Synthesis, antioxidant, hemolytic and cytotoxicity activity of AB ring core of mappicine

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Abstract

The series of AB ring cores of mappicine **5a-e** were generated by the reaction of 2-chloro-quinoline-3-carbaldehyde **4a-e** with $NaBH_4$ through eco-friendly route. All the synthons were characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR, LCMS and HRMS techniques. All the synthesized compounds were screened for their antioxidant activity, *in vitro* hemolytic activity, on human erythrocytes and cytotoxicity, on HeLa and Vero cells. Out of all synthons, **5e** exhibit good antioxidant as well as cytotoxicity activities, where **5d** showed promising hemolytic activity, and compound **5a** (2-chloro-quinolin-3-yl)-methanol displayed good cytotoxicity with noteworthy selectivity towards HeLa cells.

Keywords: mappicine, montmorillonite K-10, antioxidant, erythrocytes, HeLa, Vero

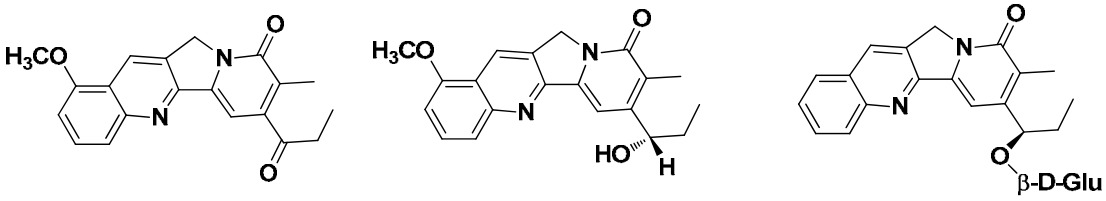
Introduction

Free radicals play a major role in several diseases including diabetes, cirrhosis, cancer and cardiovascular diseases.[1-2] Compounds that can scavenge free radicals have a vital role in the improvement of these diseased conditions. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, which results in oxidative stress leading to cellular damage. Investigation on natural anticancer agents is an important subject of current research world wide, as cancer is a growing public health menace and has been a major killer in most developed, developing and underdeveloped countries. The search for new anticancer drugs from nature continues to be a fruitful activity, as evidenced by the successes of natural products as pharmaceutical agents. Mappicine and Camptothecin, are chemically novel pyrrole [3, 4-b] quinoline alkaloids that show remarkable antitumour and antileukaemic activities.[3] Mappicine is also a potential agent in the AIDS chemotherapy.[4] Camptothecin as such was not ideal for pharmaceutical development, mostly due to its toxicity, poor solubility and the unstable nature of the lactone ring, which opens rapidly to an inactive hydroxy acid under physiological conditions.

Chemical modifications of camptothecin afforded several analogues. Two semi synthetic analogues, irinotecan[5] and topotecan[6] have been introduced in the clinic. Irinotecan showed broad spectrum activity and it was approved in Japan for treatment of lung, cervical and breast cancers.

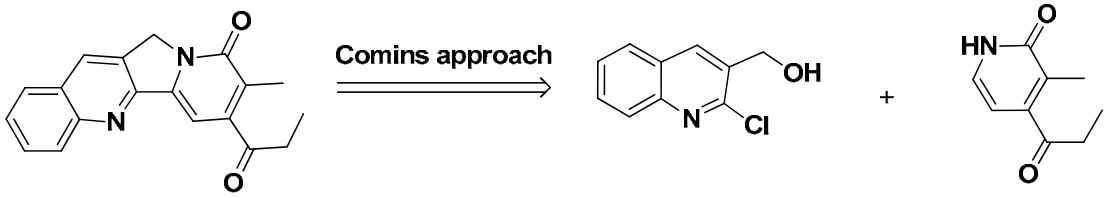
In the present work, the chemistry and bioactivity of the AB ring core of mappicine and its analogues is reported. All the AB ring cores of mappicine and their analogues were evaluated for their antioxidant, hemolytic against on human erythrocytes and cytotoxic activities against a HeLa and Vero cell lines.

Inorganic clay[7] materials such as montmorillonite K-10 have been used as catalysts for organic reactions and are superior then organic solvent medium due to their strong acidity, non-corrosive nature, cheapness, mild reaction conditions, high yields and selectivity and the ease of set-up and work-up. In recent years, use of solvent-free organic synthesis and microwave assisted reactions have shown a great impact. Solvent-free organic reactions have directed important organic syntheses, both in the laboratory and in the industry, more effectively and more eco-friendly than conventional reactions, in reducing hazardous explosions and removal of high boiling aprotic solvents from reaction mixtures.[8-10] Substituted quinolines are common structural features in a number of biologically active alkaloids, for example 9-methoxymappicine ketone **1**,[11] S-9-methoxymappicine **2**,[12] mappicine 20-O-β-D-glucopyranoside **3**[13a]



1 **2** **3**

The reaction of the AB ring core of mappicine with the D ring (Scheme 1) in a Comins[13b] approach has been used by several groups for the assembly of mappicine.



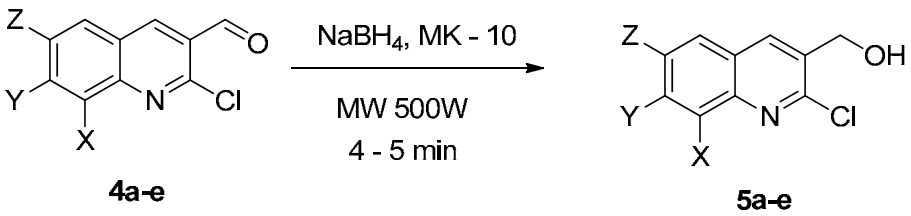
1a **AB ring** **D ring**

Scheme 1

We wish to report, a synthesis of the AB ring core of mappicine under solvent-free conditions via combination of supported reagents and eco-friendly techniques.

Result and Discussion

In this work, montmorillonite has a Lewis acid character, it seems that in the presence of montmorillonite K-10 the reaction was quite successful. After the reaction, catalyst can be filtered easily from mixture and may be reused after activation. The reaction proceeds efficiently in high yields through non conventional method of microwave irradiation for 4 to 5 minutes in 500W, and it proceeds without involvement of toxic and expensive material (Scheme 2).



X, Y, Z = -H (4a and 5a)
X = -CH_3, Y, Z = -H (4b and 5b)
Y = -CH_3, X, Z = -H (4c and 5c)
X = -OCH_3, Y, Z = -H (4d and 5d)
Z = -OCH_3, X, Y = -H (4e and 5e)

Scheme 2

Treatment of 2-chloro-quinoline-3-carbaldehyde **4**, sodiumborohydride and montmorillonite K-10 under microwave irradiation 500W for 4 to 5 minutes afforded AB ring core of the mappicine **5**, The IR spectra of all the compounds showed a broad peak at 3415 to 3415 cm^{-1} (the hydroxy stretching) and disappearance of peak at 1750 cm^{-1} (the aldehyde carbonyl stretching) gives conformation of the formation of AB ring core of mappicine The LC mass spectra confirmed the composition and the complete structures were assigned by detailed ^1H NMR experiments. For **5a**, hydroxy protons appear as a singlet at 2.27 ppm. The CH_2-2H protons of side chain showed as a singlet at 4.94 ppm.

Biological results

Antioxidant activity. The radical scavenging activity was determined as described by Roopan *et al.*[7, 14] (2008). Briefly, 1 mL of 0.15 mM alcoholic solution of DPPH was added to 3 mL of the synthesized samples **5a-e**, at different concentration (0.02, 0.05, 0.1, 0.15, 0.2 mM). The samples were kept in the dark for 30 min after which the optical density was measured at 517 nm. The radical scavenging activity was determined by the literature method[7].

Unlike other free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with absorption maximum at 517 nm. The purple colour generally fades when an antioxidant such as butylated

hydroxyl toluene, ascorbic acid were added. In the present study AB ring core of the mappicine can quench DPPH free radical and convert them to a colourless intermediate, resulting in decrease in absorbance at 517 nm. As the absorbance decreases the more potent the antioxidant activity of the AB ring core of mappicine.

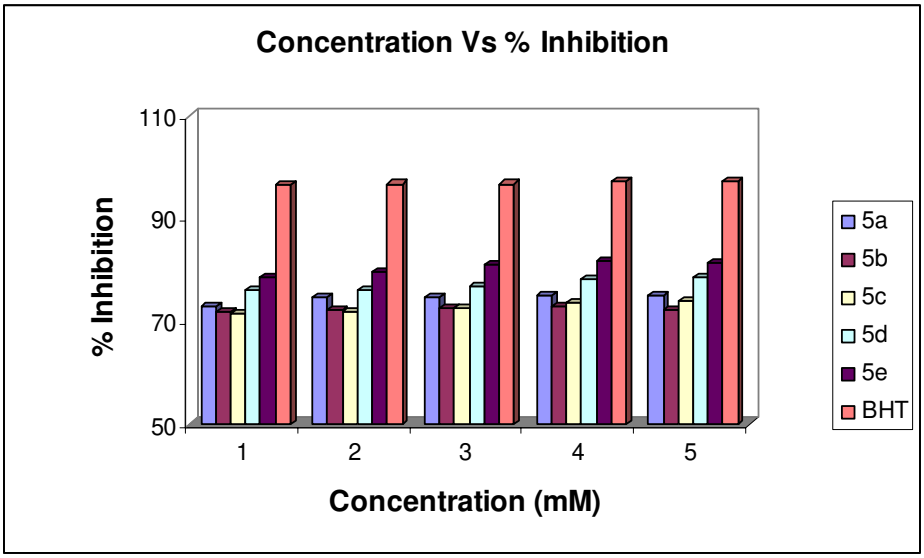


Figure 1. Antioxidant activity of AB ring core of mappicine derivatives.

All the synthesized AB ring cores of mappicine show free radical scavenging properties at all the five concentrations studied. The results obtained are shown in Fig. 1. All the compounds **5a-e** showed satisfactory effect in inhibiting DPPH. At a concentration of 0.1 mM to 0.2 mM, the scavenging effects of **5e** showed good effect. The results showed that among the entire ring core analyzed for DPPH scavenging activity, **5e** showed higher radical inhibition activity due to the presence of $–OCH_3$ group in the aromatic ring where as in the presence of $–CH_3$ group in the benzene ring exhibit less activity compare to **5a**, **5d** and **5e**.

Hemolytic activity and cytotoxic activity

Preparation of stock solution. 1 mg of all synthesized AB ring of the mappicine analogues **5a-e** were dissolved in 1 mL of DMSO solution. The appropriate concentrations of the compounds were made by serial dilution.

***In vitro* hemolytic assay.** Hemolytic effect of the compounds on human erythrocytes was evaluated by using washed erythrocytes (RBCs). For the preparation of mouse and human erythrocytes the method of Suthindhiran et.al[15]., Blood samples from the rats were collected from Charles foster strain, (each weighing 130-180 g) in citrated tubes. The cells were then washed three times with 20 mM Tris-HCl containing 144 mM NaCl (pH 7.4) and 2% erythrocyte suspension was prepared. Human erythrocytes were obtained from the peripheral blood (O^+) of healthy volunteer. The blood was used within 24 h after bleeding and washed three times in 9

volumes of sterile 0.9 % NaCl saline solution. After each washing, cells were centrifuged 150 *X* g for 5 min and the supernatant was discarded. The final pellet was diluted 1:9 (v/v) in sterile 0.9 % NaCl saline solution then 1:24 (v/v) in sterile Dulbecco's phosphate buffer saline (D-PBS), pH 7.0 containing 0.5 mM boric acid and 1 mM calcium chloride.

The hemolytic activities of the compounds were tested by the method of Malagoli under *in vitro* conditions in 96-well plates. Each well received 100 μL of 0.85 % NaCl solution containing 10 mM $CaCl_2$. The first well served as negative control contained only water, and in the second well, 100 μl of compound of various concentrations (5 to 500 μg/mL) were added. The last well served as positive control containing 20 μL of 0.1% Triton X- 100 in 0.85 % saline. Then, each well received 100 μL of a 2 % suspension of mouse and human erythrocytes in 0.85 % saline containing 10 mM $CaCl_2$. After 30 min incubation at room temperature, centrifuged and the supernatant was used to measure the absorbance of the liberated hemoglobin at 540 nm. The average value was calculated from triplicate assay.

Cell culture. HeLa and Vero cell lines were obtained from ATCC and maintained in DMEM and RPMI 1640 (Himedia, Mumbai, India) medium supplemented with 10 % FBS (v/v) and 100 mg/L streptomycin and 100 IU/mL penicillin (Himedia, India) at 37°C in a CO_2 incubator with 5 % CO_2.

MTT cell proliferation assay. The cytotoxic activity of the compounds (diluted in DMSO 0 to100 μg/mL) on HeLa and Vero cells (1 X 10^5 cells/well) were tested by using the CellQuanti-MTT cell viability assay kit (Bioassay Systems). The wells with only culture medium or cells treated with 0.1 % of DMSO served as control. The graph was plotted with cell viability against the time period in hours at increasing concentrations of secondary metabolite. The mean and the IC_{50} value were calculated by non-linear regression analysis using the data analysis software (Prism) from three independent experiments.

Trypan blue dye exclusion assay. Cell viability assay was done by Trypan blue dye exclusion assay. The cells were incubated with or without compound. After 24 h of incubation cells were trypsinised, centrifuged for 5 min at 100 *X* g and the pellet was resuspended in 1 mL PBS. Trypan blue (0.4 %) 10 μL was added with 10 μL of cell suspension and incubated for 3-5 min. Trypan blue/cell mixture (10 μL) was placed in a haemocytometer and a total of 100 cells were counted and the number of viable and non-viable cells was recorded. The assay was done in triplicates.

Table 1. *In vitro* hemolytic on human erythrocytes and cytotoxicity on HeLa and Vero cells of compounds **5a-e**

Compound	Hemolytic activity	Cytotoxicity on HeLa	Cytotoxicity on Vero
	EC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
5a	>500	26.0	67.0
5b	298.1	>100	>100
5c	345.6	>100	>100
5d	126.2	>100	>100
5e	405.5	42.5	47.4
Camptothecin	429.9	0.28	58.0

The total hemolytic was obtained with 20μL of Triton X-100 (0.1%) and 1h incubation. The $EC_{50,}$ IC_{50} and 95 % confidence interval (CI 95%) was obtained by non-linear regression analyses. EC_{50} value lower than 250 μg/mL and was considered as active for hemolytic activity. As shown in tables 1, compound **5d** showed good hemolytic activity while other compounds **5b, 5c, and 5e** displayed moderate hemolytic activities. Compounds **5a** only had inactive against hemolytic activity against human erythrocytes. IC_{50} value lower than 50 μg/mL was considered as active for cytotoxicity on HeLa and Vero cells. As shown in tables 1, compound **5e** showed good cytotoxic activity on HeLa and Vero cells while others compounds **5a** displayed showed moderate cytotoxicity against on HeLa cells. Compounds **5b, 5c & 5d** had inactive on both cells.

Experimental Section

General. Melting points were taken in open capillary tubes and are corrected with reference to benzoic acid. UV in Hitachi U-2800 spectrophotometer was used measure the absorbance. IR spectra in KBr pellets were recorded on Nucon Infrared spectrophotometer. Nuclear Magnetic Resonance (^{1}H and ^{13}C) spectra were recorded on a Bruker Spectrospin Avance DPX400 Ultra shield (400 MHz) spectrometer. Chemical shifts are reported in parts per million (δ) downfield from an internal tetramethylsilane reference.

General procedure for synthesis of AB ring core of mappicine analogues

2-Chloro-quinoline-3-carbaldehydes **4a-e** was prepared by the reported procedure[16]. A mixture of 2-chloro-quinoline-3-carbaldehyde **4a-e,** sodium borohydride, and montmorillonite K-10 were mixed well and then irradiated in the microwave oven for 4 to 5 minutes gave (2-chloro-quinolin-3-yl)-methanol derivatives, **5a-e**. (Scheme 2) After completion of reaction, ethyl acetate was added to reaction mixture and catalyst was recovered by filtration. Filtrate was washed with saturated NH_4Cl solution to remove any unreacted sodium borohydride and further washed with

water to remove any inorganic materials. The organic layer was dried, solvent evaporated to get products.

Synthesis of (2-chloro-quinolin-3-yl)-methanol 5a

2-Chloro-quinoline-3-carbaldehyde **4** (5 mmol), sodium borohydride (5 mmol), and catalytic amount of montmorillonite K-10 were placed in a glass mortar, mixed well and irradiated with 800 W for 4 - 5 min. The reaction was monitored by TLC, and after completion of reaction, work up was performed as above to give crude product. Pure (2-chloro-quinolin-3-yl)-methanol **5a** was obtained by washing with ethylacetate, filter it and evaporate the solvent. Yield was determined (Table 1, compound **5a**).

A similar procedure was followed to obtain other derivatives **5b-e** from different 2-chloro-quinoline-3-carbaldehyde derivatives **4b-e** (Scheme 2). The products were characterized by FTIR, ^{1}HNMR, ^{13}CNMR and LCMS spectral techniques.

(2-Chloroquinolin-3-yl)methanol 5a. Yield: 94.2%; m.p.161°C (Lit. 162-163°C)[17]; IR (KBr pellets, cm^{-1}) ν: 3416, 2917, 1622; ^{1}H NMR ($CDCl_3$) δ: 8.3 (s, 1H, C-4H), 8.04-8.01 (d, 1H, *J*= 8.43Hz), 7.87-7.84 (d, 1H, *J*= 8.07Hz), 7.76-7.71 (t, 1H, *J*= 7.02, 8.4 Hz), 7.60-7.55 (t, 1H, *J*= 7.02 Hz, 8.01 Hz,), 4.94 (s, 2H, -CH_2), 2.27 (s, 1H, -OH); ^{13}C NMR ($CDCl_3$) δ: 146.87(N=*C*-Cl), 136.16, 132.25, 130.18, 128.16, 2 X 127.48, 127.29, 127.15, 61.96(-*C*H_2); LCMS: m/z, 194.4 (M+1) $C_{10}H_8ClNO$ (mol. wt. 193.63).

(2-Chloro-8-methylquinolin-3-yl)methanol 5b. Yield: 92%; m.p. 137 °C; IR (KBr pellets, cm^{-1}) ν: 3434, 2923, 1627, 1477; ^{1}H NMR ($CDCl_3$) δ: 8.23 (s, 1H, C-4H), 7.68-7.68 (d, 1H, *J*= 8.0Hz), 7.57-7.55 (d, 1H, *J*= 6.8 Hz), 7.47-7.43 (t, 1H, *J*= 7.6 Hz), 4.92 (s, 2H), 2.77 (s, 3H), 2.16 (s, 1H, -OH); ^{13}C NMR ($CDCl_3$) δ: 148.05, 146.26, 136.50, 136.47, 131.89, 130.32, 127.42, 126.93, 125.44, 62.18(-*C*H_2), 17.81(-*C*H_3); HRMS m/z 207.5216 (M^+); LCMS: m/z, 208.2 (M+1) $C_{11}H_{10}ClNO$ (mol. wt. 207.66).

(2-Chloro-7-methylquinolin-3-yl)methanol 5c. Yiled: 89%; m.p. 146 °C; IR (KBr pellets, cm^{-1}) ν: 3342, 1654, 1437; ^{1}H NMR ($CDCl_3$) δ: 8.22 (s, 1H, C-4H), 7.77 (s, 1H), 7.71-7.69 (d, 1H, *J*= 8.4Hz), 7.39-7.37 (d, 1H, 8.4 Hz), 4.90 (s, 2H), 2.54 (s, 3H), 2.48 (s, 1H, -OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.07, 147.22, 140.82, 136.08, 131.37, 129.43, 127.24, 127.15, 125.39, 62.06(-*C*H_2), 21.89(-*C*H_3); HRMS m/z 207.1000 (M^+); LC-MS m/z 208.2 (M+1) $C_{11}H_{10}ClNO$ (mol. wt. 207.66).

(2-Chloro-8-methoxy quinolin-3-yl)methanol 5d. Yield: 86%; m.p. 190°C; IR (KBr pellets, cm^{-1}) ν: 3464, 2920, 1634, 1462; ^{1}H NMR ($CDCl_3$) δ: 8.23 (s, 1H, C-4H), 7.48-7.44 (t, 1H, *J*= 8.0Hz), 7.38-7.36 (d, 1H, *J*= 8.0Hz), 7.06-7.04 (d, 1H, *J*= 7.6 Hz), 4.90 (s, 2H), 4.16 (s, 3H, -OCH_3), 2.57 (s, 1H, -OH); ^{13}C NMR ($CDCl_3$) δ: 154.48, 148.14, 138.59, 136.04, 133.06, 128.57, 127.47, 119.22, 108.47, 61.97(-*C*H_2), 56.05(-*C*H_3). HRMS m/z 223.6556 (M^+); LCMS: m/z, 224.0 (M+1) $C_{11}H_{10}ClNO_2$ (mol. wt. 223.66).

(2-Chloro-6-methoxy quinolin-3-yl)methanol 5e. Yield: 83%; m.p. 124°C (Lit. 120-122°C)[18]; IR (KBr pellets, cm^{-1}) ν: 3423, 2923, 1623, 1499; ^{1}H NMR ($CDCl_3$) δ: 8.17 (s, 1H, C-4H), 7.91-7.89 (d, *J*= 9.2Hz, 1H), 7.37-7.34 (m, 1H), 7.08 (s, 1H), 4.91 (s, 2H), 3.92 (s, 3H, -OCH_3), 2.31-

2.28 (m, 1H, -OH); HRMS m/z 223.6558 (M^+); LCMS. m/z 224.0 (M+1) $C_{11}H_{10}ClNO_2$ (mol. wt. 223.66).

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