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Synthesis of New Cytosine Derivatives and binding with ctDNA

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Abstract:
The reaction is divided into two main parts. The first part involves the synthesized compounds derived from cytosine. The second part involves binding some synthesized compounds with Calf Thymus DNA. To clarify the work more broadly-The first type a series of Mannich bases for cytosine were synthesized by react of cytosine with diethylamine in the presence of acetaldehyde for synthesized of (A_1), after that react of (A_1) with indole in presence of acetaldehyde for synthesized of (A_2). Since the react of (A_2) with (diethylamine, morpholine, diethanoleamine and diphenylamine) for synthesized of (A_3, A_4, A_5 and A_6). All reactions were followed by using Thin Layer chromatography, the derivatives have been purified by column Chromatography and characterized on the basis of IR and N.M.R .The second part was selecting mannich derivatives and study interaction with ctDNA by studying the effect of binding on ctDNA structure, by using FT-IR and UV-Visible techniques.

1- INTRODUCTION:

Mannich bases, beta-amino ketones carrying compounds, are the end products of Mannich reaction [1, 2]. Mannich reaction is a nucleophilic addition reaction which involves the condensation of a compound with active hydrogen(s) with an amine (primary or secondary) and formaldehyde (any aldehyde) [3]. The schematic representation of general Mannich reaction is given in Scheme (1). Mannich bases also act as important pharmacophores or bioactive leads which are further used for synthesis of various potential agents of high medicinal value which possess aminoalkyl chain. The examples of clinically useful Mannich bases which consist of aminoalkyl chain are cocaine, fluoxetine, atropine, ethacrynic acid, trihexyphenidyl, procyclidine, ranitidine, biperiden [4–6], and so forth. Mannich bases are known to play a vital role in the development of synthetic pharmaceutical chemistry.

The literature studies revealed that Mannich bases are very reactive and can be easily converted to other compounds, for example, reduced to form physiologically active amino alcohols [7]. Mannich bases are known to possess potent activities like anti-inflammatory [8, 9], anticancer [10, 11], antifilarial [8], antibacterial [12,13], antifungal [13, 14], anticonvulsant [15], anthelmintic [16], antitubercular [17, 18], analgesic [19], anti-HIV [17], antimalarial [20], antipsychotic [21], antiviral [22] activities and so forth. Along with biological activities.

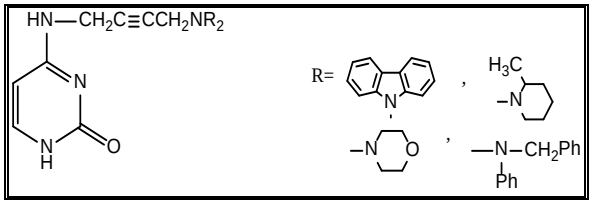
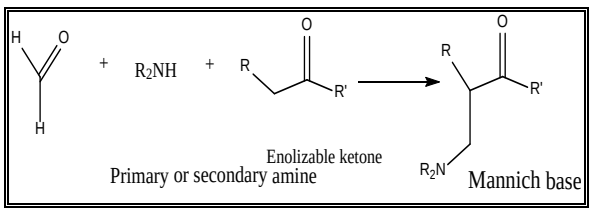


Figure 1: some examples on mannich bases of cytosine.



SCHEME 1: General Mannich Reaction .

Due to their antiviral and anticancer properties, 5-substituted pyrimidine nucleosides, including 5-substituted derivatives of cytosine have been the subject of increasing interest for many years.[24] On the other hand, there is continuing interest in the synthesis of nucleoside analogues in which the carbon in the heterocyclic base is linked to the nitrogen of the secondary cyclic amines.[25] In view of the above, we prepared a series of new 5-substituted derivatives of cytosine, which may have anticancer and/or antivirus activity. This letter reports the synthesis of (A_1, A_2, A_3, A_4, $A_5$and A_6)in which the cyclic amines were introduced through a methylene bridge at position-5 of cytosine via a Mannich reaction.

The interactions of various low-molecular weight substances with DNA are naturally relevant mechanisms in the cellular cycle and so also used in medicinal treatment. Depending on the particular drug structure, DNA-binding modes like groove binding, intercalating and/or stacking, give rise to

supramolecular assemblies of the polynucleotides, as well as influence the DNA-protein binding.

i) intercalation:

The intercalating drugs are a large group of compounds with various kinds of structures, but all combining planar aromatic chromophores (important structure units of common intercalators, see Fig. (2). These planar polycyclic tracts are capable of horizontal “sliding” into the interstacked gap of the DNA receptor helix between the base pairs [26-28]. Charge-transfer forces, hydrogen bonds, hydrophobic and sometimes also electrostatic interactions would hold the drug in [29], whereby, aromatic charge-transfers between the chromophore and the bases often display essential stabilizing factors. This process, called intercalation, interferes with normal DNA function (like transcription,replication) and therefore imparts pharmaceutical properties to drugs capable of intercalating. Lerman first postulated the intercalation model for complexes of native DNA with acridine derivatives[30].

ii) Groove binding:

The two grooves of the B-DNA double helix offer highly differentiated and to a certain degree even flexible potential receptor sites for crescent-shaped drugs complementarily mimicking the shape of the helix [31-34]. Modeling the

more unspecific introductory contacts of proteins within the minor and their comparatively specific reading of nucleic acids codes within the major groove, small molecule effectors might profit from hydrophilic contacts to the outside sugars and phosphates, and likewise more specific hydrogen-bond highlighted hydrophobic interactions with the inside bases. Here groove binding molecules that choose sequence selectively those recognition sites are of major interest, example Methyl green. Whereas the major groove often is occupied by the highly specific recognition patterns of the protein interactions, the more unspecific contacts with the minor groove , example Netropsin could additionally be untilized for drug targeting.Even though most of the DNA binding drugs interact in the minor groove, the major groove might be not only useful to support the minor groove interaction, e.g. with electrostatic interactions of water molecules and counterions.

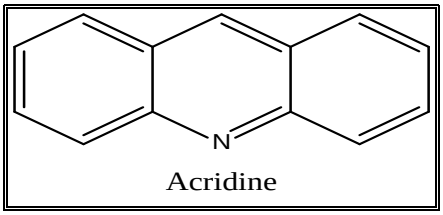


Figure 1: Chemical structures of common intercalating chromophore units .

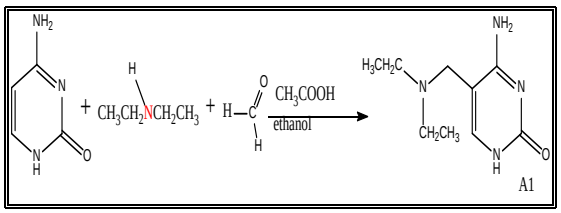
2.EXPERIMENTAL MATERIALS AND METHODS :

Cytosine , Indole, diethylamine , Morpholine , diethanolamine and diphenylamine were purchased from Fluka and were used as recived.

Melting points were determined with a Electro thermal Melting Point Apparatus Stuart-SMP11 and are uncorrected . IR spectra (KBr disc or liquid Smear) were recorded on a Fourier-transform infrared (FT-IR) Spectrophotometer NICOLT100 in Kirkuk university Collage of Science Chemistry dep. ^{1}HNMR and ^{13}CNMR were taken in Islamic Republic Of Iran using dimethyl sulfoxide (DMSO) as solvent and tetra methyl silan (TMS) as internal standard. Thin Layer Chromatography were performed with aluminum sheets protected with silica-gel supplied by Merck . UV spots were detected with Iodine vapor. Using the compound that we prepared in above (drug stock) and highly polymerized type-1 Calf thymus DNA was procured from Sigma Aldrich,USA and used as supplied.

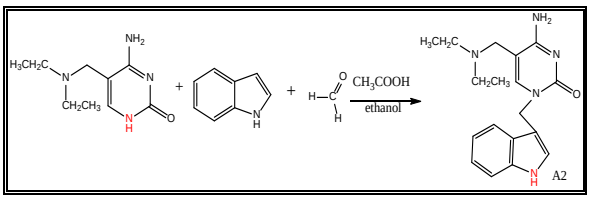
2.1 Synthesis:

i) Synthesis of (4-amino-5-((diethylamino)methyl)pyrimidin-2(1H)-one) A_1:



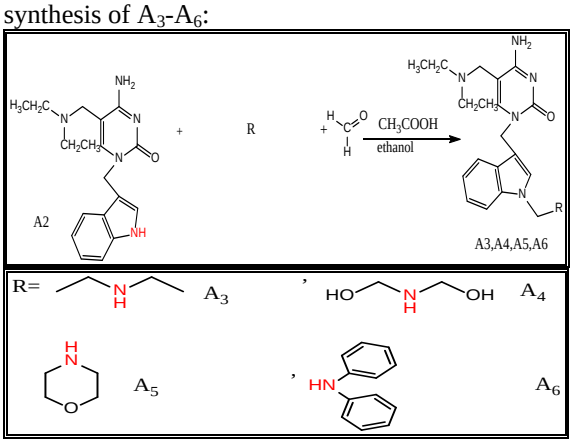
(0.999gm, 9mmol) of Cytosine , (0.657gm, 9mmol) diethyl amine and (0.27gm, 9mmol) formaldehyde were suspended in ethanol (99.8% , 20-25 ml). Glacial acetic acid (1-1.5ml) was added dropwise over 0.5 h to the boiling solution until the Cytosine dissolved[41] . The resulting reaction solution was refluxed for 7-9h . After the completion of the reaction as established by TLC (diethylether-ethanol 8:2), the solvent was evaporated under vacuum and the mixture was kept under reduced pressure. After cooling ,the gummy oil was shaken with dry benzene until a solid precipitate appears. The precipitated solid was filtered off and crystallized from ethanol[35]. And the final product was purified by column chromatography.

ii) synthesis of (1-((1H-indol-3-yl)methyl)-4-amino-5-((diethylamino)methyl) pyrimidin-2(1H)-one)A_2:



(1.773gm, 9mmol) of (A_1), (1.053gm, 9mmol) of Indole and (0.27gm, 9mmol) of formaldehyde were suspended in ethanol (99.8%,20-25ml) and then glacial acetic acid (1-1.5ml)was added dropwise over 0.5h to the boiling solution until the precipitate was dissolved , The resulting reaction solution was refluxed for 7-9h . After the completion of the reaction as established by TLC (diethylether-ethanol 8:2), the solvent was evaporated under vacuum and the mixture was kept under reduced pressure. After cooling ,the gummy oil was shaken with dry benzene until a solid precipitate appears. The precipitated solid was filtered off and crystallized from ethanol[35]. And the final product was purified by column chromatography.

iii) synthesis of A_3-A_6:



Mannich bases were prepared according to literature with some modifications [36]:

Formaldehyde (0.001 mol) was added to a mixture of Cytosine derivative (A_2)(0.001mol) and 3ml glacial acetic acid. The mixture was cooled to 5 °C, then one of the secondary amines (di ethylamine, di ethanol amine, di phenylamine and morpholine) (0.001 mol) was added to the mixture. The solution was

heated under reflux for 7-13hr, 5% NaOH was added to the reaction mixture, amorphous precipitate was formed and recrystallized from suitable solvent.

2.2 Binding with DNA:

Preparation of stock solutions: Stock solution of calf thymus DNA was prepared by dissolving (0.01gm) of DNA in 100µL Tris HCl buffer (PH=7.4). The DNA solution was kept at(8°C) for (24h) and was continuously stirred at frequent intervals to ensure the formation of homogenous solution . The final concentration of DNA stock solution was estimated as (600µl) . Drug stock solution was prepared in mixture of ultrapure water and ethanol (99.8%) with the ratio of (6:4) and stored at 8°C until used. Solution of drug stock with DNA were prepared by mixing varying concentration of drug stock with constant concentration of DNA. For UV absorption and FTIR studies different ratios of drug/DNA were taken by adding drug stock drop by drop to the DNA solution. Drug/DNA volume ratios (r) of (1/100, 1/50, 1/20, 1/10 and 1/5) were prepared with constant DNA volume of(600µL) for FTIR studies . UV-visible studies were carried out using the same range drug/DNA from FTIR[38].

3- RESULTS AND DISCUSSION:

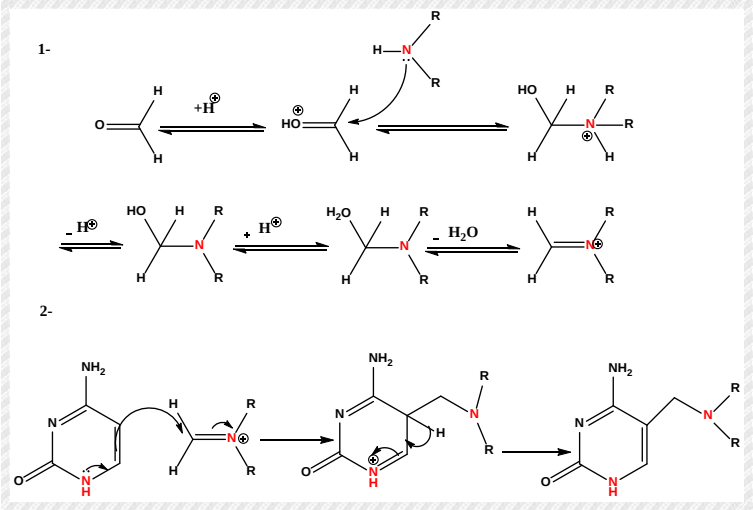
From the Cytosine we prepared some derivative and identified them by FTIR, ^{1}H.N.M.R, ^{13}C.N.M.R TLC and M.P. Some the derivatives binding with Ct-DNA and studied by UV-Visible and FTIR.

i) Identification mannich bases derived from cytosine:

Derivative (A_1)was prepared by the reaction between cytosine and diethyl amine in present of formaldehyde. And the mechanism[39] of preparation mannich bases is explained in Schim(2).

The IR spectrum for (A_1) show band absorption for (C-H) str cytosine in (3163.04 cm^{-1}) disappeared and bands absorption in (2910 cm^{-1}) appeared back to (C-H)str aliphatic (-CH_2CH_3) .All the values were corresponding to literatures. The $_1$H.N.M.R for (A_1) showed signal in the chemical shift (σ 2.5 ppm, s)back to proton of the Solvent (DMSO). Another signal appeared at chemical shift (σ5.63 ppm, 2H,d) the other signals are shown in Fig(8). Derivative (A_2) was prepared by the reaction between (A_1) and indole in present of formaldehyde . The IR spectrum shown in the table NO.2

Derivatives (A_3, A_4, A_5 and A_6) was prepared by the reaction between (A_2) and some secondary amines (di ethyl amine, di ethanol amine, morpholine and di phenylamine) in present of formaldehyde.



SCHEME (2):General mechanism for mannich reaction.

Table no. (1): Some physical properties of synthesized mannich bases:

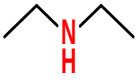
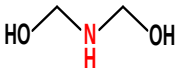
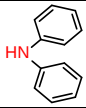
Comp.N.O	R	Name of the compounds	Colour	M.P C°	Yild %
A1	-----	4-amino-5-((diethylamino) methyl)pyrimidin-2(1H)-one)	Yellow	99°-101°	67%
A2	-----	(1-((1H-indol-3-yl)methyl)-4-amino-5-((diethylamino)methyl) pyrimidin-2(1H)-one)	Light Orange	139°-142°	55%
A3		4-amino-5-((diethylamino) methyl)-1-((1-((diethylamino) methyl)-1H-indol-3-yl)methyl) pyrimidin-2(1H)-one	Misty Rose	79°-82°	55%
A4		4-amino-1-((1-((bis(2-hydroxy ethyl)amino)methyl)-1H-indol-3-yl)methyl)-5-((diethylamino) methyl)pyrimidin-2(1H)-one	Dark Brown	81°-84°	59%
A5		4-amino-5-((diethylamino) methyl)-1-((1-(morpholinomethyl)-1H-indol-3-yl)methyl)pyrimidin -2(1H)-one	Brown	86°-89.°	53%
A6		4-amino-5-((diethylamino) methyl)-1-((1-((diphenylamino)methyl)-1H-indol-3-yl)methyl)pyrimidin-2(1H)-one	Dark Magenta	102°-105°	58%

Table No. (2): FTIR spectral data for compounds (A_1-A_6)

Comp.NO.	ν N-H cm^{-1}	ν C-H cm^{-1} Aromatic	ν C-H cm^{-1} Aliphatic	ν C=C cm^{-1} ν C=N cm^{-1}	ν C-N cm^{-1}
A_1	3429.25	------	2910	1412 1641	1145
A_2	3394	3055	2829	1455 1657	1228
A_3	3422	3051.99	2931	1460 1644	1224.66
A_4	3402	3051.37	2873.84	1458 1640	1153
A_5	3421	3047	2853.17	1461 1628	1125
A_6	3399	3082.13	2924.31	1461 1623	1153

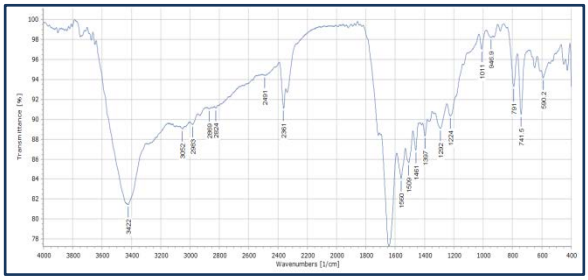


Figure (3): FTIR for A_3.

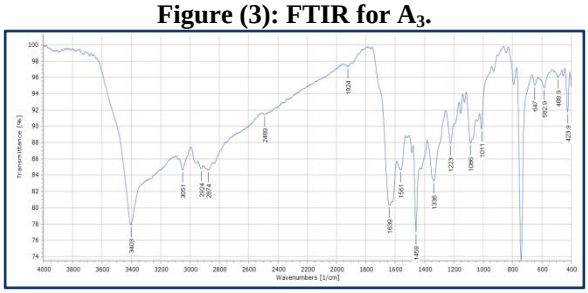


Figure (4): FTIR for A_4.

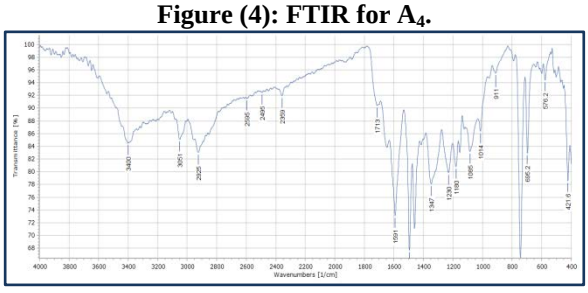


Figure (5): FTIR for A_6.

^{1}H.N.,M.R

The ^{1}H.N.M.R spectrum of (A_1) in DMSO showed a signal at (σ1.14ppm,T,3H) and a signal at (σ2.9ppm,q,2H) for CH_3 and CH_2 of the ethyl group respectively, while a signal appeared at (σ5.63ppm,s,2H) for CH_2 that connect the cytosine to the amine group. The signal of NH_2 proton appeared at (σ7.39ppm,s,2H) , shown in Fig (6).

The ^{1}H.N.M.R spectrum for compound (A_4) in DMSO showed a signal at (σ 1.11ppm,T,3H) and a signal at (σ 2.79ppm,q,2H) for CH_3 and CH_2 protons of ethyl group ,and a signal at (σ4.27ppm,s2H) for CH_2 proton that links the cytosine to indole . The NH_2 proton appears at (σ 7.55ppm,s,2H) and a signal at (σ 4.16ppm,s1H) for the OH proton. shown in Fig (7).

$_1$H.N.M.R spectrum of (A_6)showed a signal at (σ 1.05ppm,T,3H) and a signal at (σ2.5ppm,q,2H) for CH_3 and CH_2 protons of ethyl group respectively. The phenyl protons signal at (σ 7.33ppm,m,1H) while a signal at at (σ7.55ppm, s,2H) appeared a signed to the NH_2 proton. Shown in Fig (8).

^{13}C.N.M.R

The ^{13}C.N.M.R spectrum of (A_1) were recorded in DMSO, showed a characteristic signal in (11.41p) for carbon (a) and a signal at (93.26p) for C (d) and a signal at (167p)for C (e) and in (143.48p) a signal appeared for C (f). The other signals are shown in Fig (9).

The ^{13}C.N.M.R spectrum of (A_4) were recorded in DMSO showed a signal at (22.57p) for C (a) and a signal at (110.54p) for C (j) and a signal at (111.77p) for C(i1), and for C (h5) showed a signal at (136.88p).The other signals are shown in Fig (10).

^{13}C.N.M.R of compound (A_6) showed a signal at (15.27p) a signed to the C(a) and signal at (111.75p) for C(o) , and a signal at (110.53p) for C(c). Signal for C(q) showed at 136.86p) and in (147.09p) showed signal for C(e). The other signals shown in Fig (11).

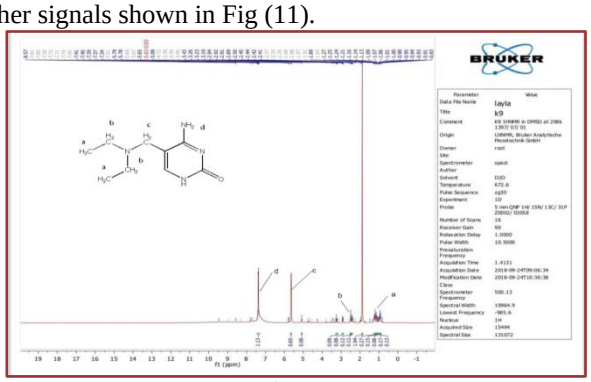


Figure (6): ^{1}H.N.M.R of A_1

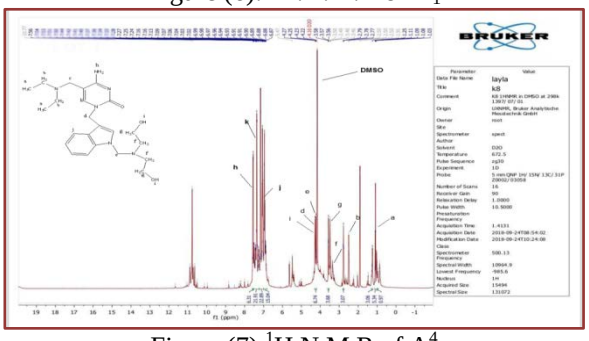


Figure (7):^{1}H.N.M.R of A^4

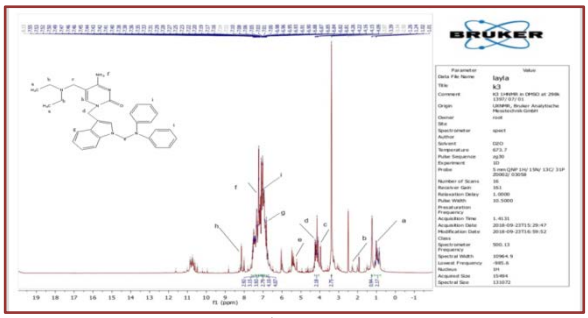


Figure (8) : ^{1}H.N.M.R of A_6.

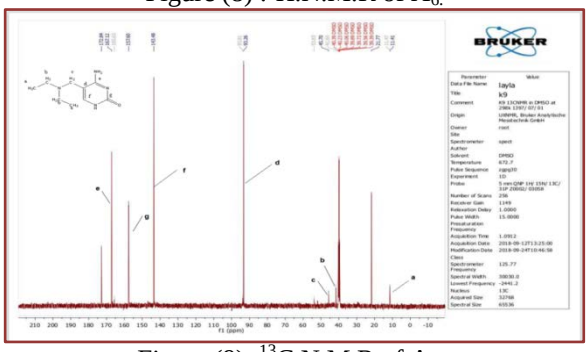


Figure (9): ^{13}C.N.M.R of A_1.

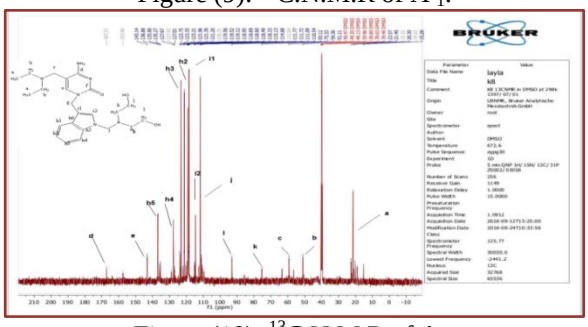


Figure (10): ^{13}C.N.M.R of A_4.

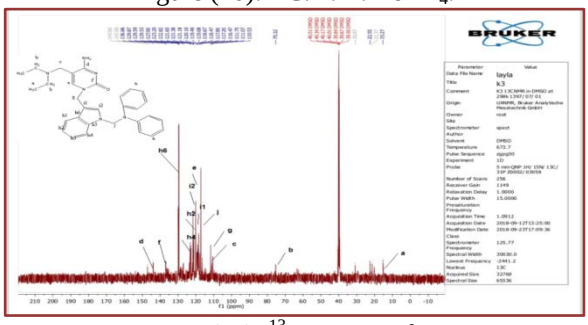
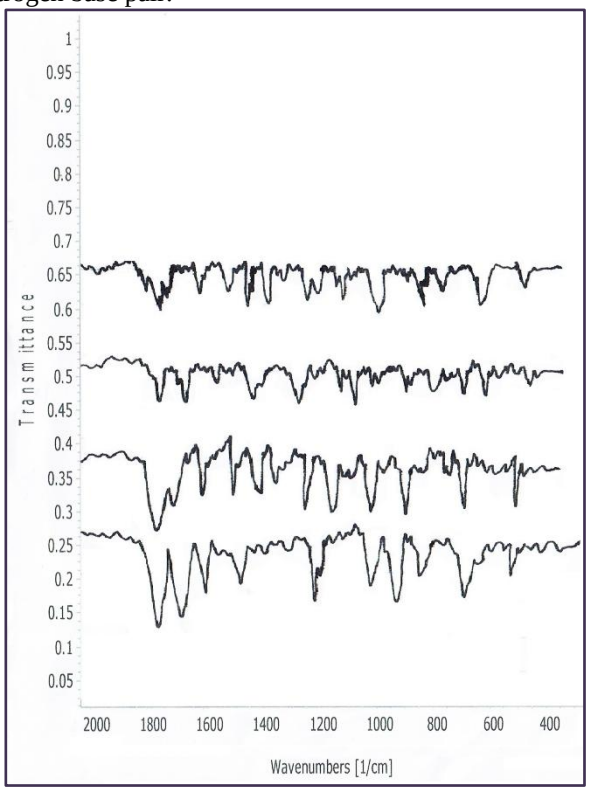


Figure (11): ^{13}C.N.M.R of A_6.

ii) Binding some derivatives with DNA

UV-Visible spectra of free DNA and (A_2) complexes are shown in (Fig13). Increase in the intensity of absorption was observed for all DNA-Complexes as compared to free DNA. Proportional increase in the absorption intensity is seen with increasing concentration of (A_2) in DNA-Complexes. This hyperchromic effect indicates toward intercalative binding of the (A_2) with DNA which might result in extension [37], unwinding and stiffness of DNA helix. This unwinding exhibits increased exposure of nitrogenous bases which inturn causes increase in absorption maxima (A_{260}) of DNA. The absorption maxima of cytosine derivatives also shift towards the longer wavelength as compared to free DNA. This bathochromic shift (red shift) is also an indication of the formation of an intercalating complex between (A_2) and DNA.

b) FTIR spectra of free DNA and it Cytosine derivatives with different molar ratios of (A_2) are shown in fig(12) . Peak assignments are in accordance with literature[40]. Shifts are observed for many prominent IR bands of DNA when derivatives(A_2) is added. Guanine band at (1713 cm^{-1}) shifts to (1711-1709 cm^{-1}). Thymine band at (1669 cm^{-1}) shifts to 1662-1661cm^{-1}). Adenine band at 1606 cm-1 shifts to 1601-1600 cm^{-1}). Cytosine band at (1492cm^{-1})shifts to (1495-1496cm^{-1}), in the spectra of DNA complexes. In different spectra ,slight intensity increase is evident for guanine and thymine vibrations whereas intensity decrease is observed for adenine and cytosine vibrations. These shifting with minor intensity variations are indicative of intercalative mode of binding of (A_2) with calf thymus DNA. Intercalation occurs through hydrogen bonding with nitrogen base pair.



Figure(12): FTIR spectra of Binding (A_2)with ctDNA.

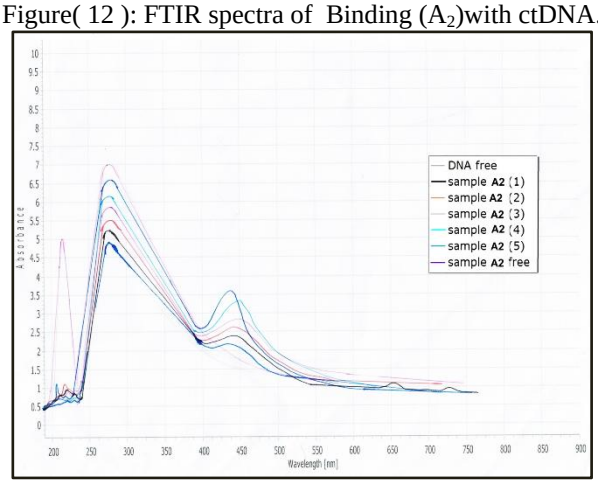


Fig (13): UV-Visible of sample (A_2) Binding with DNA.

CONCLUSION

Many mannich derivatives synthesized by substitution on C-H atom in the cytosine by many methods, also mannich derivatives synthesized by substitution on N-H in the cytosine derivatives . Studied the binding of some mannich with ct DNA by FTIR and U.V visible. The result shown the derivatives interaction happen with ct DNA from minor groove side according on the literature.

Acknowledgement

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