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# Synthesis of 2-Methyl -3,4,5,10-Tetrahydro-Pyrrolo [3,2-A] Carbazole-Ethyl Carboxylate Derivatives and Evaluation of their Binding Affinity with CT-DNA

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## Abstract

*A new class of heterocyclic compound, substituted 2-methyl-3,4,5,10-tetarahydro-pyrrolo [3,2-a]carbazole-1-ethyl carboxylate derivatives have been prepared from 1-oxo-1,2,3,4,-tetrahydrocarbazoles. The structural features were determined by analytical and spectral techniques. DNA binding study of the compounds was investigated by absorption titration experiments which indicated that the compounds showed good binding affinity to CT-DNA via intercalation.*

***Keywords:*** *1-oxo-1,2,3,4-tetrahydrocarbazole; DNA binding; Ethylaceto acetate; EI mass spectroscopy; NMR spectroscopy.*

## 1. INTRODUCTION

Aryl- and heteroaryl- annulated carbazoles, such as pyridocarbazoles, indolocarbazoles, and pyrrolocarbazoles have attracted growing attention since they are distributed in numerous natural products with diverse useful bioactivities. Among them, many efforts have been devoted to the design and synthesis of pyrido, pyrrolo and pyrano carbazoles have received considerable attention from both medicinal as well as synthetic chemists as these compounds display a wide range of biological activites such as anticancer, anti-HIV, DNA-action and antimicrobial activities. In particular, the pyrrolo[2,3-a] and [3,4-c] carbazoles have great importance due to their inhibiting properties towards pim kinase inhibitores and chk inhibitors, respectively (Yamuna *et al*. 2011; Viji *et al*. 2012). In addition, indole moiety is an essential part of the amino acid tryptophan and the neurotransmitter serotonin and the indole scaffold is also found in a manifold of naturally occurring plant based alkaloids. The biological, pharmacological and medicinal importances of indole heterocycles, have made indole extremely attractive and rewarding research targets, and have motivated countless researchers to study their synthesis and pharmacological properties (Yamuna *et al*. 2012). Despite of recent progress in cancer chemotherapy, high toxicity and low specificity of current medications are motivating the scientists to search for safer and more effective anticancer drugs. Chemotherapy-induced cell cycle arrest was shown to result from DNA damages caused by a variety of chemotherapeutics. In the case of ellipticine, it was suggested that the prevalent DNA-mediated mechanisms of their antitumor, mutagenic and cytotoxic activities are (i) intercalation into DNA and (ii) inhibition of DNA topoisomerase II activity (Auclair *et al*. 1987; Singh *et al*. 1994; Monnot *et al*. 1991).

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Since the intracellular target for wide range of anticancer and antibiotic drugs are deoxyribonucleic acid (DNA), the studies of interaction of drug molecules with DNA have become an active area of research in recent years. These studies can greatly help to understand drug–DNA interactions and design of new and promising drugs for clinical use.

In our present study, we have synthesized a series of 2-methyl-3,4,5,10-tetrahydro-pyrrolo [3,2-a]carbazole-1-ethyl carboxylate derivatives (4a-b) by a simple synthetic route (Scheme 1). The structure of the products was deduced from their physico-chemical and spectroscopic studies. Additionally, a comparative study of the interaction of the carbazole derivatives with CT-DNA have been employed in order to investigate the potential mechanism of their biological activities using UV-visible spectroscopic technique.

## 2. EXPERIMENTAL

### 2.1 Materials, instruments & methods

All the chemicals used were chemically pure and AR grade. Solvents were purified and dried according to the standard procedure (Vogel, 1991). 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 . $^{1}H$ 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. Melting points were measured with veego VMP-Ds heating table.

### 2.2 Synthesis of pyrrolo carbazole derivatives

2-methyl-7methoxy-3,4,5,10-tetarahydro-pyrrolo[3,2-a]carbazole-1-ethylcarboxylate (4a). 1-oxo-1,2,3,4-tetrahydrocaarbazole 2a (1.85 g, 0.01 mol) is mixed with 5.00 ml of glacial acetic acid, Cool the solution in ice salt mixture to 5 $^{f}C$ and $NaNO_2$ solution is added (0.68 g, 0.01mol) in drop wise, then the solution is stirred for 30 min, further it is stirred at room temperature for 4 h.

The above solution is stirred vigorously then Zinc powder (0.65 g, 0.1 mol) and acetic acid solution of ethyl acetoacetate (1.26 ml, 0.01 mol) are added then refluxed the mixture for 1 h. The reaction mixture is then cooled and poured into crushed ice. The resulting precipitate that separated out is collected by filtration and purified by using column chromatography with light petroleum ether: ethyl acetate (10:2 v/v). The product formed 4a was recrystallised by using methanol solution. The compounds, 4b-e is prepared in a similar manner as described to compound 4a.

2-methyl-7-mthoxyl-3,4,5,10-tetarahydro-pyrrolo[3,2-a]carbazole-1-ethylcarboxylate (4b). Brown solid; Yield: 65%; m.p. 197 $^{o}C$ (DMSO); IR (KBr, $cm^{-1}$), γ: 3277 (N-H), 2921 (C-H), 1639 (C=O); $^{1}H$-NMR (500 MHz,DMSO-$d_6$, ppm), δ2.74 (s, 3H, $C_7$-$CH_3$); 2.62 (s, 3H, $C_2$-$CH_3$); 2.15 (q, 2H $C_1$-$COOCH_2$, $J$ = 6.00 Hz); 2.50 (t, 3H, $C_1$-$COOCH_3$, $J$ = 2.00 Hz); 2.54 (t, 2H, $C_4$-$CH_2$, $J$ = 6.00 Hz); 2.91 (t, 2H, $C_5$-$CH_2$, $J$ = 6.00 Hz); 3.73 (s, 3H, $C_7$-$OCH_3$); 7.1-7.4 (m, 3H, $C_6$, $C_8$, $C_9$-H); 10.50 (s, 1H, 3-NH) 11.45 (s,1H, 10-NH). EI-Mass Spectrum, *m/z*: 325 [$M^+$]. Found, %: C, 70.35; H, 6.21; N, 8.64%. $C_{19}H_{20}N_2O_3$. Calculated, %: C, 70.33; H, 6.20; N, 8.66.

2,9-Dimethyl-3,4,5,10-tetarahydro-pyrrolo[3,2-a]carbazole-1-ethyl carboxylate (4c). Brown solid; Yield: 81 %; m.p. 204 $^{o}C$ (DMSO); IR (KBr, $cm^{-1}$), γ :3278 (N-H), 2924 (C-H), 1649 (C=O); $^{1}H$-NMR (500 MHz,DMSO-$d_6$, ppm), δ 2.74 (s, 3H, $C_9$-$CH_3$); 2.62 (s, 3H, $C_2$-$CH_3$); 2.15 (q, 2H $C_1$-$COOCH_2$, $J$ = 6.00 Hz); 2.53 (t, 3H, $C_1$-$COOCH_3$, $J$ = 2.00 Hz); 2.52 (t, 2H, $C_4$-$CH_2$, $J$ = 6.00 Hz); 2.91 (t, 2H, $C_5$-$CH_2$, $J$ = 6.00 Hz); 7.1-7.4 (m, 3H, $C_6$, $C_7$, $C_8$-H); 10.58 (s, 1H, 3-NH); 11.44 (s,1H, 10-NH). EI-Mass spectrum, *m/z*: 308 [$M^+$] (308.25). Found, %: C, 74.00; H, 6.54; N, 9.08. $C_{19}H_{20}N_2O_2$. Calculated, %: C, 74.03; H, 6.49; N, 9.09.

### 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 $dm^3mol^{-1}cm^{-1}$ 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. Adsorption 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.

## 3. RESULTS & DISCUSSION

### 3.1 Chemistry

1-oxo-1,2,3,4-tetrahydrocarbazole (1a) was reacted with sodium nitrite in glacial acetic acid in ice cold condition afforded the intermediate (2a). The intermediate formed (2a) was refluxed with ethyl acetoacetate (3) and zinc powder in glacial acetic acid yielded 2-methyl-7-methoxy-3,4,5,10-tetarahydro-pyrrolo [3,2-a]arbazole-1-ethyl carboxylate (4a) (Scheme 1).

The oxime functionality in compound 2a, which is obtained by reaction of 1-oxo-1,2,3,4-tetrahydrocarbazole with nitrous acid undergoes nitrosation on the active methylene carbon. This reaction introduced the nitrogen atom that will appear in the target pyrrole. Condensation of 2a with 3 completes the formation of pyrrole ring in the Carbazole derivative 4a.

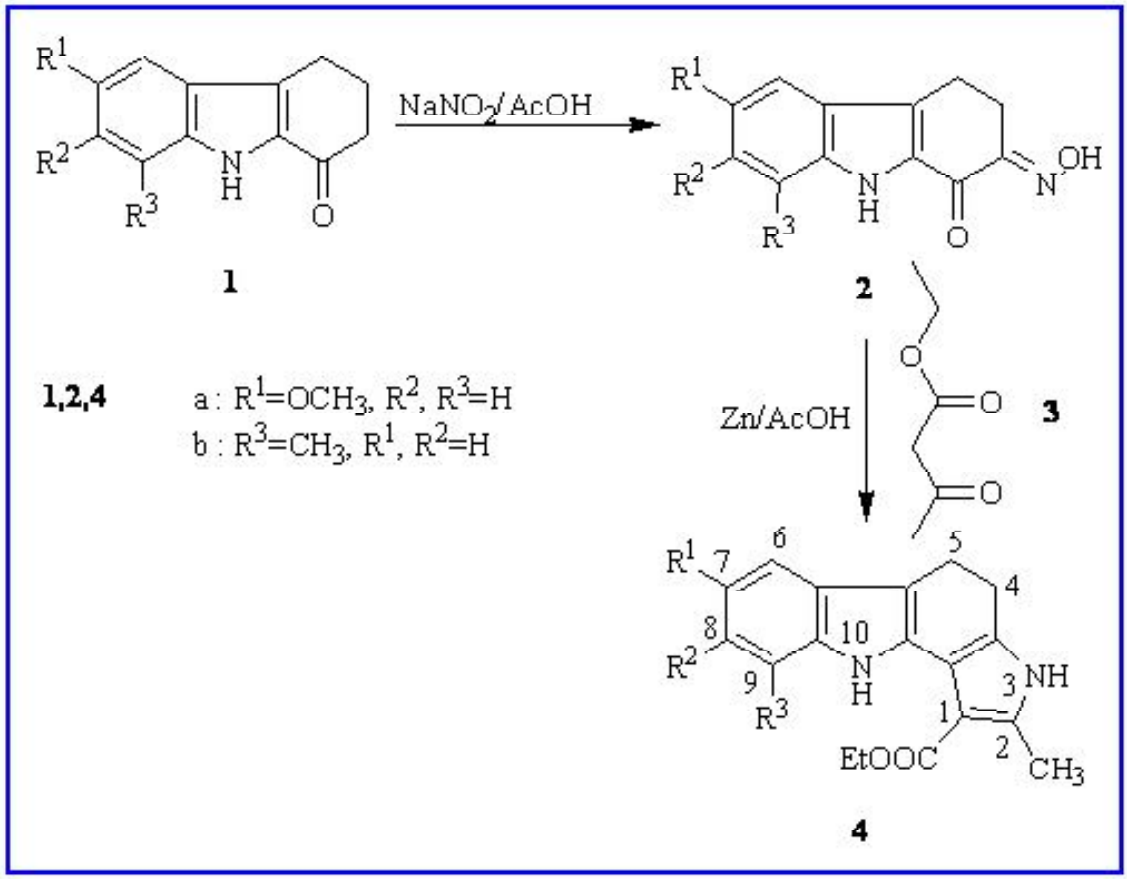
### 3.2 Spectroscopic measurements

The IR, $^1H$ and $^{13}C$ NMR spectra were used to ascertain the structure of all the compounds and the $^1H$ NMR spectra of the compounds, 4a-e, are shown in Fig.1-5. The IR spectrum of the compound 4a exhibits sharp bands at 3271 and 1639 $cm^{-1}$ which are due to the presence of N-H and ester carbonyl groups respectively. The $^1H$ NMR spectrum of 4a shows the appearance of quartet at δ 2.15 ($J$ = 6.00 Hz) which account for $C_1$ substituted ethyl ester $CH_2$ protons. The three protons of $C_1$ substituted ethyl ester $CH_3$ appeared as triplet at δ 2.50 ($J$ = 2.00Hz). The protons at $C_4$ carbon appeared as triplet at δ 2.54 ($J$ = 6.00Hz). A three proton singlet at δ 2.62 accounts for the methyl protons at the $C_2$ position. The two protons on $C_5$ carbon also appears as triplet at δ 2.91 ($J$ = 6.00 Hz). A three proton singlet at δ 2.3.73 accounts for the methoxy protons at the $C_7$ position. The aromatic protons appeared as a multiplet in the region δ 7.1-7.4. The appearance of two broad singlets at δ 10.50 and 11.45 which accounts for pyrrolo-NH and indol-NH protons respectively. The $^{13}C$ NMR spectrum reveales the presence of 19 carbons. The EI mass spectrum of the compound, 4a is in good agreement with the molecular formula, $C_{19}H_{20}N_2O_3$. The molecular ion peak, $[M^+]$ appear at $m/z$ = 325 which conforms the proposed molecular structure of the compound. The structure of the other compound 4b was also established by similar spectroscopic and analytical data.

### 3.3 Biological evaluation

#### *3.3.1 DNA binding - Titration experiments*

The size and the shape of the carbazole ring lead to an almost perfect overlapping of the aromatic ring with that of DNA base pair (Jain *et al.* 1979) therefore, the pyrrolocarbazole ring appears as an appropriate skeleton to design DNA intercalating drugs. Electronic absorption spectroscopy is one of the most




**Schema 1. Synthetic reaction of 2-methyl-3,4,5,10-tetrahydro-pyrrolo[3,2-a]carbazole-1-ethyl carboxylate derivatives**

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. 6.

The binding of the compounds have been characterized through absorbance and shift in the wavelength as a function of added concentration of DNA. Upon addition of increasing amount of CT-DNA, a significant hypochromism 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 (Wolf *et al.* 1987).

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

Where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficient $\varepsilon_a$, $\varepsilon_f$ and $\varepsilon_b$ corresponds to $A_{obs}$/ [compound], the extinction coefficient of the free compound and the extinction coefficient of the compound when fully bound to DNA,

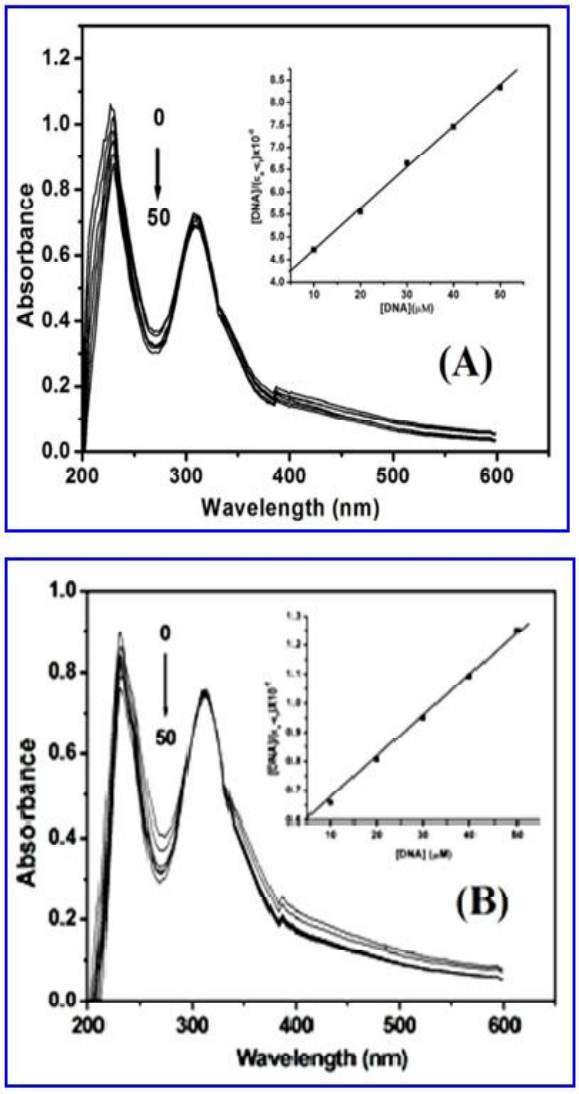



**Fig. 1: Electronic spectra of CP adduct in Tris–HCl buffer upon addition of CT-DNA. [Compound] = 25 µM, [DNA] = 0–50 µM. Arrow shows the absorption intensities decrease upon increasing DNA concentration (Inset: Plot between [DNA] and [DNA]/[$\varepsilon_a$–$\varepsilon_f$]X $10^{-8}$).**

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 4a and 4b are $2.4 \times 10^4 M^{-1}$, $2.8 \times 10^4 M^{-1}$ respectively, it is obvious that the title compound's planarity and its extended π system lead to the possibility of DNA intercalation.

## 4. CONCLUSION

In this work we have presented the synthesis of 2-methyl-3,4,5,10-tetarahydro-pyrrolo[3,2-a]carbazole-1-ethyl carboxylate derivatives. The compound was structurally characterized by IR, Mass, $^1H$ and $^{13}C$ NMR spectroscopy techniques. The spectroscopy experimental evidences strongly suggested that the compound could interact with Calf Thymus DNA (CT-DNA) through intercalation. The pyrrolo carbazole ring appears as an appropriate skeleton to design DNA interacting drugs.

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