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In vitro DNA Binding Interaction Studies of Hydrazones and Thiosemicarbazones Derived from Pyrazole Moiety

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In this work, the novel hydrazones and thiosemicarbazones derived from pyrazole moieties were synthesized by reacting 4-chloro-1-methyl-1*H*-pyrzolecarboxaldehyde with various hydrazides and thiosemicarbazides. All the synthesized compounds were characterized and confirmed with different spectroscopic techniques like FTIR, ^{1}H NMR and LC-MS. The *ct*-DNA binding interaction studies were evaluated by using UV-visible spectroscopic and fluorescence spectrophotometric techniques with calf thymus DNA. The obtained binding constants of the synthesized were found to be **7** [8.44×10^4] > **4** [3.61×10^4] > **2** [3.60×10^4] > **1** [3.18×10^4] > **8** [2.85×10^4 M^{-1}].

Keywords: Calf thymus DNA, Hydrazones, Thiosemicarbazones.

INTRODUCTION

In recent years, many biologically active hydrazones have been synthesized from different carbonyl compounds and they have been found to exhibit various interesting biological activities like anticancer, anti-inflammatory, antiviral, antiprotozoal and antimicrobial activities [1,2]. Moreover, hydrazones are used as chemotherapeutic agents such as nitrofurazone, nitrofurantoin and furazolidone [3-5]. Hydrazones attracted the medicinal chemists due to their important functional active azomethine group, which is linked to carbonyl groups having great pharmaceutical applications and makes researchers deliver novel heterocyclic molecules towards many biological applications [6,7].

In addition to this, thiosemicarbazones also have equal importance in synthetic chemistry because of their interesting applications towards pharmacological properties like antibacterial, antineoplastic, antiviral *etc.* [8,9]. In addition to *p*-acetamidobenzaldehyde thiosemicarbazone, which is commercially available as thiacetazone [10], there is another interesting property of thiosemicarbazones that they exhibit very good anticancer activity as well as antitumor activity, which is involved in the inhibition of ribonucleotide reductase, an enzyme containing diferric center and involved in the rate limiting step of DNA synthesis [11].

Many molecules have shown their antitumor activity by binding to DNA, by altering the replication of DNA and inhibiting the growth of tumour cells, which is the basis for designing novel more potential antitumor drugs [12]. Their activity depends on the mode of interaction of binding. Binding the interaction of DNA with small heterocyclic molecules provides useful information in the development of novel molecular probes and novel therapeutic entities [13-18]. Thus, all these emerging biological and pharmacological applications of hydrazones, semicarbazones and thiosemicarbazones motivated us to synthesize hydrazones and thiosemicarbazones and studied their interaction behaviour with calf thymus DNA (*ct*-DNA) by using UV-visible absorption and fluorescence spectroscopy.

EXPERIMENTAL

All the chemicals and *ct*-DNA were obtained from Sigma-Aldrich, while the pUC18 was obtained from Bangalore GeNei. The melting points of the synthesized compounds were recorded by the open capillary method and are uncorrected. The FTIR spectra was recorded with Bruker Alpha using the KBr pellet

method and the ^{1}H NMR spectra was recorded using Agilent with an ATB probe in DMSO-d_6 solvent (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR). The LC-MS was obtained by Agilent 1200 series LC-Micromass ZQ spectrometer, Fluorescence studies were performed using a F-2300 Spectrophotometer (Hitachi Japan, equipped with a 1 cm quartz cell at 298 K) and electronic absorption studies were carried out using an Elico SL-159 UV-visible Spectrophotometer at room temperature.

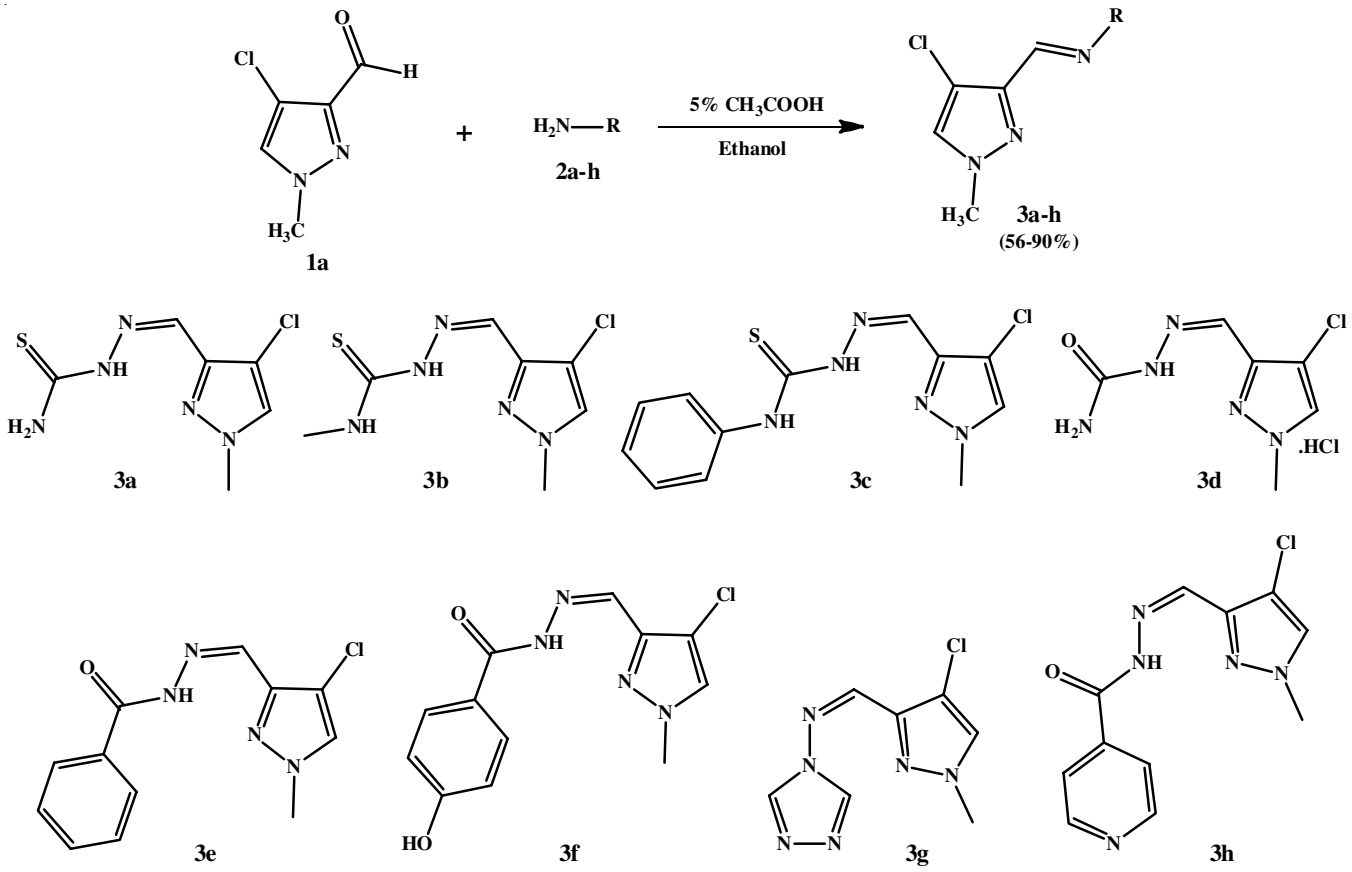
Synthesis of hydrazones and thiosemicarbazones (3a-h): A hot ethanolic solution of 4-chloro-1-methyl-pyrazolecarboxaldehyde (**1a**) was added to a warm aqueous amide (**2a-h**) solution in the presence of 5% glacial acetic acid and refluxed for about 30-60 min. The obtained solid product was collected by filtration and recrystallized with alcohol [19,20] (**Scheme-I**).

(*E*)-2-((4-Chloro-1-methyl-1*H*-pyrazol-3-yl)methylene)-hydrazinecarbothioamide (3a): m.p.: 223-228 ºC; IR (KBr, ν_{max}, cm^{-1}): 3421 (N-H *str.*), 3138-3262 (NH_2), 3039 and 2986 (C-H *str.*, aromatic, aliphatic), 1655 (C=N), 1537 (C=C Ar), 1086 (C-N *str.*), 824 (C-S *str.*), 654 (C-Cl *str.*); ^{1}H NMR (400 MHz, DMSO-d_6): 3.81 (3H, s, CH_3), 7.33 (1H, s, C-H arom.), 7.98 (s, 1H), 8.35 (s, 2H, NH_2), 11.54 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 178.43, 141.90, 135.76, 131.63, 107.70, 40.53: LCMS (*m/z*) calculated for $C_6H_8N_5SCl$: 217.673, found 217.90. Elemental analysis calcd. (found) %: C, 33.11 (33.12); H, 3.70 (3.71); Cl, 16.29 (16.28); N, 32.17 (32.18); S, 14.73 (14.69).

(*E*)-2-((4-Chloro-1-methyl-1*H*-pyrazol-3-yl)methylene)-*N*-methylhydrazinecarbothioamide (3b): m.p.: 214-218 ºC; IR (KBr, ν_{max}, cm^{-1}): 3365 (N-H *str.*), 3337 (NH_2), 3180 (arom. C-H *str.*), 1542 (C=N), 1472 (C=C arom.), 1086 (C-N, *str.*), 1240 (C=S *str.*), 637 (C-Cl *str.*); ^{1}H NMR (400 MHz, DMSO-d_6): 2.9-3.0 (d, 3H, CH_3), 3.81 (s, 3H, CH_3), 7.76-7.77 (1H, d C-H, azomethine), 7.99 (1H, s, C-H, arom.), 8.05 (s, 1H, N-H), 11.54 (s, 1H, N-H); ^{13}C NMR (100 MHz, DMSO-d_6): 178.26, 141.98, 134.95, 131.42, 108.00, 40.57, 31.36; LCMS (*m/z*): Calculated for $C_7H_{10}N_5SCl$: 231.669, found 232. Elemental analysis calcd. (found) %: C, 36.29 (36.29); H, 4.35 (4.35); Cl, 15.30 (15.30); N, 30.23 (30.23); S, 13.84 (13.84).

(*E*)-2-((4-Chloro-1-methyl-1*H*-pyrazol-3-yl)methylene)-*N*-phenylhydrazinecarbothioamide (3c): m.p.: 177-182 ºC; IR (KBr, ν_{max}, cm^{-1}): 3289-3210 (N-H *str.*), 1593 (C=N), 1541 (C=C arom.), 1182 (C=S *str.*), 1088 (C-N *str.*), 819 (C-S *str.*), 688 (C-Cl *str.*); ^{1}H NMR (400 MHz-DMSO-d_6): 3.84 (CH_3, s, 3H), 7.13-7.17 (t, 1H, arom.), 7.32-7.36 (t, 2H, arom.), 7.64-7.66 (d, 2H, arom.), 8.05 (s, 1H, C-H azomethine), 8.15 (s, 1H, C-H), 9.52 (s, 1H, N-H), 11.98 (s, 1H, N-H); ^{13}C NMR (100 MHz, DMSO-d_6): 175.63, 141.80, 138.96, 135.94, 131.81, 128.88, 125.50, 123.95, 107.67, 40.62; LCMS (*m/z*): Calculated for $C_{12}H_{12}N_5SCl$: 293.765, found 293.95. Elemental analysis calcd. (found) %: C, 49.06 (49.04); H, 4.12 (4.11); Cl, 12.07 (12.08); N, 23.84 (23.84); S, 10.91 (10.91).

(*E*)-2-((4-Chloro-1-methyl-1*H*-pyrazol-3-yl)methylene)-hydrazinecarboxamide hydrochloride (3d): m.p.: 224-228 ºC; IR (KBr, ν_{max}, cm^{-1}): 3454 (N-H *str.*), 3218-3270 (NH_2), 3130 & 3014 (C-H *str.*, aromatic, aliphatic), 1688 (C=O), 1583 (C=N), 1088 (C-N *str.*), 690 (C-Cl *str.*); ^{1}H NMR (400 MHz,



Scheme-I: Synthesis of heterocyclic Schiff bases

DMSO-d_6): 3.79 (s, 3H, CH_3), 6.0-6.5 (s, 2H, NH_2), 7.82 (s, 1H, C-H azomethine), 7.95 (s, 1H, arom.), 10.32 (s, 1H, N-H); ^{13}C NMR (100 MHz, DMSO-d_6): 156.84, 142.39, 132.64, 131.38, 106.95, 40.54; LCMS (*m/z*): Calculated for $C_6H_9N_5OCl_2$: 238.067, Found 238. Elemental analysis calcd. (found) %: C, 30.27 (30.25); H, 3.81 (3.82); Cl, 29.78 (29.79); N, 29.42 (29.42); O, 6.72 (6.72).

(*E*)-*N*′-((4-Chloro-1-methyl-1*H*-pyrazol-3-yl)methylene)-benzohydrazide (3e): m.p.: 158-162 ºC; IR (KBr, ν_{max}, cm^{-1}): 3228 (N-H *str.*), 3099 (C-H *str.*, arom.), 1687 (C=O *str.*), 1552 (C=N, *str.*), 1489 (C=C *str.*), 1279 (C-N, *str.*), 685 (C-Cl *str.*); ^{1}H NMR (400 MHz, DMSO-d_6): 4.0 (s, CH_3, 3H), 7.4-7.6 (5H, aromatic, m), 7.62 (C-H azomethine, d, 1H), 8.24 (1H, s, C-H, aromatic), 13.0 (s, 1H, N-H); ^{13}C NMR (100 MHz, DMSO-d_6): 163.63, 163.11, 142.30, 141.42, 140.39, 133.83, 133.13, 132.82, 132.19, 131.44, 131.22, 130.40, 129.57, 128.92, 128.03, 127.56, 109.76, 40.57; LCMS (*m/z*): Calculated for $C_{12}H_{11}N_4OCl$: 262.686, Found 263. Elemental analysis calcd. (found) %: C, 54.87 (54.88); H, 4.22 (4.21); Cl, 13.50 (13.52); N, 21.33 (21.31); O, 6.09 (6.09).

(*E*)-*N*′-((4-Chloro-1-methyl-1*H*-pyrazol-3-yl)methylene)-4-hydroxybenzohydrazide (3f): m.p.: 278-282 ºC; IR (KBr, ν_{max}, cm^{-1}): 3259 (O-H *str.*), 3218 (NH *str.*), 3094 & 2924 (C-H *str.*, aromatic, aliphatic), 1669 (C=O), 1606 (C=N), 1510 (C=C arom.), 1277 (C-N *str.*), 846 (C-Cl *str.*); ^{1}H NMR (400 MHz, DMSO-d_6): 4.01 (s, 3H, CH_3), 6.88-6.91 (d, 2H, aromatic), 7.42 (s, 1H, azomethine C-H), 7.74-7.77 (aromatic, d, 2H), 8.23 (s, 1H, aromatic C-H), 10.26 (s, 1H, O-H phenolic), 12.87 (s, 1H, N-H); ^{13}C NMR (100 MHz, DMSO-d_6): 161.61, 141.51, 131.13, 129.74, 129.36, 123.59, 116.09, 109.52, 40.55; LCMS (*m/z*) Calculated for $C_{12}H_{11}N_4O_2Cl$: 278.686, found 279.05. Elemental analysis calcd. (found) %: C, 51.72 (51.73); H, 3.98 (3.97); Cl, 12.72 (12.72); N, 20.10 (20.11); O, 11.48 (11.47).

(*E*)-*N*-((4-Chloro-1-methyl-1*H*-pyrazol-3-yl)methylene)-4*H*-1,2,4-triazol-4-amine (3g): m.p.: 198-202 ºC; IR (KBr, ν_{max}, cm^{-1}): 3432 (N-H *str.*), 3263-3332 (NH, *str.*), 3114 & 2961 (C-H *str.*, aromatic, aliphatic), 1614 (C=N), 1509 (C=C arom.), 1048 (C-N, *str.*), 665 (C-Cl *str.*); ^{1}H NMR (400 MHz DMSO-d_6): 3.90 (s, 3H, CH_3), 8.14 (s, 1H, azomethine), 8.94 (s, 1H, aromatic), 9.14 (s, 2H, triazole ring); ^{13}C NMR (100 MHz, DMSO-d_6): 150.54, 140.60, 139.37, 132.32, 109.45, 40.60; LCMS (*m/z*): Calculated for $C_7H_7N_6Cl$: 210.168, found 210.95. Elemental analysis calcd. (found) %: C, 39.92 (39.92); H, 3.35 (3.36); Cl, 16.83 (16.84); N, 39.90 (39.90).

(*E*)-*N*′-((4-Chloro-1-methyl-1*H*-pyrazol-3-yl)methylene)-isonicotinohydrazide (3h): m.p.: 202-206 ºC; IR (KBr, ν_{max}, cm^{-1}): 3207 & 3124 (N-H *str.*), 3041 (C-H *str.*, arom.), 1703 (C=O *str.*), 1550 (C=N), 1409 (C=C, arom.), 1062 (C-N, *str.*), 639 (C-Cl *str.*); ^{1}H NMR (400 MHz, DMSO-d_6): 4.02 (s, 3H, CH_3), 7.56 (s, 1H, C-H), 7.78-7.79 (d, 2H, aromatic), 8.26 (s, 1H, aromatic), 8.81-8.82 (2H, aromatic, d); ^{13}C NMR (100 MHz, DMSO-d_6): 161.73, 151.37, 141.28, 140.18, 131.71, 131.32, 121.38, 110.05, 40.63; LCMS (*m/z*): Calculated for $C_{11}H_{10}N_5OCl$: 263.675, found 263.95. Elemental analysis calcd. (found) %: C, 50.10 (50.11); H, 3.82 (3.81); Cl, 13.45 (13.45); N, 26.56 (26.57); O, 6.07 (6.06).

DNA interaction studies

DNA binding interactions studies by UV-spectrophotometer: A mixture of *ct*-DNA in 50 mM NaCl/50 mM Tris-HCl of pH 7.0 gives a ratio of UV absorbance reading at A_{260}/A_{280} of 1.8-1.9, reveals that the DNA was sufficiently free of proteins. DNA standard solutions were prepared in 50 mmol Tris-HCl/50 mmol NaCl in double distilled water (pH 7.0) and the *ct*-DNA concentration was determined per nucleotide by considering the absorption coefficient (6600 dm^3 mol^{-1} cm^{-1}) at 260 nm. Stock solution of *ct*-DNA was prepared by dissolving in suitable buffers and kept overnight at 4 ºC for complete dissolution and was used within 4 days. Solutions were prepared by mixing the complex and *ct*-DNA in DMF medium. The spectra were recorded after attains equilibrium, against an analogous blank solution containing the similar concentration of DNA. The absorption maxima (usually corresponding to the changes at maximum absorption) were recorded. The results were then fit to the following formula given to determine the intrinsic binding constant (K_b):

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

where ε_a, ε_b and ε_f correspond to apparent, bound and free metal complexes extinction coefficients, respectively. A plot of [DNA]/(ε_a-ε_f) *vs.* [DNA] gave a slope of 1/(ε_b-ε_f) and a Y-intercept equal to 1/K_b (ε_b-ε_f); K_b is the ratio of the slope to the Y-intercept. The obtained binding constants are shown in Table-1.

DNA binding studies by fluorescence spectrophotometer: The *ct*-DNA binding studies of all the synthesized compounds and the fluorescence probe (ethidium bromide) with *ct*-DNA was examined by measuring the change of fluore-

TABLE-1
UV-SPECTRAL RESULTS WITH ADDITION OF *ct*-DNA TO COMPOUNDS **3a-h**

Compound	λ_{max} (nm)		$\Delta\lambda_{max}$ (nm)	H (%)	K_b (M^{-1})	-ΔG
	Free	Bound				
3a	238	237	1	1.42	3.18×10^4	2.56×10^4
3b	238	237	1	4.80	3.60×10^4	2.59×10^4
3c	–	–	–	–	–	–
3d	240	240	0	4.23	3.61×10^4	2.60×10^4
3e	241	240	1	2.38	2.82×10^4	2.53×10^4
3f	–	–	–	–	–	–
3g	240	239	1	4.84	8.44×10^4	2.81×10^4
3h	241	240	1	1.74	2.85×10^4	2.54×10^4

H%[b] = [A_{free} – A_{bound}/A_{free}] × 100, H= '–' Hypochromism and '+' Hyperchromism.

scence intensity of the reaction mixtures containing (1.0606 × 10^{-5}) of *ct*-DNA and (1.0606 × 10^{-5}) EtBr then 50 mM NaCl and Tris·HCl buffer (pH 6.9-7.01) were pretreated with 50 µL of each solution (50 µM of NaCl and 50 µM of Tris·HCl at final concentrations) for every 2 min and the contents were analyzed using fluorescence measurement. Then, the gradually enhancing concentrations of the compound (0, 2, 4, 6, 8 and 10 µL final concentrations) were effectively added and the difference in the fluorescence intensity was recorded. The excitation and emission slits were fixed at 5 nm. All the measurements were carried out by excitation at 500 nm in the range 520-700 nm for EtBr and by excitation at 350 nm in the range 390-600 nm for the compounds. The control was EB-*ct*-DNA solution with respect to the corresponding compounds were studied the resulting fluorescence quenching data were measured as per eqn. 2:

$$\frac{I_0}{I} = 1 + K_{SV}[Q]$$

where I_0 and I indicates the fluorescence intensities of probe-*ct*-DNA in without and with presence compound respectively, K_{SV} is quenching rate constant and [Q] is the concentration ratio of the Compound to DNA ([**1**]/[CT-DNA]). The K_{SV} value was obtained from the plot of I_0/I *versus* [Q] [21].

DNA binding studies by fluorescence spectroscopic method: The ethidium bromide quenching Assay studies carried by fluorescence method was used to investigate further to confirm the binding mode between thiadiazole and DNA. In present studies, on increase in concentration of compounds (**3a-h**, except **3c** & **3f**), decreased the fluorescence intensity of DNA bound EtBr between 594-601.5 nm (for compound **3a** = 601.5 nm, **3b** = 594.0 nm, **3d** = 600 nm, **3e** = 600 nm, **3g** = 601.5 nm, **3h** = 601 nm) indicates the quenching of EtBr molecules due to binding of thiadiazole moiety to *ct*-DNA [22]. If thiadiazoles act as quenchers, the quenching of fluorescence intensity of *ct*-DNA bound EtBr can be described by the linear Stern-Volmer equation (eqn. 3). From Table-2, the linear Stern-Volmer quenching constant (K_{SV}) (1.20 × 10^4–2.18 × 10^4 M^{-1}) indicates the moderate quenching efficiency between thiadiazole and DNA. A linear Stern-Volmer plot indicates quenching process gives the bimolecular quenching rate constant (K_q) values and are found to be greater than (in the order of 10^{12} M^{-1} s^{-1}) the fluorescence lifetime of the biomolecule (10^{-8} s) indicates the quenching process is static rather than dynamic [23,24].

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (3)$$

The intrinsic binding constant (K_b) and number of binding cites (n) were calculated from eqn. 4. From Table-2, the number of binding cites (n) close to 1, which indicates the existence of single binding cite between thiadiazole and DNA. The DNA binding constant and free energy change are close to the values obtained using absorption spectroscopic method.

$$\log\left(\frac{(F_0 - F)}{F}\right) = \log K_b + n\log[Q] \quad (4)$$

RESULTS AND DISCUSSION

Heterocyclic Schiff bases (**3a-h**) were synthesized according to procedure given in literature [19,20] by using 4-chloro-1-methyl-pyrazolecarboxaldehyde. The newly synthesized compounds were obtained in good yield, air stable, non-hygroscopic in nature and are readily soluble in common organic solvents. The structural confirmation was done by spectroscopic techniques (FTIR, ^{1}H NMR and LCMS), which suggested that the analytical data of the synthesized compounds were confirmed and good agreement with proposed molecular structures.

In infrared spectrum of the synthesized compounds (**3a-h**), the stretching frequency of primary amines was observed at 3400-3072 cm^{-1}, the azomethine (C=N) functional group stretching frequency observed between 1614-1542 cm^{-1}, the band appears between 1703-1687 and 1280-1240 cm^{-1} is mainly due to ketonic (C=O) and thioketonic (C=S) stretching frequency, respectively. The stretching frequency for (C-N), (C-S) groups and aromatic C-H, group observed between 1279-1048, 824-819 and 3040-2946 cm^{-1}, respectively. The C=C double bond stretching frequency observed between 1509-1498 cm^{-1} and for carbon and halogen stretching frequency was observed at 690-637 cm^{-1}.

In ^{1}H NMR of the synthesized heterocyclic Schiff bases (**3a-h**), CH_3 protons appeared at δ 3.809-4.023 ppm and aromatic protons are at δ 6.7-7.5 ppm. The NH protons of the hydrazones/thiosemicarbazones were observed in the downfield range of δ 9.5-13.0 ppm where as aromatic NH proton gives the signal at the range of δ 7.6-8.04 ppm. The LC-MS of the synthesized compounds (**3a-h**) confirms molecular mass coincides with the molecular ion of the compounds and also the chromatogram in LCMS indicates purity of the compounds.

DNA binding studies: The DNA binding studies for the Schiff bases (**3a-h**) (except **3c** and **3f**) was performed by using Elico-SL-159 spectrophotometer with fixed concentration the synthesized compounds (41.14-59.55 µM) and varying the

TABLE-2
FLUORIMETRIC SPECTRAL RESULTS WITH ADDITION OF *ct*-DNA TO COMPOUNDS **3a-h**

Compound	K_{sv} × 10^4 (M^{-1})	K_q × 10^{12} (M^{-1} S^{-1})	$R^{2(a)}$	K_b (M^{-1})	N	$R^{2(b)}$	-ΔG (J/mol)
3a	1.57	1.57	0.80	1.93 × 10^4	1.03	0.99	2.44 × 10^4
3b	2.18	2.18	0.99	0.59 × 10^4	0.91	0.98	2.15 × 10^4
3c	–	–	–	–	–	–	–
3d	1.94	1.94	0.99	7.48 × 10^4	1.16	0.99	2.78 × 10^4
3e	1.20	1.20	0.98	8.25 × 10^4	1.23	0.88	2.80 × 10^4
3f	–	–	–	–	–	–	–
3g	1.73	1.73	0.99	2.58 × 10^4	1.04	0.99	2.51 × 10^4
3h	1.20	1.20	0.99	0.07 × 10^4	1.18	0.69	1.62 × 10^4

[a]R, [b]R are the correlation coefficient for the K_{sv} K_b values respectively ΔG = Gibbs free energy change.

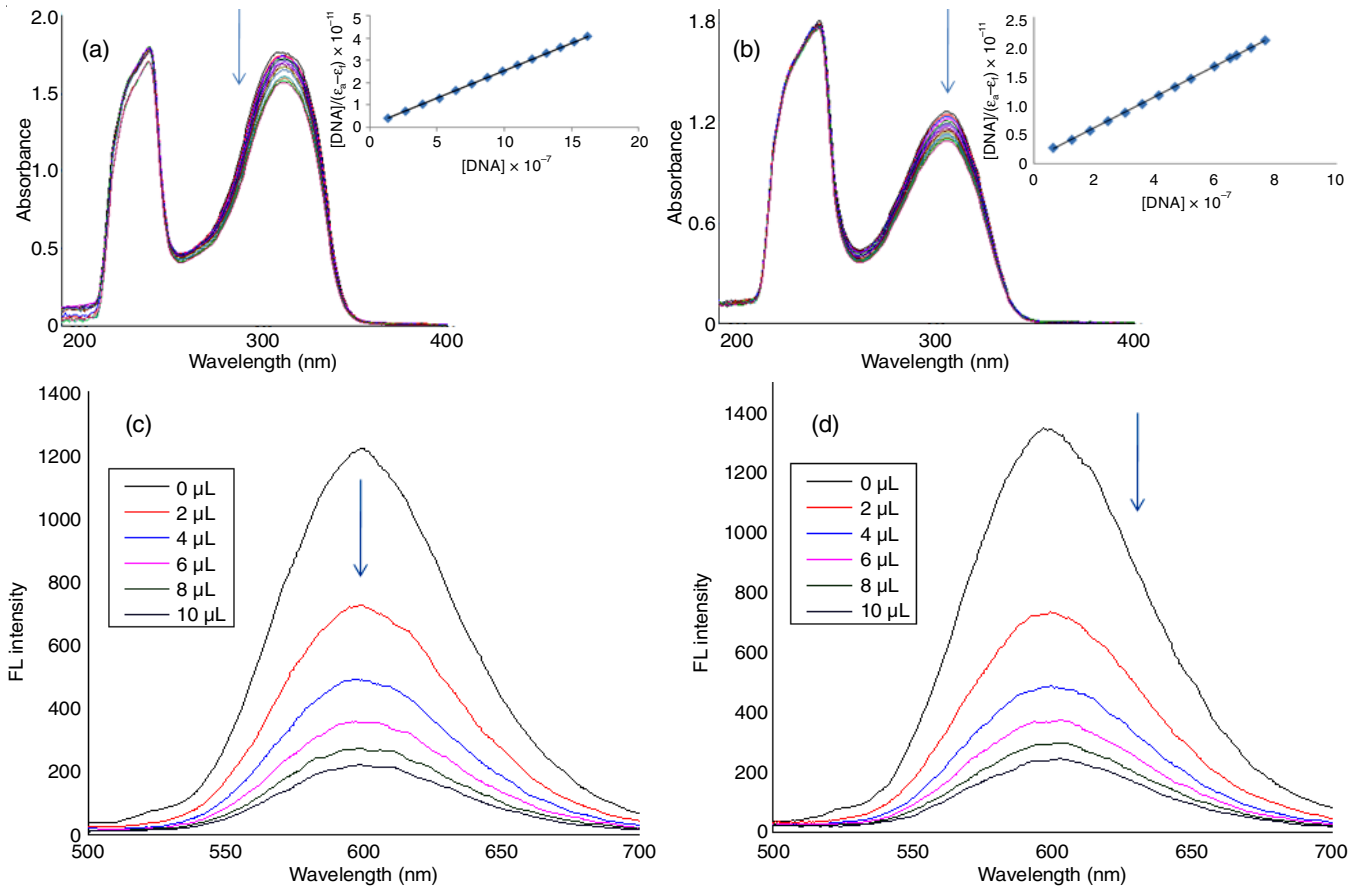


Fig. 1. DNA interactions using electronic absorption (a & b) spectra of compounds **3b** (a) and **3e** (b) in the absence and presence of the increasing amount of CT-DNA and fluorescence titration (c & d) of CT-DNA and EB (intercalator) in the presence of **3g** (c) and **3e** (d) addition of 0-10 µL

concentration of *ct*-DNA from (0 to 350 µL of stock *ct*-DNA solution of 1-5 × 10^{-5} M) at pH 6.9-7.01. From Table-1, it is observed that on addition of DNA, without/negligible red/blue shift along with 1.42-4.84% hyperchromism (for **3a**-1.42%, **3b**-4.80%, **3d**-4.23%, **3e**-2.38%, **3g**-4.84% and **3h**-1.74%) (Fig. 1) indicates the non-intercalative binding mode between Schiff base and *ct*-DNA. The binding efficiency of the molecule was determined by calculating intrinsic binding constant, K_b by plotting graph between [DNA]/(ε_a–ε_f) *versus* [DNA] and K_b values for **3a**: 3.18 × 10^4, **3b**: 3.60 × 10^4, **3c**: 3.61 × 10^4, **3g**: 8.44 × 10^4 and **3h**: 2.85 × $10^4 M^{-1}$. Hence the binding constant fallows in the order of **3g** [8.44 × 10^4] > **3d** [3.61 × 10^4] > **3b** [3.60 × 10^4] > **3a** [3.18 × 10^4] > **3h** [2.85 × 10^4 M^{-1}].

Based on the binding constant (K_b), the Gibb's free energies (ΔG) were calculated using the classical van't Hoff's equation:

$$\Delta G = -RT \ln K_b$$

where R = universal gas constant (8.314 J K^{-1} mol^{-1}) and T = temperature in K (298 K).

Negative free energies of the heterocyclic Schiff bases indicated that there is a spontaneous interaction between *ct*-DNA and heterocyclic Schiff bases during adducts formation. The binding efficiency of the molecule was determined by calculating intrinsic binding constant (K_b) by plotting graph between [DNA]/(ε_a–ε_f) *vs*. [DNA] and K_b values for **3a**: 3.18 × 10^4, **3b**: 3.60 × 10^4, **3e**: 3.61 × 10^4, **3g**: 8.44 × 10^4 and **3h**: 2.85 × 10^4 M^{-1}.

DNA cleavage studies: From Figs. 2 and 3, it was found that synthesized heterocyclic Schiff bases do not show any cleavage activity in the absence of H_2O_2 but in the presence of H_2O_2, the activity enhanced moderately from compounds **3a-d** whereas for compounds **3e-h** there is no such cleavage activity was observed. The results indicated that the heterocyclic Schiff base molecules were able to convert super-coiled DNA into open circular DNA and cleavage process may be closely related to the oxidative type of cleavage.

Conclusion

A series of novel heterocyclic Schiff bases derived from 4-chloro-1-methyl-pyrazolecarboxaldehyde and hydrazones/

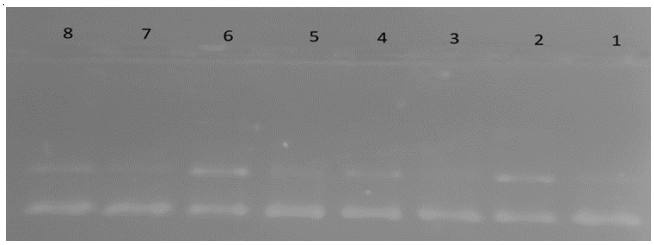


Fig. 2. DNA cleavage pattern for compounds **1-4** in presence and absence of H_2O_2 [Lane-1: Control DNA; Lane-2: Control DNA with H_2O_2; Lane-3: 100 µm of compound **1** + DNA + buffer; Lane-4: 100 µm compound **1** + DNA + buffer + H_2O_2; Lane-5: 100 µm compound **2** + DNA + buffer; Lane-6: 100 µm compound **2** + DNA + buffer + H_2O_2; Lane-7: 100 µm compound **4** + DNA + buffer; Lane-8: 100 µm compound **4** + DNA + buffer + H_2O_2]

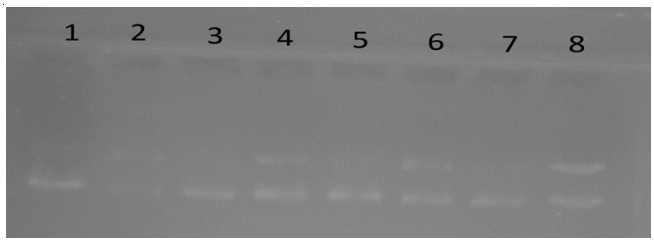


Fig. 3. DNA cleavage pattern of compounds **3a-h** in presence and absence of H_2O_2 [Lane-1: Control DNA, Lane-2: Control DNA + H_2O_2, Lane-3: 100 µm of compound **5** + DNA + buffer, Lane-4: 100 µm compound **5** + DNA + buffer + H_2O_2, Lane-5: 100 µm compound **7** + DNA + buffer, Lane-6: 100 µm compound **7** + DNA + buffer + H_2O_2, Lane-7: 100 µm compound **8** + DNA + buffer, Lane-8: 100 µm compound **8** + DNA + buffer + H_2O_2]

4N-substituted thiosemicarbazones were synthesized and characterized with FT-IR, NMR and LC-MS studies, which suggested that the synthesized compounds were in good agreement with proposed molecular structures. Further synthesized molecules were tested for their interaction against *ct*-DNA interaction by using absorption titrations and the intrinsic binding constant (K_b) was evaluated for all the compounds **3a-h**, except **3c** and **3f**, which were obtained in the range of $2.82 \times 10^4 - 8.44 \times 10^4$ further competitive DNA binding studies by fluorescence titrations. Among the tested compounds, compound **3g** showed the highest K_b value of 8.44×10^4 M^{-1}. According to the experimentally findings, the synthesized compounds have a strong affinity for binding *ct*-DNA in the non-intercalative state.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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