.6 (CH-8), 110.3 (C-3), 109.5 (C-1), 25.7 ($CH_3$-4′), 23.6 ($CH_2$-1′), 18.1 ($CH_3$-5′); HRMS $m/z$ 295.1438 $[M + H]^+$ (calcd. for $C_{18}H_{18}N_2O_2$ + H, 295.1447).

*4.3.4.2. 7-Methoxyheptaphylline oxime (**IIh**).* 16.6 mg, 73% yield, dark yellow amorphous solid; mp 201–203 °C, IR (KBr) $\nu_{max}$ $cm^{-1}$: 3442, 2911, 2534, 1616, 1434, 1319, 1234, 1171, 993; $^1H$ NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 8.26 (1H, s, CH-oxime), 7.71 (1H, d, $J$ = 8.5 Hz, H-5), 7.50 (1H, s, H-4), 6.84 (1H, s, H-8), 6.73 (1H, dd, $J$ = 8.5, 2.1 Hz, H-6), 5.26 (1H, t, $J$ = 6.8 Hz, H-2′), 3.81 (3H, s, $OCH_3$), 3.56 (2H, d, $J$ = 6.8 Hz, H-1′), 1.81 (3H, s, H-5′), 1.67 (3H, s, H-4′); $^{13}C$ NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 158.2 (C-7), 153.2 (CH-oxime), 152.7 (C-2), 141.3 (C-1a), 141.2 (C-8a), 133.2 (C-3′), 122.0 (CH-2′), 120.0 (CH-5), 119.6 (CH-4), 117.5 (C-5a), 116.6 (C-4a), 110.5 (C-3), 109.4 (C-1), 107.9 (CH-6), 95.3 (CH-8), 55.6 ($OCH_3$), 25.6 ($CH_3$-4′), 23.5 ($CH_2$-1′), 18.0 ($CH_3$-5′); HRMS $m/z$ 325.1544 $[M + H]^+$ (calcd. for $C_{19}H_{20}N_2O_3$ + H, 325.1546).

*4.3.5. Addition with N-bromosuccinamide*

To a solution of carbazole **I** (0.09 mmol) in EtOAc (2 mL) was added dropwise the solution of NBS (0.10 mmol) in MeCN : $H_2O$ (2 mL) at 0 °C and stirred at 0 °C for 1 h and keep stirring at room temperature for 1 h. The entire reaction mixture was poured into cold water and extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with water, saturated NaCl, dried over anhydrous $Na_2SO_4$ and evaporated to give solid products. After purification by PLC, carbazole derivatives **Ii** and **Ij** were obtained. The reaction of **II** with NBS was examined in the same procedure as described above and then **IIi** and **IIj** were obtained.

*4.3.5.1. 1-(2′-Bromo-3′-hydroxy-3′-methylbutyl)mukonal (**Ii**).* 21.7 mg, 64% yield, pale yellow solid; mp 130–132 °C, IR (KBr) $\nu_{max}$ $cm^{-1}$: 3408, 2978, 1614, 1474, 1384, 1330, 1230, 743; $^1H$ NMR ($CDCl_3$) $\delta$ 11.74 (1H, s, OH-2), 9.93 (1H, s, CHO), 8.65 (1H, br s, NH), 8.11 (1H, s, H-4), 7.99 (1H, d, $J$ = 7.7 Hz, H-5), 7.39–7.45 (2H, m, H-7 and H-8), 7.29 (1H, m, H-6), 4.59 (1H, dd, $J$ = 10.6, 2.0 Hz, H-2′), 3.83 (1H, dd, $J$ = 14.9, 2.2 Hz, H-1′a or b), 3.23 (1H, dd, $J$ = 14.9, 10.6 Hz, H-1′b or a), 2.29 (1H, br s, OH-3′), 1.55–1.56 (6H, s, H-4′ and H-5′); $^{13}C$ NMR ($CDCl_3$) $\delta$ 195.4 (CHO), 158.5 (C-2), 145.3 (C-1a), 140.3 (C-8a), 126.0 (CH-7), 125.9 (CH-4), 123.7 (C-5a), 120.9 (CH-6), 119.8 (CH-5), 117.6 (C-4a), 115.2 (C-3), 111.0 (CH-8), 107.3 (C-1), 73.2 (C-3′), 68.1 (CH-2′), 30.4 ($CH_2$-1′), 27.4 ($CH_3$-4′ or 5′), 26.5 ($CH_3$-5′ or 4′); HRMS $m/z$ 398.0368 $[M + Na]^+$ (calcd. for $C_{18}H_{18}BrNO_3$ + Na, 398.0368).

*4.3.5.2. 2-Bromo-5-formyl-3,3-dimethylpyrano[3,2-a]carbazole (**Ij**).* 11.3 mg, 35% yield, pale yellow solid; mp 232–234 °C, IR (KBr) $\nu_{max}$ $cm^{-1}$: 3255, 2980, 1664, 1601, 1457, 1334, 1241, 1175, 1129, 737; $^1H$ NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 10.33 (1H, s, CHO), 8.38 (1H, s, H-6), 7.91 (1H, d, $J$ = 7.6 Hz, H-7), 7.36 (1H, d, $J$ = 7.8 Hz, H-10), 7.30 (1H, t, $J$ = 7.4 Hz, H-9), 7.16 (1H, t, $J$ = 7.4 Hz, H-8), 4.37 (1H, dd, $J$ = 8.7, 5.8 Hz, H-2), 3.50 (1H, dd, $J$ = 16.7, 5.8 Hz, H-1a or b), 3.30 (1H, dd, $J$ = 16.7, 8.8 Hz, H-1b or a), 1.58 (3H, s, H-1′ or H-2′), 1.48 (3H, s, H-2′ or H-1′); $^{13}C$ NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 190.0 (CHO), 154.0 (C-4a), 143.3 (C-11a), 140.6 (C-10a), 125.6 (CH-9), 123.8 (C-7a), 120.4 (CH-8), 119.9 (CH-7), 119.7 (CH-6), 117.9 (C-5), 117.3 (C-6a), 111.0 (CH-10), 101.7 (C-1a), 77.8 (C-3), 51.2 (CH-2), 30.1 ($CH_2$-1), 26.7 ($CH_3$-1′ or 2′), 21.6 ($CH_3$-2′ or 1′); HRMS $m/z$ 381.0342 $[M + H + Na]^+$ (calcd. for $C_{18}H_{17}BrNO_2$ + Na, 381.0340).

*4.3.5.3. 1-(2′-Bromo-3′-hydroxy-3′-methylbutyl)-6-bromo-7-methoxymukonal (**IIi**).* 13.1 mg, 30% yield, brown solid; mp 118–120 °C, IR (KBr) $\nu_{max}$ $cm^{-1}$: 3363, 2918, 1619, 1466, 1363, 1291, 1201, 1168, 1038; $^1H$ NMR ($CDCl_3$) $\delta$ 11.64 (1H, s, OH), 9.84 (1H, s, CHO), 8.59 (1H, br s, NH), 8.03 (1H, s, H-4), 7.89 (1H, s, H-5), 6.91 (1H, s, H-8), 4.50 (1H, dd, $J$ = 10.6, 2.4 Hz, H-2′), 3.91 (3H, s, $OCH_3$), 3.74 (1H, dd, $J$ = 14.9, 2.4 Hz, H-1′a or b), 3.13 (1H, dd, $J$ = 14.9, 10.6 Hz, H-1′b or a), 1.49 (3H, s, H-5′), 1.48 (3H, s, H-4′); $^{13}C$ NMR ($CDCl_3$) $\delta$ 195.4 (CHO), 158.1 (C-7), 154.7 (C-2), 145.3 (C-1a), 140.5 (C-8a), 124.9 (CH-4), 124.1 (CH-5), 118.1 (C-5a), 116.8 (C-4a), 115.4 (C-3), 107.6 (C-1), 104.8 (C-6), 95.0 (CH-8), 73.2 (C-3′), 67.9 (CH-2′), 56.5 ($OCH_3$), 30.4 ($CH_2$-1′), 27.4 ($CH_3$-4′ or 5′), 26.5 ($CH_3$-5′ or 4′); HRMS $m/z$ 483.9763 $[M + H]^+$ (calcd. for $C_{19}H_{19}Br_2NO_4$ + H, 483.9763).

*4.3.5.4. 2,8-Dibromo-5-formyl-3,3-dimethyl-7-methoxypyrano[3,2-a]carbazole (**IIj**).* 25.2 mg, 60% yield, pale yellow solid; mp 260 °C (dec.), IR (KBr) $\nu_{max}$ $cm^{-1}$: 3414, 2976, 1665, 1593, 1444, 1200, 1169; $^1H$ NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 10.29 (1H, s, CHO), 8.20 (1H, s, H-6), 8.01 (1H, s, H-7), 6.94 (1H, s, H-10), 4.37 (1H, dd, $J$ = 8.5, 5.8 Hz, H-2), 3.88 (3H, s, $OCH_3$), 3.49 (1H, dd, $J$ = 17.0, 5.7 Hz, H-1a or b), 3.24–3.31 (1H, m, H-1b or a), 1.57 (3H, s, H-1′ or H-2′), 1.47 (3H, s, H-2′ or H-1′); $^{13}C$ NMR ($CDCl_3$ + $CD_3OD$) $\delta$ 190.0 (CHO), 154.4 (C-9), 153.6 (C-4), 143.6 (C-11a), 141.1 (C-10a), 124.0 (CH-7), 118.8 (CH-6), 118.3 (C-7a), 118.1 (C-5), 116.5 (C-6a), 104.1 (C-8), 103.5 (C-1a), 95.0 (CH-10), 77.8 (C-3), 56.3 ($OCH_3$), 51.1 (CH-2), 30.0 ($CH_2$-1), 26.6 ($CH_3$-1′ or 2′), 21.7 ($CH_3$-2′ or 1′); HRMS $m/z$ 487.9475 $[M + Na]^+$ (calcd. for $C_{19}H_{17}Br_2NO_3$ + Na, 487.9473).

*4.4. Cytotoxicity assay*

Cytotoxicity assay against human epidermoid carcinoma of oral cavity (KB), and human small cell lung cancer (NCI-H187) cell lines were performed employing Resazurin Microplate Assay (REMA) [33]. In brief, cells at a logarithmic growth phase are harvested and diluted to $7 \times 10^4$ cells/mL for KB and $9 \times 10^4$ cells/mL for NCI-H187, in fresh medium. Successively, 5 μL of test sample diluted in a 5% DMSO, and 45 μL of cell suspension are added to 384-well plates, incubated at 37 °C in 5% $CO_2$ incubator. After the incubation period (3 days for KB and 5 days for NCI-H187), 12.5 μL of 62.5 μg/mL resazurin solution is added to each well, and the plates are then incubated at 37 °C for 4 h. The fluorescence signal is measured using SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at the excitation and emission wavelengths of 530 nm and 590 nm. Percent inhibition of cell growth is calculated by the following equation: % Inhibition = $[1-(FU_T/FU_C)] \times 100$, where $FU_T$ and $FU_C$ are the mean fluorescent units from treated and untreated conditions, respectively. Dose response curves are plotted from 6 concentration of 2-fold serially diluted test compounds and the sample concentrations that inhibit cell growth by 50% ($IC_{50}$) can be derived using the SOFTMax Pro software (Molecular Devices, USA).

Cytotoxicity assay against Vero cells (African green monkey kidney) was performed by Green Fluorescent Protein (GFP) based assay [34]. The GFP-expressing Vero cell line was generated in-house by stably transfecting the African green monkey kidney cell line (Vero, ATCC CCL-81), with pEGFP-N1 plasmid (Clontech). The

cell line is maintained in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate and 0.8 mg/mL geneticin, at 37 °C in a humidified incubator with 5% $CO_2$. The assay is carried out by adding 45 μL of cell suspension at $3.3 \times 10^4$ cells/mL to each well of 384-well plates containing 5 μL of test compounds previously diluted in 0.5% DMSO, and then incubating for 4 days in 37 °C incubator with 5% $CO_2$. Fluorescence signals are measured using SpectraMax M5 microplate reader (Molecular Devices, USA) in the bottom-reading mode with excitation and emission wavelengths of 485 nm and 535 nm. The fluorescence signal at day 4 is subtracted with background fluorescence at day 0. The percentage of cytotoxicity is calculated by the following equation, where $FU_T$ and $FU_C$ represent the fluorescent unit of cells treated with test compound and untreated cells, respectively. % Cytotoxicity $= [1-(FU_T/FU_C)] \times 100$. $IC_{50}$ values are derived from dose–response curves, using 6 concentrations of 2-fold serially diluted samples, by the SOFTMax Pro software (Molecular Devices, USA). Ellipticine and 0.5% DMSO are used as a positive and a negative control, respectively.

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