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Microwave assisted synthesis of pyrido[2,3-a]carbazoles; investigation of *in vitro* DNA binding/cleavage, antioxidant and cytotoxicity studies

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ABSTRACT

We have developed an effective microwave assisted *p*-TsOH catalyzed synthesis of pyrido[2,3-a]carbazoles *via* a one pot reaction of ethanolamine and 1-chloro-2-formyl carbazoles. The structure has been characterized by spectroscopic methods. The electronic spectroscopic experimental evidence strongly showed that the compounds could interact with calf thymus DNA (CT-DNA) through intercalation with a binding constant value of $1.2–3.0 \times 10^4\ M^{-1}$. All the compounds showed weak to moderate capacity of scavenging with DPPH. The cytotoxicity has been evaluated by MTT assay against MCF-7 cell line and compared with standard drug cisplatin.

1. Introduction

Carbazole and its derivatives are an important type of nitrogen containing aromatic heterocyclic compounds endowed with various pharmacological activities such as anti-cancer, antimicrobial, antiviral, anti-inflammatory and antioxidant activity [1–3]. Carbazole scaffold is present in many drugs such as carvedilol and carproten. Carvedilol is an antihypertensive drug act as a non-specific β-adrenergic antagonist. Carproten is a non-steroidal anti-inflammatory drug (NSAID) and a selective COX-2 inhibitor [4,5]. Also, carbazole ring is present in a variety of naturally occurring medicinal active substances, such as ervatamine [6], 20-epiervatamine [7], 16-episilicine [8], eruistine [9], caulersine [10], homoarcyriaflavin [11], and are having highly interesting pharmacological properties.

In particular, several pyridocarbazole derivatives has been reported to exhibit anti-cancer and anti-HIV activities [12–16]. In addition ellipticine and its regioisomeric annulated indole and carbazole derivatives with pyrido[4,3-b]carbazole framework constitute an interesting class of antitumor activity drugs [17–20]. Among the ellipticine compounds, the 9-methoxyellipticine shows activity against a verity of human tumor cell lines, especially agaius leukaemia where as the quaternary pyridinium salt ellipticinium acetate was developed against metastatic breast cancer [19,21]. Apart from their utility for several disease targets, pyrido[2,3-a]carbazole derivatives has shown an enormous synthetic value in the preparation of various bio-active molecules.

To encourage by the varied biological activities of pyrido carbazole derivatives, we used an alternative green reaction (Microwave) for the synthesis of pyrido carbazole derivatives. On the other hand, for the stringent and growing environmental regulations, organic chemists are requested to develop environmentally benign synthetic methodologies. Microwave-assisted heating has been shown to be an invaluable technology in synthesis since it highly reduces reaction times, typically from days or hours to minutes or even seconds. It can also provide pure products in quantitative yields. Solvent-free reaction techniques were successfully coupled with the microwave method because they avoid the use of the low boiling point and high vapour pressure solvents, which may sometimes lead to explosions. Additionally, it can also avoid the use of poisonous and expensive solvents, and as such can be environmentally benign, and make manipulations much easier [22,23].

Considering the above mentioned fact, herein we wish to report a simple, convenient microwave assisted synthesis of pyrido[2,3-a]carbazoles from the reaction of ethanolamine and 1-chloro-2-formyl carbazoles in the presence of *p*-TsOH as the catalyst with shorter reaction times and good yield. The structures of the products were deduced from their elemental analysis data, and from their IR, mass, ^1H and ^{13}C NMR spectra. Furthermore, the newly synthesized compounds were evaluated for their *in vitro* DNA binding/cleavage affinity, antioxidant assay and cytotoxicity against MCF-7 breast cancer cell line.

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2. Experimental protocols

2.1. Materials, instruments and methods

All the chemicals used were chemically pure and AR grade. Solvents were purified and dried according to the standard procedure [24]. Elemental analysis (C, H and N) was performed on a vario EL 111 CHN analyzer. IR spectra were recorded by KBr pellet technique in the range 400–4000 cm^{-1} region using a Perkin Elmer FT-IR 8000 spectrophotometer model. ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 111 500 MHz instrument using TMS as internal reference. Electron ionization mass spectra of the compounds were recorded on a JEOL GCMATEII mass spectrometer. DNA cleavage studies were carried out using Gelstan – Gel documentation system. Antioxidant and anticancer studies were carried out at the Kovai Medical Centre and Hospital Pharmacy College, Coimbatore, Tamil Nadu, India.

2.2. General procedure for synthesis of compound **3a–g**

A mixture of 1-chloro-2-formyl carbazole (**1**, 1 mmol) and ethanolamine (**2**, 1 mmol) and *p*-TsOH (20 mol%) was heated at 90 °C for 10 min under microwave irradiation (Biotage microwave oven, 90 °C). The crude product obtained was purified by column chromatography, over silica gel using petroleum ether: ethyl acetate (98:2) as an eluent to get a yellow solid 2,3-dihydro-1H,11H-pyrido[2,3-a]carbazol-4-one**(3a).** The product formed **3a** was recrystallised by using methanol solution. The compounds, **3b–g** is prepared in a similar manner as described for compound **3a.**

2.2.1. 2,3-Dihydro-1H,11H-pyrido[2,3-a]carbazol-4-one ***(3a)***

Golden yellow solid; yield: 77.8%; M.P. 165 °C. Anal. Calcd. for $C_{15}H_{12}N_2O$ (%): C, 76.25; H, 5.12; N, 11.86. Found (%): C, 76.26; H, 5.12; N, 11.88. IR (KBr, cm^{-1}): 3392, 3271 ν (N—H), 3011 ν (C—H), 1639 ν (C=O); ^1H NMR ($CDCl_3$, ppm), δ 1.02 (t, 2H, C_3—CH_2, J = 7.8 Hz); 2.60 (t, 2H C_2—CH_2, J = 9.00 Hz); 7.35–8.65 (m, 6H,C_5, C_6, C_7, C_8, C_9, C_{10}—H); 5.68 (s, 1H, 1-NH); 9.80 (s, 1H, 11-NH). ^{13}C NMR ($CDCl_3$, ppm), δ 12.45, 26.68, 106.88, 110.59, 113.31, 119.93, 122.88, 124.66, 126.37, 128.71, 129.41, 136.28, 136.86, 142.68, 189.24. MS: *m/z* (%) 237 (M^+, 100).

2.2.2. 8-Methyl-2,3-dihydro-1H,11H-pyrido[2,3-a]carbazole-4-one ***(3b)***

Yellow solid; yield: 79.4%; M.P. 185 °C. Anal. Calcd. for $C_{16}H_{14}N_2O$ (%): C, 76.78; H, 5.64; N, 11.19. Found (%): C, 76.78; H, 5.63; N, 11.20. IR (KBr, cm^{-1}): 3380, 3271 ν (N—H), 3013 ν (C—H), 1645 ν (C=O); ^1H NMR ($CDCl_3$, ppm), δ 1.05 (t, 2H, C_3—CH_2, J = 7.8 Hz); 2.50 (s, 3H, C_8—CH_3); 2.62 (t, 2H C_2—CH_2, J = 9.00 Hz); 7.35–8.20 (m, 5H,C_5, C_6, C_7, C_9, C_{10}—H); 5.72 (s, 1H, 1-NH); 9.75 (s, 1H, 11-NH). ^{13}C NMR ($CDCl_3$, ppm), δ 21.31, 12.46, 26.68, 106.88, 110.59, 113.31, 119.93, 122.88, 124.66, 126.37, 128.71, 129.41, 136.28, 136.86, 142.68, 189.24. MS: *m/z* (%) 251 (M^+, 100).

2.2.3. 9-Methyl-2,3-dihydro-1H,11H-pyrido[2,3-a]carbazole-4-one ***(3c)***

Yellow solid; yield: 56.4%; M.P. 189 °C. Anal. Calcd. for $C_{16}H_{14}N_2O$ (%): C, 76.78; H, 5.64; N, 11.19. Found (%): C, 76.77; H, 5.61; N, 11.20. IR (KBr, cm^{-1}): 3393, 3273 ν (N—H), 3017 ν (C—H), 1642 ν (C=O); ^1H NMR ($CDCl_3$, ppm), δ 1.03 (t, 2H, C_3—CH_2, J = 7.8 Hz); 2.49 (s, 3H, C_9—CH_3); 2.62 (t, 2H C_2—CH_2, J = 9.00 Hz); 7.35–8.10 (m, 5H,C_5, C_6, C_7, C_8, C_{10}—H); 5.69 (s, 1H, 1-NH); 9.81 (s, 1H, 11-NH). ^{13}C NMR ($CDCl_3$, ppm), δ 12.45, 21.51, 26.68, 106.88, 110.59, 113.31, 119.93, 122.88, 124.66, 126.37, 128.71, 129.41, 136.28, 136.86, 142.68, 189.24. MS: *m/z* (%) 251 (M^+, 100).

2.2.4. 10-Methyl-2,3-dihydro-1H,11H-pyrido[2,3-a]carbazole-4-one ***(3d)***

Yellow solid; yield: 52.0%; M.P. 194 °C. Anal. Calcd. for $C_{16}H_{14}N_2O$ (%): C, 76.78; H, 5.64; N, 11.19. Found (%): C, 76.78; H, 5.62; N, 11.19. IR (KBr, cm^{-1}): 3399, 3271 ν (N—H), 3011 ν (C—H), 1654 ν (C=O); ^1H NMR ($CDCl_3$, ppm), δ 1.03 (t, 2H, C_3—CH_2, J = 7.8 Hz); 2.51 (s, 3H, C_{10}—CH_3); 2.63 (t, 2H C_2—CH_2, J = 9.00 Hz); 7.35–8.00 (m, 5H,C_5, C_6, C_7, C_8, C_9—H); 5.70 (s, 1H, 1-NH); 9.73 (s, 1H, 11-NH). ^{13}C NMR ($CDCl_3$, ppm), δ 12.45, 16.51, 26.68, 106.88, 110.59, 113.31, 119.93, 122.88, 124.66, 126.37, 128.71, 129.41, 136.28, 136.86, 142.68, 189.24. MS: *m/z* (%) 251 (M^+, 100).

2.2.5. 8-Chloro-2,3-dihydro-1H,11H-pyrido[2,3-a]carbazol-4-one ***(3e)***

Brown solid; yield: 65.8%; M.P. 216 °C. Anal. Calcd. for $C_{15}H_{11}ClN_2O$ (%): C, 66.55; H, 4.10; N, 10.35. Found (%): C, 66.55; H, 4.11; N, 10.34. IR (KBr, cm^{-1}): 3354, 3282 ν (N—H), 3010 ν (C—H), 1641 ν (C=O); ^1H NMR ($CDCl_3$, ppm), δ 1.05 (t, 2H, C_3—CH_2, J = 7.8 Hz); 2.58 (t, 2H C_2—CH_2, J = 9.0 Hz); 7.35–8.00 (m, 6H,C_5, C_6, C_7, C_8, C_9, C_{10}—H); 5.71 (s, 1H, 1-NH); 9.83 (s, 1H, 11-NH). ^{13}C NMR ($CDCl_3$, ppm), δ 12.45, 26.68, 106.88, 110.59, 113.31, 119.93, 122.88, 124.66, 126.37, 128.71, 129.41, 136.28, 136.86, 142.68, 189.24. MS: *m/z* (%) 271 (M^+, 100).

2.2.6. 8-Bromo-2,3-dihydro-1H,11H-pyrido[2,3-a]carbazol-4-one ***(3f)***

Yellow solid; yield: 61.5%; M.P. 227 °C. Anal. Calcd. for $C_{15}H_{11}BrN_2O$ (%): C, 57.16; H, 3.52; N, 8.89. Found (%): C, 57.16; H, 3.50; N, 8.86. IR (KBr, cm^{-1}): 3357, 3277 ν (N—H), 3015 ν (C—H), 1646 ν (C=O); ^1H NMR ($CDCl_3$, ppm), 1.07 (t, 2H, C_3—CH_2, J = 7.8 Hz); 2.59 (t, 2H C_2—CH_2, J = 9.0 Hz); 7.35–8.10 (m, 5H,C_5, C_6, C_7, C_9, C_{10}—H); 5.72 (s, 1H, 1-NH); 9.83 (s, 1H, 11-NH). ^{13}C NMR ($CDCl_3$, ppm), δ 12.45, 26.68, 106.88, 110.59, 113.31, 119.93, 122.88, 124.66, 126.37, 128.71, 129.41, 136.28, 136.86, 142.68, 189.24. MS: *m/z* (%) 315 (M^+, 100).

2.2.7. 8-Methoxy-2,3-dihydro-1H,11H-pyrido[2,3-a]carbazol-4-one ***(3g)***

Brown solid; yield: 63.2%; M.P. 173 °C. Anal. Calcd. for $C_{16}H_{14}N_2O$ (%): C, 76.78; H, 5.64; N, 11.19. Found (%): C, 76.78; H, 5.63; N, 11.20. IR (KBr, cm^{-1}): 3395, 3271 ν (N—H), 3019 ν (C—H), 1639 ν (C=O); ^1H NMR ($CDCl_3$, ppm), δ 1.05 (t, 2H, C_3—CH_2, J = 7.8 Hz); 3.73 (s, 3H, C_8—OCH_3); 2.62 (t, 2H C_2—CH_2, J = 9.0 Hz); 7.35–8.20 (m, 5H,C_5, C_6, C_7, C_9, C_{10}—H); 5.72 (s, 1H, 1-NH); 9.75 (s, 1H, 11-NH). ^{13}C NMR ($CDCl_3$, ppm), δ 12.45, 26.68, 56.00, 106.88, 110.59, 113.31, 119.93, 122.88, 124.66, 126.37, 128.71, 129.41, 136.28, 136.86, 142.68, 189.24. MS: *m/z* (%) 267 (M^+, 100).

2.3. Biological evaluation

2.3.1. DNA binding – titration experiments

The binding affinities with CT-DNA of all the compounds were carried out in doubly distilled water with Tris(hydroxymethyl)-aminomethane (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.2 with hydrochloric acid. A solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.8–1.9 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar extinction coefficient value of 6600 $dm^3\ mol^{-1}\ cm^{-1}$ at 260 nm. The compounds were dissolved in a mixed solvent of 5% DMSO and 95% Tris HCl buffer for all the experiments. Stock solutions were stored at 4 °C and used within 4 days. Spectrophotometric titration experiments

were performed with fixed concentration of the compounds (25 μM) with varying concentration of DNA (0–50 μM). While measuring the absorption spectra, an equal amount of DNA was added to the all test solutions and the reference solution to eliminate the absorbance of DNA itself.

2.3.2. Nuclease activity using gel electrophoresis

The DNA cleavage activity of the pyrido[2,3-a]carbazole derivatives was monitored by agarose gel electrophoresis on CT DNA. The cleavage was monitored by 30 μM of CT DNA and 25, 50 μM of each compounds (**3e and 3f**) in 5% DMSO and 95% Tris-HCl buffer (5 mM, pH 7.2) with 50 mM NaCl.

The samples with sufficient buffer were incubated for 2 h at 37 °C. After incubation, 1 μL of loading buffer (0.25% bromophenol blue, 0.25% xylene cynol and 60% glycerol) was added to the reaction mixture and loaded onto a 1% agarose gel containing 1.0 μg/mL of ethidium bromide. The electrophoresis was carried out for 2 h at 50 V in Tris-acetic acid EDTA buffer. The bands were visualized under UV light and photographed.

2.3.3. Antioxidant activity

The 2,2-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of the compounds was measured according to the method of Elizabeth and Rao [25]. The DPPH radical is a stable free radical having a λ_{max} at 517 nm. As this electron becomes paired off in the presence of free radical scavenger, the absorption vanishes and the resulting decolourisation is stoichiometric with respect to the number of electron taken up. A fixed concentration of the experimental compound (100 μL) was added to a solution of DPPH in methanol (0.3 mM, 1 mL) and the final volume was made up to 4 mL with double distilled water. DPPH solution with methanol was used as a positive control and methanol alone acted as a blank. The solution was incubated at 37 °C for 30 min in dark. The decrease in absorbance of DPPH was measured at 517 nm. The tests were run in triplicate, and various concentrations (20–100 μg/mL) of the compounds used to fix a concentration at which the compounds showed 50% of activity. In addition, the percentage of activity was calculated using the formula, % of suppression ratio = $[(A_0–A_c)/A_0] \times 100$. A_0 and A_c are the absorbance in the absence and presence of the tested compounds respectively. The 50% activity (IC_{50}) can be calculated using the percentage of activity.

2.3.4. In vitro anticancer activity assay

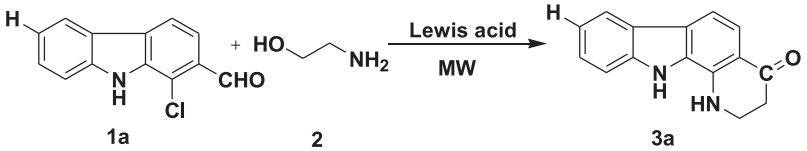
Cytotoxicity of the compounds were carried out on human breast cancer cell line (MCF-7). It was obtained from National Centre for Cell Science (NCCS), Pune, India. Cell viability was carried out using the MTT assay method [26]. MCF-7 cell was grown in eagles minimum essential medium (EMEM) containing 10% fetal bovine serum (FBS). For the screening experiment, the cells were seeded onto 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to the addition of the compounds. The compounds were dissolved in DMSO and diluted in the respective medium containing 1% FBS. After 24 h the medium was replaced with the respective medium with 1% FBS containing the compounds at various concentrations and incubated at 37 °C under conditions of 5% CO_2, 95% air and 100% relative humidity for 48 h. Triplication was maintained and the medium not containing the compounds served as control. After 48 h, 15 μL of MTT (5 mg mL^{-1}) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was then removed and the formed formazan crystals were dissolved in 100 μL of DMSO. The absorbance was then measured at 570 nm using micro plate reader and % cell inhibition was determined. Using the following formula and a graph was plotted with the percentage of cell inhibition *versus* concentration. From this, the IC_{50} was calculated: % cell inhibition = [mean OD of untreated cells (control)/mean OD of treated cells (control)] × 10.

3. Results and discussion

3.1. Chemistry

The focus of this work is on the synthesis of pyrido[2,3-a] carbazoles by microwave assisted synthetic method. The reactants 1-chloro-2-formyl carbazole **1a** and ethanolamine **2** were reacted in the presence of *p*-TsOH as the catalyst with shorter reaction time and good yield. The reaction was attempted in the presence of various catalysts like tin chloride, zinc chloride, aluminium chloride and ferric chloride (Table 1) in micro oven, when these metal chlorides were replaced by *p*-TsOH (p-toluenesulfonic acid) the reaction proceeded smoothly, comparatively required shorter reaction time and gave higher yield than the other metal chloride catalysts. Thus *p*-TsOH as the most efficient catalyst compared with other catalysts tested (Table 2). Using this method 1-chloro-2-formyl Carbazole **1a** was reacted with ethanolamine **2** to produce the corresponding 2,3-dihydro-1H,11H-pyrido[2,3-a] carbazol-4-one **3a**. The IR spectrum of **3a** displayed absorption bands at 3392, 3271 (N—H, Stretch), 3011 (C—H, Aromatic Stretch), 1639 (C═O, Stretch). In its ^{1}H NMR spectrum (Fig. 1–7) of the aliphatic amino proton resonated at δ 5.68. The total number of protons matched perfectly with its structure. The ^{13}C NMR spectrum revealed the presence of 15 carbons. The structures of all compounds were confirmed by elemental and spectral analysis. The same reaction was carried out with various substituted

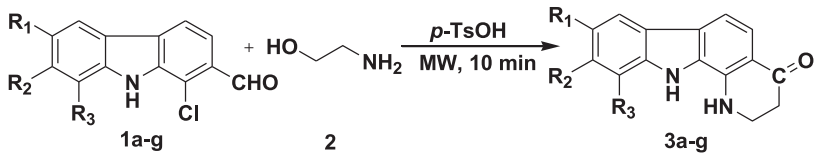
Table 1
Reaction of 1-chloro-2-formyl carbazole with ethanol amine, under various conditions.



1a 2 3a

Entry	Catalyst	Time (min)	Conditions (°C)	Yield (%) **3a**
1	p-TsOH	10	90	78
2	$ZnCl_2$	25	90	40
3	$FeCl_3$	20	90	51
4	$AlCl_3$	25	90	54
5	$SnCl_4$	15	90	61

Table 2
Reaction of 1-chloro-2-formyl carbazole with ethanol amine, under various conditions.



Entry	R_1	R_2	R_3	Product	Yield
1	H	H	H	**3a**	78
2	CH_3	H	H	**3b**	79
3	H	CH_3	H	**3c**	56
4	H	H	CH_3	**3d**	52
5	Cl	H	H	**3e**	67
6	Br	H	H	**3f**	62
7	OMe	H	H	**3g**	63

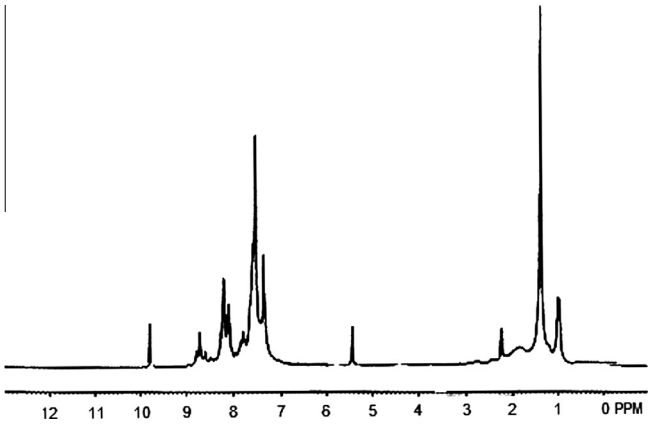


Fig. 1. ^{1}H NMR spectrum of compound **3a**.

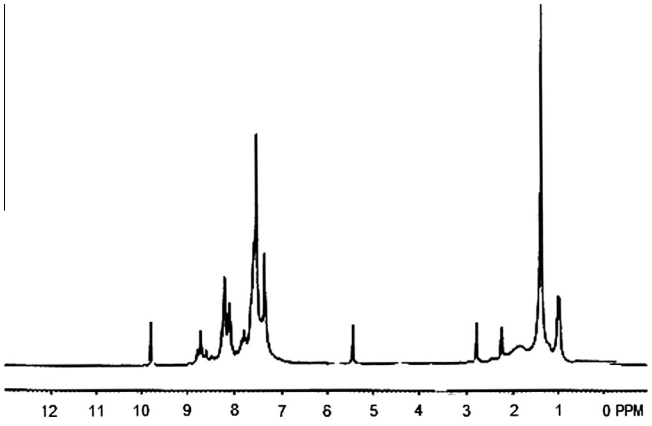


Fig. 3. ^{1}H NMR spectrum of compound **3c**.

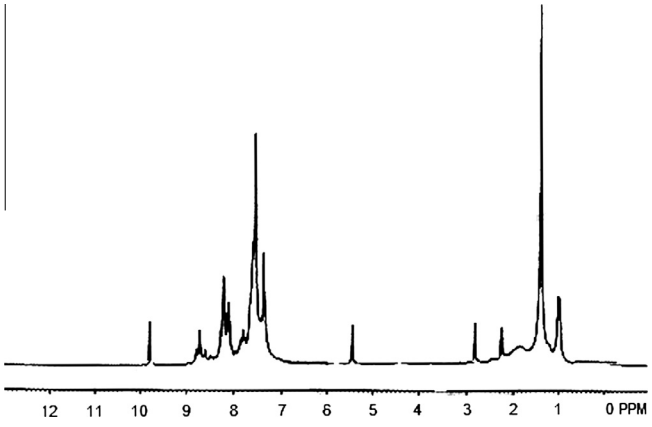


Fig. 2. ^{1}H NMR spectrum of compound **3b**.

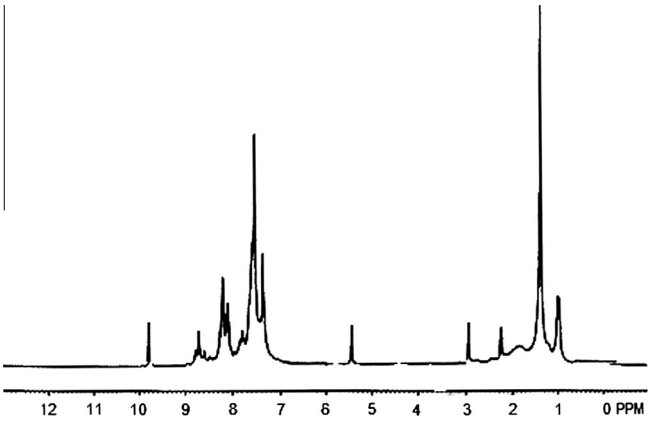


Fig. 4. ^{1}H NMR spectrum of compound **3d**.

1-chloro-2-formyl carbazoles, which are represented in Scheme 1 and Table 2.

3.2. Pharmacology

3.2.1. DNA binding – titration experiments

DNA molecule is a target for plethora of anticancer drugs that form covalent and non-covalent adducts with major or minor groove of DNA [27]. Because, DNA act as a significant intracellular receptor and many chemicals exhibit their antitumor effects by binding to DNA. Pharmacological actions of these compounds were mediated by means of change in replication of DNA leading to inhibit growth of tumor cells and their effectiveness depends upon the mode and affinity of their DNA binding [28]. Therefore, the binding studies of hetero compounds to DNA are considered to be highly important in the development of new anticancer drugs.

The size and the shape of the carbazole ring leads to an almost perfect overlapping of the aromatic ring with that of DNA base pair [29] therefore, the pyrido carbazole ring appears as an appropriate

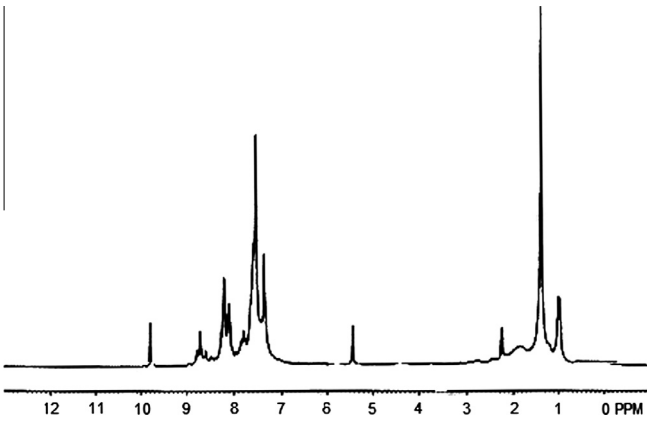


Fig. 5. ^{1}H NMR spectrum of compound **3e**.

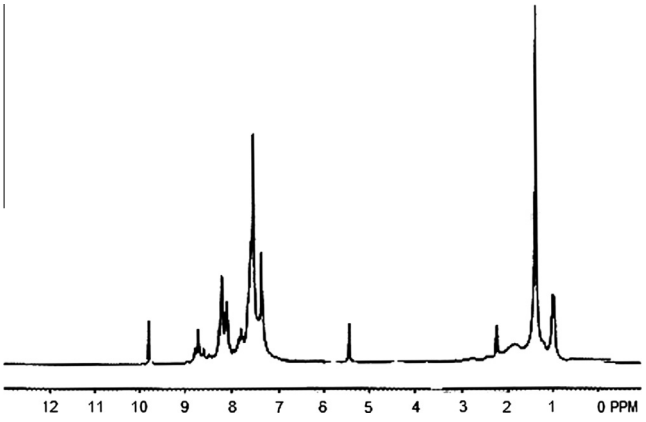


Fig. 6. ^{1}H NMR spectrum of compound **3f**.

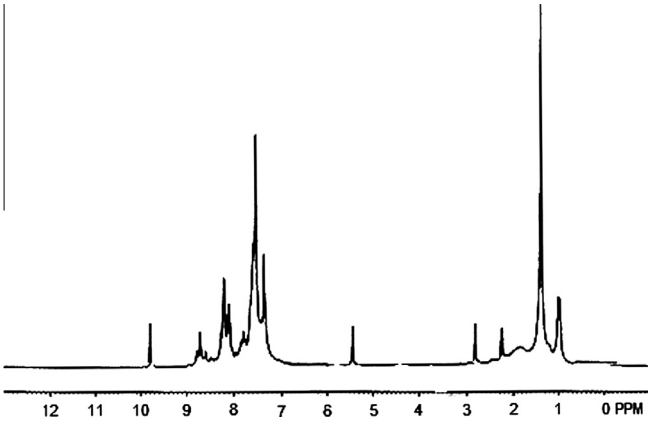


Fig. 7. ^{1}H NMR spectrum of compound **3g**.

skeleton to design DNA intercalating drugs. Electronic absorption spectroscopy is one of the most common techniques for the investigation of the mode of the interaction of compounds with DNA. Absorption spectra of the compounds in the absence and presence of CT-DNA is given in Fig. 8. The absorbance spectroscopy revealed the shift in the wavelength as a function of concentration of DNA. This confirms the biding of the compounds with DNA. Upon addition of increased amount of CT-DNA, a significant hypochromism and a red shift of about 3–5 nm is observed in the band at 228–315 nm. This can be attributed to a strong interaction between DNA and compounds, and it also likely that this compounds bind to the DNA helix *via* intercalation. In order to illustrate quantitatively the consequence, the absorption data was analyzed to evaluate the intrinsic binding constant (K_b), which can be determined from the following equation [30],

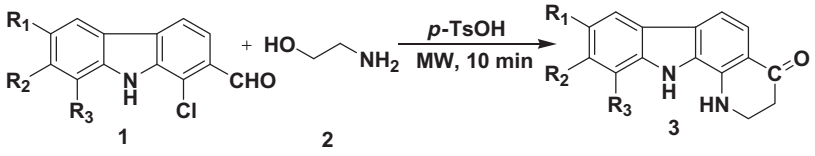
$$\mathrm{DNA}/(\varepsilon_a - \varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficient ε_a, ε_f and ε_b corresponds to A_{obs}/[compound], the extinction coefficient of the free compound and the extinction coefficient of the compound when fully bound to DNA, respectively. From the plot of DNA/($\varepsilon_a - \varepsilon_f$) *versus* [DNA], K_b is calculated by the ratio of slope to the intercept. The magnitude of intrinsic binding constant (K_b) values for compounds **3a, 3b, 3c, 3d, 3e, 3f** and **4g** are $2.8 \times 10^4\ M^{-1}$, $2.6 \times 10^4\ M^{-1}$, $2.4 \times 10^4\ M^{-1}$, $1.2 \times 10^4\ M^{-1}$, $3.0 \times 10^4\ M^{-1}$, $2.9 \times 10^4\ M^{-1}$ and $2.8 \times 10^4\ M^{-1}$ respectively. Further, the observed binding constant values are in comparable with the classical intercalator ethidium bromide [31]. Hence, **3d** shows weaker binding towards CT-DNA than the six compounds, and **3e** shows highest binding affinity. From the above DNA binding results, it can be concluded that the title compound's planarity and extended π system lead to the possibility of DNA intercalation.

3.2.2. DNA cleavage activity

A number of studies have shown that the clinical efficacies of many drugs correlate with their ability to induce enzyme – mediated DNA cleavage. The inhibitory potency of the test compounds was assessed by comparing the cleavage of DNA by control and title compound. Also, the ability of the compounds to perform DNA cleavage is generally monitored by agarose gel electrophoresis and in the present work CT-DNA was chosen to investigate its cleavage activity of **3e** and **3f.**

It was observed from the photograph that compounds **3e** and **3f** after treatment with DNA (calf-thymus) have cleaved completely. In **3e** lane, with chloro substituent on the para position of the carbazole ring, whole band of the DNA is missing indicating the complete cleavage of DNA. This cleavage may be because of the intercalation of the carbazole units with the DNA strands as the molecules are reported to bind AT sequences [32]. Further, DNA treated with **3f,** with bromo substituted on the para position of the carbazole ring. It is also showed good cleavage activity was observed as shown in Figs. 9 and 10. The intercalation of the compounds has destabilized the CT–DNA.



Scheme 1. Synthesis of 2, 3-dihydrol-1H,11H-pyrido [2,3-a]carbazole-4-one using *p*-TsOH.

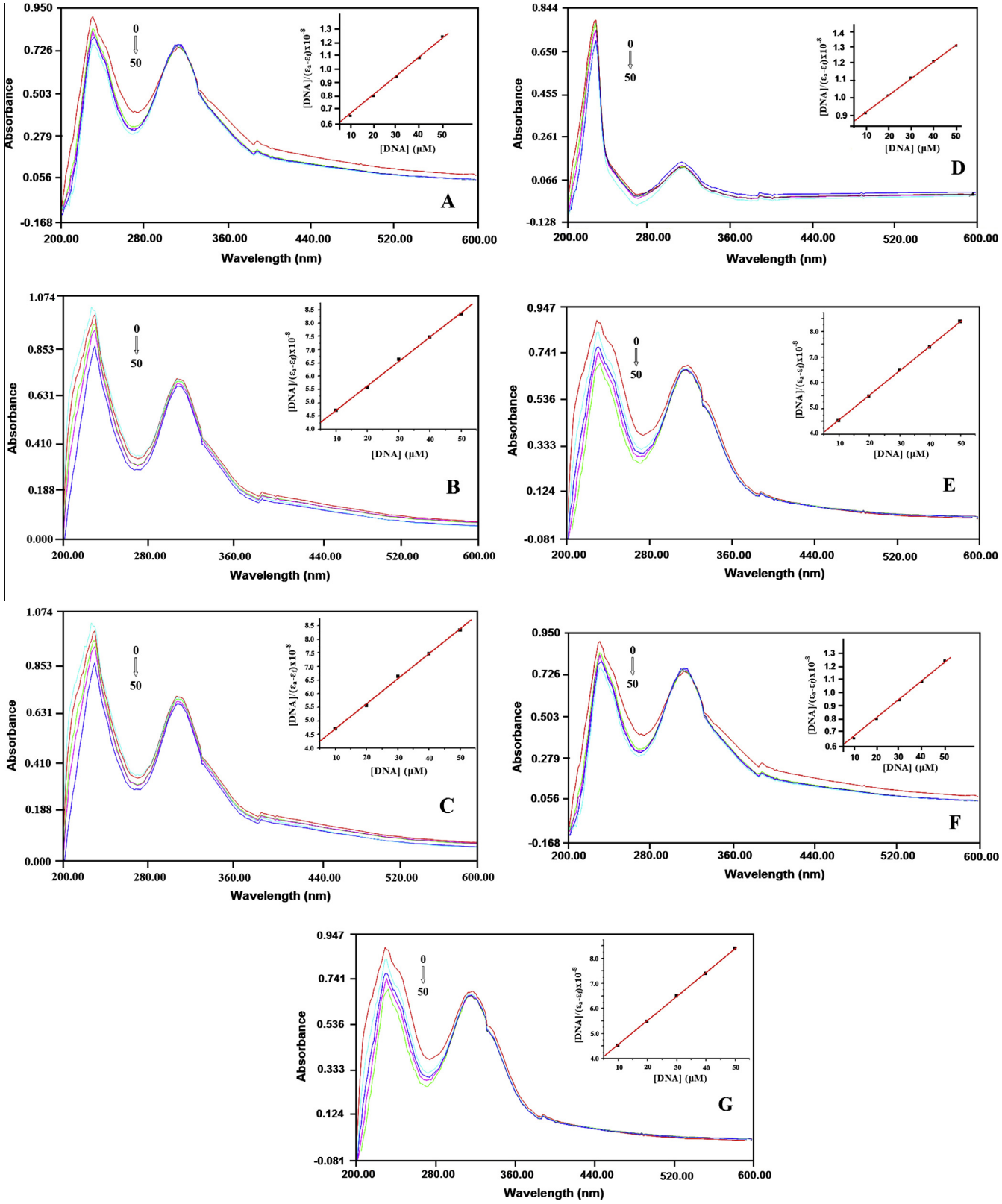


Fig. 8. Electronic spectra of compounds **3a** (A), **3b** (B), **3c** (C), **3d** (D), **3e** (E), **3f** (F) and **3g** (G) in Tris-HCl buffer upon addition of CT-DNA. [Compound] = 25 μM, [DNA] = 0–50 μM. Arrow shows the absorption intensities decrease upon increasing DNA concentration (inset: plot between [DNA] and [DNA]/[$\varepsilon_a - \varepsilon_f$] × 10^{-8}).

3.2.3. Antioxidant activity

The DPPH radical has been used to test the ability of compounds as free radical scavengers or hydrogen donors to evaluate the antioxidant activity. Hence, we carried out experiments to explore the free radical scavenging ability of pyrido[2,3-a]carbazoles with DPPH radical and compared with those of the positive control, ascorbic acid (Aca). Nearly all the tested compounds showed weak to moderate capacity for scavenging DPPH. The IC_{50} values

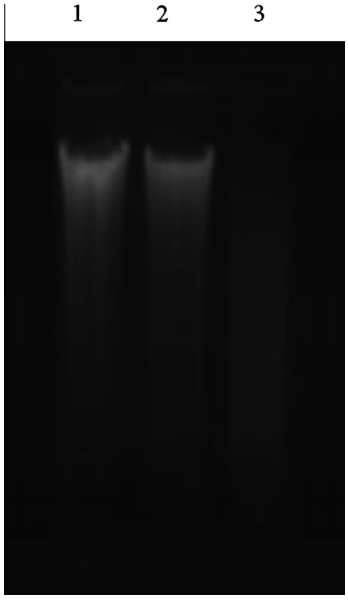


Fig. 9. Gel electrophoresis diagram showing the cleavage of CT-DNA (30 μM) by compound **3e** in 5% DMSO and 95% 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2. Lane 1: control DNA (untreated sample). Lane 2, 3: compound **3e** (25, 50 μM) + DNA.

Fig. 10. Gel electrophoresis diagram showing the cleavage of CT-DNA (30 μM) by compound **3f** in 5% DMSO and 95% 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2. Lane 1: control DNA (untreated sample). Lane 2, 3: compound **3f** (25, 50 μM) + DNA.

indicated that the compounds showed antioxidant activity in the order of **3e > 3f > 3a > 3g > 3b.** All the compounds showed scavenging capacity beyond 110 μm/mL. Among the compounds **3c** and **3d** did not shows any radical scavenging capacity up to 600 μm/mL. The IC_{50} values are expressed in μm/ml of all compounds except **3c** and **3d** as represented in Fig. 11.

3.2.4. In vitro anticancer activity evaluation

The positive result obtained from DNA binding encouraged us to test the cytotoxicity against cancer cell. cytotoxicity of newly synthesized compounds, against human breast cancer cell line (MCF-7) was evaluated by means of MTT assay method. This method measures mitochondrial dehydrogenase activity as an indication of cell viability (corresponding to the reductive activity), and

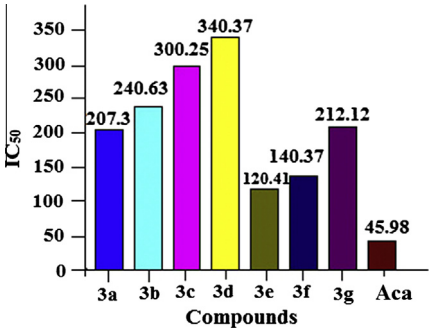


Fig. 11. Antioxidant activity of compounds.

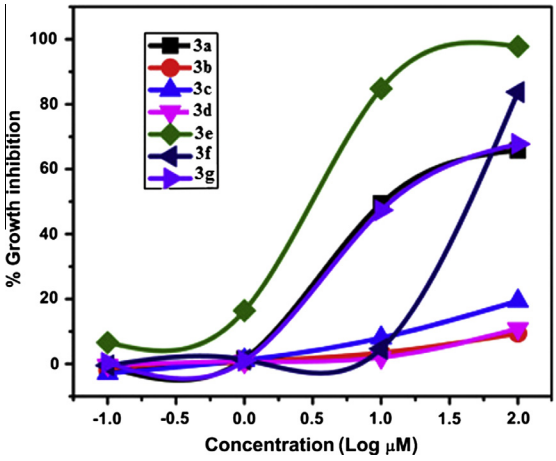


Fig. 12. % Growth inhibition of MCF-7 cell line as a function of concentration of the compounds **3a–g.**

Table 3
Cytotoxic activity of the compounds against the cancer cell line, MCF-7.

Compounds	IC_{50} (μM)
3a	98.82
3b	>100
3c	>100
3d	>100
3e	44.26
3f	47.15
3g	58.04
Reported value (cisplatin)	12.75

is proportional to the production of purple formazan, which is measured spectrophotometrically [33]. The results are analyzed by means of cell viability curves and expressed with IC_{50} (the concentration that cause a 50% reduction of the cell growth) values in concentration range from 0.1 to 100 μM. The activity that corresponds to the inhibition of cancer cell growth at a minimum level is shown in Fig. 12. The results are summarized in Table 3.

As shown in Table 3, some of the tested compounds showed good anticancer activity against tested cancer cell line. Among the compounds, **3e** showed higher cytotoxicity effect followed by **3f**. Compounds, **3a** and **3g** showed significant IC_{50} values but the cytotoxic effect of these compounds is less when compared to the other two compounds, **3e** and **3f.** Compounds, **3b, 3c** and **3d** does not show any significant activity (IC_{50} > 100 μM), against MCF-7 cancer cell line. It is observed from the results that the electron withdrawing group could enhance the anticancer activity of the compounds [34]. It is interesting to note that, chloro and bromo derivatives (**3e, 3f**) showed better cytotoxic activity than the

methyl and methoxy derivatives. Also, from the results, it is inferred that, **3e** and **3f** shows better results but still these derivatives does not reach the potential of cisplatin whose IC_{50} value is 12.75 μM [35].

4. Conclusions

We have synthesized pyrido[2,3-a]carbazoles by *p*-TsOH catalyzed microwave reaction of 1-chloro-2-formyl carbazole with ethanolamine. The structure of the newly synthesized compounds is established by spectroscopic methods. The DNA binding ability of the compounds were assessed by absorption spectra which inferred an intercalative mode of binding the experimental result suggested that the compound **3e** can bind to DNA more strongly than the other compounds. The binding constant value of **3e** is $3.0 \times 10^4\ M^{-1}$. In case of DNA cleavage activity we observed that the compounds **3e** and **3f** exhibited complete cleavage of DNA. All the compounds showed weak to moderate capacity for free radical scavenging. Cytotoxicity studies were carried out by MTT assay method against human breast cancer cell line MCF-7 and showed significant activity. It may be noted that the performance of compounds **3e** and **3f** was much better activity against MCF-7 cell line and compounds **3a** and **3g** showed moderate activity against MCF-7, than the other three compounds.

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