5-[Substituted]-1, 3, 4-thiadiazol-2-amines: Synthesis, Spectral Characterization, and Evaluation of their DNA interactions

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CHRONICLE	ABSTRACT
	The presence of heterocyclic moiety in diverse compounds, strongly indicative of the desired effect on physiological activity, and it reflects on efforts to find useful synthetic drugs. In this connection, here reporting the synthesis and characterization of 5-[substituted]-1, 3, 4-thiadiazol-2-amines (**1-7**). All the prepared compounds were characterized by spectroscopic methods *viz*. ^{1}H-NMR, ^{13}C{^{1}H}-NMR, FT-IR, and LC-MS. The results of the DNA binding interactions using absorption and fluorescence spectroscopy reveal that the compounds are avid binders to DNA. A DNA cleavage study with pUC18 DNA using gel electrophoresis indicates the compounds are able to cleave DNA in presence of oxidant H_2O_2.

1. Introduction

Recently interest in the synthesis and investigation of heterocyclic compounds forms major part of organic chemistry may be due to their vital role in the development of therapeutic drugs, industrial catalysts etc. Literature survey[1-6]reveals that heterocyclic compounds containing sulphur and nitrogen have been under investigation due to their remarkable biological and industrial applications.

Among, thiadiazoles, a five-membered heterocyclic compounds containing two nitrogenand one sulphur atoms as the heteroatoms, and are exist in different isomeric forms *viz*.(a)1,2,3-thiadiazole (b)1,2,5-thiadiazole (c) 1,2,4-thiadiazole and (d) 1,3,4-thiadiazole[7]. Among, 1,3,4-thiadiazoles having more applications and exhibiting potential biological activities like insecticidal[8, 9], fungicidal[10, 11],herbicidal activity[12], potent anti-cancer[13, 14],anti-proliferative activity[15, 16], Antiviral[17], inhibitors of acetyl cholinesterase (AChE) and butyrylcholinesterase (BuChE)[18], Alzheimer[19, 20], and antimicrobial activities[21]. 1,3,4-thiadiazoles also used in electrical and optical[22], liquid crystal[24-26], corrosion inhibitors[27], in dye preparation[28]. The literature survey reveals that various thiadiazoles are the part of many potential drugs (**Fig. 1**) and exhibiting the wide spectrum of pharmacological activities and the biological activity of 1,3,4-thiadiazole moieties is may due to the presence of the =N–C–S moiety. Synthesis by either ferric chloride or acids catalyzed oxidative cyclization of thiosemicarbazide derivatives and biological studies of similar 5-[Aryl]-1, 3, 4-thiadiazol-2-amines were reported[29, 30]. However the detailed spectral characterization and DNA studies of the 5-[Aryl]-1, 3, 4-thiadiazol-2-not reported so far. In consideration of diverse biological properties of these heterocyclic scaffolds and
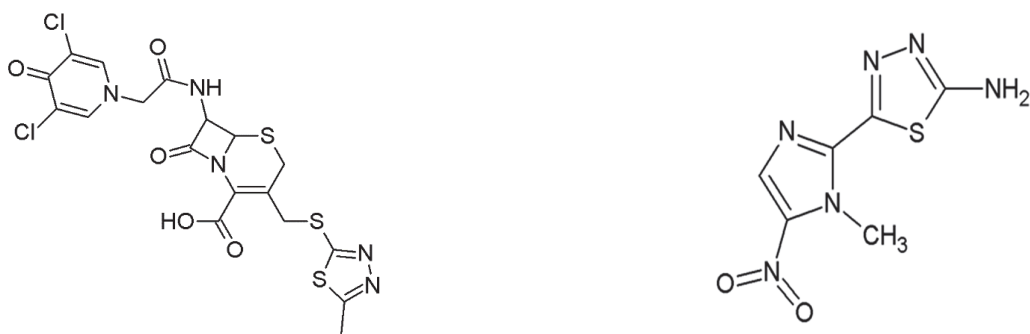
our continued interest on sulphur and nitrogen containing derivates[31-37], prompted us to design and synthesize heterocyclic thiadiazole moieties and to study their DNA studies.

(a) Cefazolin Sodium   (b) Megazol

Fig. 1. Biologically active 1,3,4-thiadiazole containing drugs

2. Results and Discussion

2.1. Synthesis and Characterization

As shown in **Scheme 1**, the 5-[substituted]-1, 3, 4-thiadiazol-2-amines (**1-7**) were prepared through the cyclization of thiosemicarbazones. The isolated compounds were obtained in good to excellent yield and are stable at room temperature, non-hygroscopic in nature and almost insoluble in water and readily soluble in common organic solvents like methanol, ethanol, DMSO and DMF. The analytical data of the prepared compounds are in good agreement with the proposed formulae of the ligands. The structural elucidation of the compounds were done by FT-IR, UV-Vis, ^{1}H-NMR, ^{13}C-NMR and LC-MS spectroscopy techniques and data are compiled in the synthesis part.

IR spectra of the compounds were recorded in the 4000-400cm^{-1}region using Bruker Alpha FT-IR spectrometer by KBr pellet method. The FT-IR spectra of compounds are shown in **Figs. S1-S7**. The stretching vibrational frequency of primary amine (N-H) was observed 3072-3400cm^{-1}. The sharp and moderately intense stretching vibrational bands between 2946-3040 cm^{-1}are assigned to aromatic C- H stretching. The most characteristic band, the C=N stretching vibration pertaining to the thiadiazole ring is present 1590–1636 cm^{-1}in the range[38], and stretching vibration for C-S-C of thiodiazole moiety observed in the range of 812-854 cm^{-1}. In compounds **4-6**, the C-X, where X= F or Cl stretching vibration observed in the range of 681-687 cm^{-1}.

The NMR spectra of all compounds were obtained using Agilen with ATB probe NMR spectrometer (400MHz for ^{1}H and 100MHz for ^{13}C) at room temperature in DMSO- d$_6$. In ^{1}H-NMR spectra (**Figs. S8-S14**), the aromatic protons resonate at 6.7– 7.5 ppm, and the thiadiazole amine protons appeared at 7.6-8.04 ppm. In ^{13}C-NMR spectra (**Figs. S15-S20**), it is clearly indicate that the 1,3,4-thiadiazole ring was formed on cyclization reaction by thiosemicarbazones were confirmed by observing -C=N group between 148-169 ppm. The aromatic carbon atoms of the compounds resonate at 112-130 ppm The LC-MS data were obtained by Agilent 1200 series LC-MicromasszQ spectrometer. In mass spectra (**Figs. S21-S27**), The molecular ion peak of the thiadiazole compounds matching with the calculated values.

2.2. DNA binding studies

2.2.1. DNA-Binding Studies by Electronic absorption spectral studies

The electronic spectroscopy is most useful technique, which is commonly used for study DNA binding interaction with small molecules[39]. Generally, when molecules bind to DNA with strong interaction such as intercalation, the intensity of absorption decreases and red shift is observed. If a ligand binds through non-intercalative or electrostatically with DNA, may result in either hyperchromism or hypochromism[40]. The DNA binding efficiency of prepared compounds (except **1** and **7**) was monitored by comparing the their absorption spectra with and without CT-DNA. The absorption titrations of compounds carried out at fixed concentration of thiadiazole compound (1.36-6.65mM) with varying DNA concentrations (25-350 μL of 2.273×10^{-6} molL^{-1} solutions of stock CT-DNA) under physiological conditions of pH 7.01. The resultant spectral graphs are given in **Fig. 2 and Figs. S28-S31.**

Table 1. Electronic absorption spectral data with addition of CT-DNA to compounds, **1-7**

Compound	λ_{max} (nm)		$\Delta\lambda_{max}$ (nm)	% H	K_b (M^{-1})	ΔG (kJ/mol)
	Free	Bound				
1	-	-	-	-	-	-
2	240	240	0	2.69	2.072×10^7	- 41.746
3	241	241	0	4.91	3.792×10^7	-43.244
4	238	239	1	-0.82	1.408×10^7	-40.788
5	300	303	3	-1.05	3.397×10^7	-42.971
6	300	300	0	1.48	2.084×10^7	-41.760
7	-	-	-	-	-	

The presence of Isosbestic point in the spectra indicates that no other species were present in the reaction except thiadiazole and DNA at equilibrium. In order to determine affinity of ligands with CT-DNA quantitatively, the intrinsic binding constant K_b for prepared compounds with CT-DNA was obtained by monitoring the changes in absorbance between 240-350nm, which attributed due to $\pi \rightarrow \pi^*$ intra-ligand transition and K_b values were evaluated in 10^7 order (1.408×10^7- 3.792×10^7 M^{-1}) of magnitude. With increase in concentration of DNA shows hyperchromism / hypochromism no/or negligible blue/red shiftindicate strong interaction of the compounds with CT DNA mainly through electrostatic or groove binding[41]. Based on the spectral change and K_b values compounds may be assigned as groove binders. The kinetics and thermodynamics of drug–DNA interaction in terms of binding constant (K_b) and Gibbs free energy change (ΔG) were evaluated by using the classical Van't Hoff's equation, $\Delta G = -2.303RT \log K_b$.
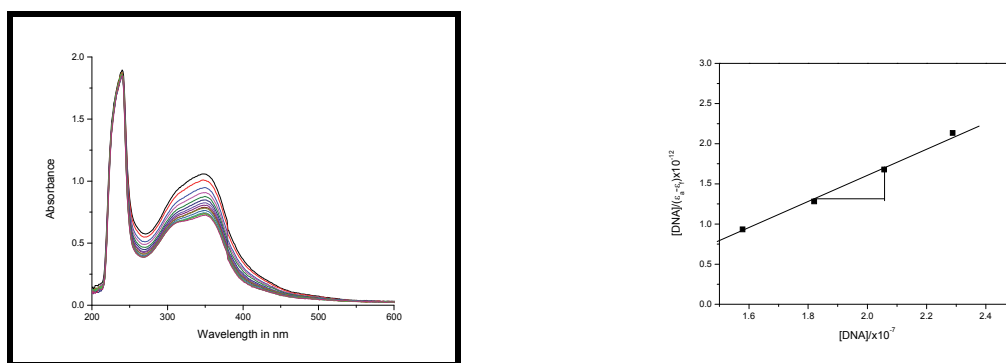
Fig. 2: The electronic absorption spectra of **2**in the absence and presence of increasing amounts of CT-DNA. Arrow shows the change in the absorbance with increase the DNA concentration. Inset: plot of [DNA]/(ε_a-ε_f) Vs[DNA].

The negative ΔG values confirmed spontaneous binding of compounds with DNA *via*. formation of stable complexes, **Table 2**. In order to further investigate the binding mode, fluorescence analyses were performed.

2.2.2. DNA-Binding Studies by Fluorescence Spectroscopy

Under similar conditions as in absorption titrations, fluorescence studies were undertaken for further proof for the binding efficiency of the compounds with DNA.The quenching assay method based on the displacement of the intercalating dye, ethidium bromide (EB), from CT-DNA was employed to investigate the interaction mode between the thiadiazole and CT-DNA. EB is a very useful DNA structural probe, which shows a significant increase in fluorescence intensity when binding to the base pair of DNA through intercalating. However, the enhanced fluorescence can be quenched if there is a second complex that can replace the bound EB or break the secondary structure of DNA[42-44]. It has been reported that the groove DNA binders can also cause the decrease in EB emission intensities. The effects were, however, only moderate[45]

Table 2. Fluorimetric spectral data with addition of CT-DNA to compounds, **1-7**

Compound	$K_{SV} \times 10^5$ (M^{-1})	$K_q \times 10^{13}$ (M^{-1} S^{-1})	r^2	K_b (M^{-1})	n	r^2	-ΔG (kJ/mol)
1	-	-	-	-	-	-	-
2	4.109	4.109	0.9905	2.6160×10⁶	1.16	0.9993	36.618
3	3.152	3.152	0.9873	3.8584×10⁶	1.22	0.9968	37.581
4	4.172	4.172	0.9952	2.1486×10⁶	1.14	0.9991	36.130
5	7.498	7.498	0.9972	2.4620×10⁷	1.30	0.9946	42.173
6	6.014	6.014	0.9890	-	1.98	0.9899	-
7	-	-	-	-	-	-	-

The fluorescence quenching of DNA-bound EB can be described by the linear Stern-Volmer equation[46] in which the synthesized compounds were the quenchers:

$$\frac{F_0}{F} = 1 + k_q \, \tau_0 [Q] = 1 + K_{SV}[Q]$$

F_0 and F represent the fluorescence intensities in the absence and presence of quencher, respectively; $K_{SV}$is a linear Stern-Volmer quenching constant; [Q] is the concentration of quencher and τ_0 is the average fluorescence lifetime of the quencher (10^{-8}s). A plot of F_0/F versus [Q] gave a slope to intercept which is equal to K_{SV}. The K_{SV} values for the tested compounds are given in **Table 2**. From K_{SV} values, compound **5** had the highestK_{SV} value, which suggested that compound bound most strongly to CT-DNA. Then, a linear Stern–Volmer plot (**Fig. 3 and S32-35**) indicates either one type of binding or quenching process is occurring by static or dynamic mechanism[47].

Further, to differentiate between the quenching processes, the bimolecular quenching rate constant, K_q is calculated. The K_q value for static quenching mechanism has been reported (10^{10}Ms). The calculated K_q values (**Table 2**) at 298K were found greater than the expected values, which indicate the quenching process is static rather than dynamic[48].

It is also calculated the intrinsic binding constant (Kb) and size of binding sites (n) compounds from the intercept and slope of plot log (F_0-F/F) versus log[Q], respectively using the following equation[49].

$$\log \frac{F - F_0}{F} = \log K_b + n \log[DNA]$$

The evaluated data of Kb and n values complemented the results obtained from obtained using absorption spectroscopy. From the values of n, *n* > 1 showed the possibility of more available sites;

hence the interactions may occur along with intercalation.

<center>(a)   (b)   (c)</center>
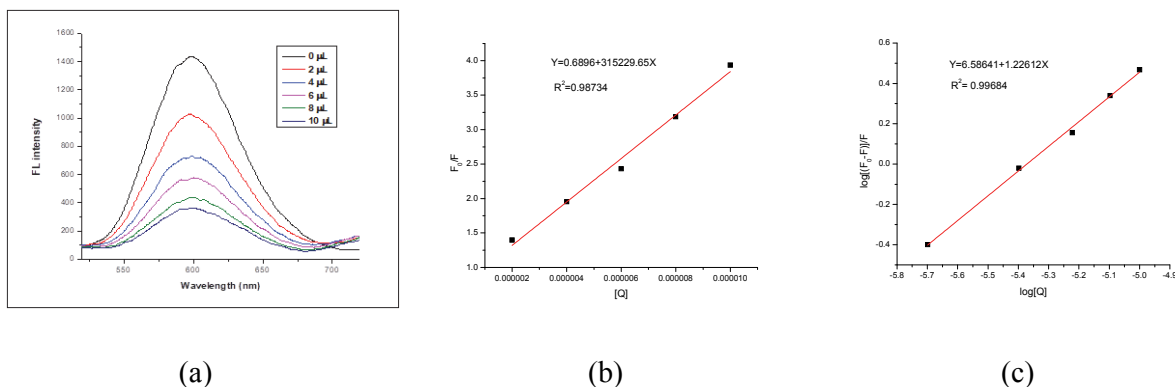
Fig. 3. (a) Fluorescence titration of CT-DNA and EB (intercalator) complex with compound 3 (0–10 µL) (b) Stern-Volmer plot for fluorescence quenching of compound **3** by EB in absence and presence of CT-DNA (c) Plot of log (F0 −F)/F as a function of log [Q].

Using binding constant values ΔG were calculated and given in **Table 2** and values are comparable with that obtained from absorption titration method. Based on fluorescence change it is possible to bind the CT-DNA and thiadiazole moieties in groove binding mode.

2.3. DNA cleavage studies

The DNA Cleavage studies of the prepared compounds were studied using Gel electrophoresis technique, which is based on the migration of DNA under the influence of an electric potential. DNA cleavage was monitored by pUC18 DNA using tris–acetic acid-EDTA (TAE) buffer (pH 8.0). The samples were incubated for 1 h at 37 ^{0}C. After incubation, 2 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 at 100 V in Tris-acetic acid- EDTA (TAE) buffer till the bromophenol blue reached 3/4th of the gel. Bands were visualized by using UV trans-illuminator and photographed. For comparison purposes, the cleavage reaction for compounds was carried out in the absence and presence of H_2O_2 and is shown in **Fig. 4.**
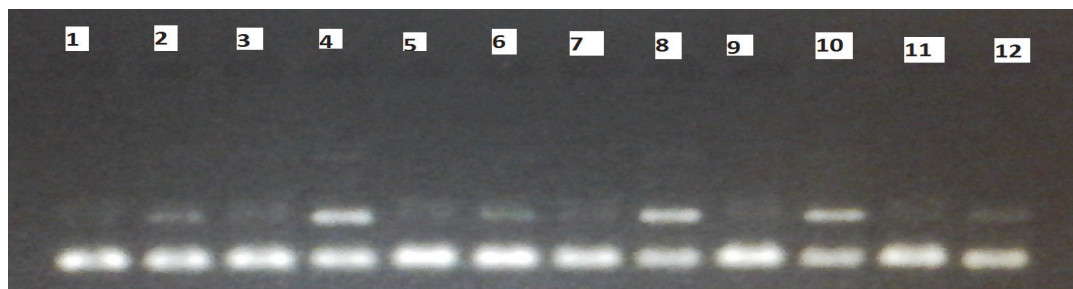
Fig. 4. Gel electrophoresis of compounds **2-6**, Lane 1: Control DNA, Lane 2: Control DNA+H_2O_2. Lane 3: 100µM of **2**+DNA+buffer, Lane 4: 100µM of 2+DNA+buffer+H_2O_2, Lane 5: 100µM of **3**+DNA +buffer, Lane 6: 100µM of **3**+DNA+buffer+H_2O_2, Lane 7: 100µM of **4**+DNA+buffer, Lane 8: 100µM of **4**+DNA+buffer+H_2O_2, Lane 9: 100µM of **5**+DNA +buffer, Lane 10: 100µM **of 5**+DNA+buffer+H_2O_2, Lane 11: 100µM of **6**+DNA +buffer, Lane 12: 100µM **of 6**+DNA+buffer+H_2O_2.

The ability of DNA cleavage was determining based on the capacity of thiadiazole moieties in conversion of open circular (OC) or nicked circular (NC) nucleic acid from its super coiled (SC) structure.

From **Fig. 4** it observed that the does show any cleavage activity in the absence H_2O_2 but in the presence of H_2O_2 the activity enhanced moderately. The results indicated that the role of thiadiazole moiety in isolated DNA cleavage reaction. The thiadiazole molecules were able to convert supercoiled DNA into open circular DNA and the results indicate that the process of DNA cleavage may be closely related to the oxidative type of cleavage.

3. Conclusion

This paper describes the synthesis and characterization of thiadiazoles. The spectral data showed that the formation of compounds. The DNA binding studies reveals that, the molecules are avid binders to CT-DNA. The DNA cleavage studies indicate that the process of DNA cleavage may be closely related to the oxidative type of cleavage.
The prepared compounds might be important biologically, and their medical research applications should be investigated.

Acknowledgements

4. Experimental methods

4.1 Materials and methods

All the starting materials, Calf thymus DNA was obtained from sigma Aldrich and PUC[18] DNA obtained from Genie, Bangalore. Melting points of the compounds were measured by open capillary method;^{1}H-NMR and ^{13}C NMR spectra were recorded on Agilent at 400MHz and 100MHz in d_6-DMSO solvent. FT-IR was recorded using Bruker alpha KBR pellet method with silicon carbide as IR source; LC-MS was obtained on Agilent 1200 series LC & Micromass Q spectrometer. Fluorescence emission spectra were recorded using a F-2300 Spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cell at 298 K). The excitation and emission slit widths were maintained at 5.0 nm, and the excitation wavelength (k_{ex}) was fixed to 500 nm in the range 520-700nm for ethidium bromide and by excitation at 350 nm in the range 390-600 nm for thidiazoles. Absorption titrations were carried on Elico SL 159 UV–visible spectrophotometer in 200–500 nm range equipped with 1.0 cm quartz cell at room temperature.

4.2 Synthesis of substituted thiadiazoles

All the titled compounds were prepared as shown below.

4.2.1. Synthesis of thiosemicarbazones

The starting precursors, thiosemicarbazones were prepared according to procedure described in literature[50]. An equimolar quantity of a warm alcoholic solution of aldehyde and 5% glacial acetic acid aqueous solution of thiosemicarbazide were mixed and refluxed for 2 hours. The reaction mixer cooled to room temperature. Then, the product was separated was collected by filtration and recrystallized in alcohol.

4.2.2. Synthesis of thiadiazoles

The thiadiazoles (**1-7**) were prepared (**Scheme 1**) according the procedure described in literature[8, 29, 30].To a suspended aqueous solution of thiosemicarbazone (0.05mol) warm aquoues solution of ferric

chloride (0.015mol) was addedslowly with constant stirring, then contents were refluxed at 80-90⁰C for 45 min. The resultant solution was filtered and added citric acid (0.11mol) and sodium citrate (0.05mol). The obtained mixture was divided in to 4 parts and each part on neutralized with 10% ammonia solution the formed amine was filtered, dried and recrystallized with alcohol.

2 - amino - 5 - aryl 1,3,4 - thiadiazole (**1-7**)
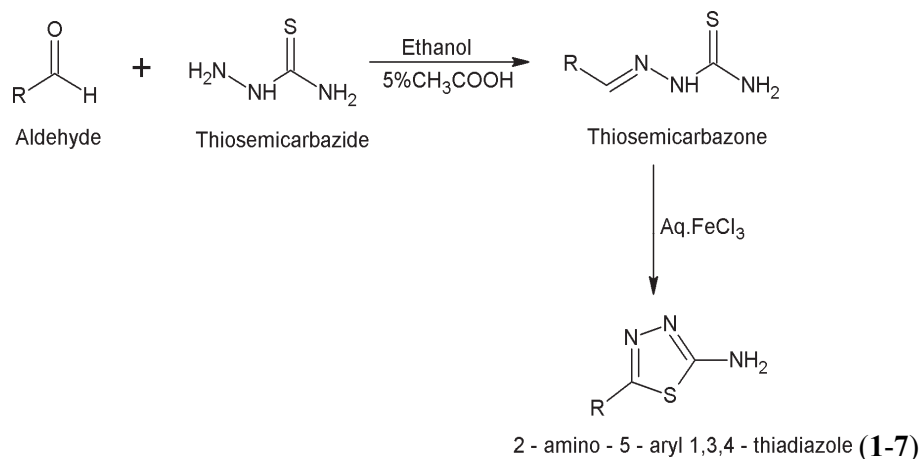
where R = 4-Isopropylbenzaldehyde (**1**), 4-Dimethylaminobenzaldehyde (**2**)

Vertraldehyde or 3,4-Dimethoxybenzaldehyde (**3),** 4-Fluoro benzaldehyde (**4**),

4-Chloro benzaldehyde (**5**), 4-Chloro-1-methyl-pyrazole carboxaldehyde (**6**),

3-Nitro benzaldehyde (**7**)

Scheme1: Synthesis of 2-amino-5-substituted thiadiazoles

4.3.Spectral data

4.3.1. **5-[4- (Propan-2yl)phenyl]-1,3,4-thiadiazol-2-amine (1)**

MP (^{0}c): 172-176, IR(cm^{-1}): 3090-3277(NH$_2$), 1626(C=N), 1509(C=Car), 2957(-CH aromatic), 823(C-S-Cstr); ^{1}H NMR: 1.16-1.21(d, 6H), 7.63(d, 2H,J=7.6Hz, aromatic),7.30(d, 2H,J=7.6Hz, aromatic),7.33(2H(s) NH$_2$), 2.87(m, 1H, -CH); ^{13}C NMR: 24ppm, 33ppm, 126ppm, 127ppm, 128ppm, 129ppm, 150ppm, 156ppm & 168ppm; LCMS(m/z) for C$_{11}$H$_{13}$N$_3$S: 219.299, Found: 220.05.

4.3.2. **5-[4-(Dimethylamino)phenyl]-1,3,4-thiadiazol-2-amine (2)**

MP (^{0}c): 135-140, IR (cm^{-1}): 3143-3248(NH$_2$), 2946(-CH aromatic), 2893(-CH), 1597(C=N), 1509(C=C), 812(C-S-C); ^{1}H-NMR: 2.7-2.9(m, 6H), 6.7-7.08(m, 4H,aromatic), 7.6-7.7(d, 2H, NH$_2$); ^{13}C-NMR: 43ppm, 111ppm, 112ppm, 127ppm, 127ppm, 129ppm, 130ppm, &131ppm; LCMS(m/z) for C$_{10}$H$_{12}$N$_4$S: 220.288, Found: 221.10.

*4.3.3.***5-(3,4-dimethoxyphenyl)-1,3,4-thiadazol-2-amine(3)**

MP (^{0}c): 148-152, IR(cm^{-1}): 3253-3346(NH$_2$), 3040 (-CH aromatic), 2954-2823(-CH), 1614(C=N), 1504(C=N), 854(C-S-C); ^{1}H-NMR: 3.74(s, 6H, dimethoxy), 6.9-7.1(m, 3H, aromatic), 7.26(s, 2H, NH$_2$); ^{13}C-NMR: 55ppm, 56ppm, 109ppm, 112ppm, 120ppm, 124ppm, 149ppm, 150ppm, 156ppm & 168ppm, LCMS(m/z) for C$_{10}$H$_{11}$N$_3$O$_2$S: 237.273, Found: 238.

*4.3.4.***5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-amine(4)**

MP (^{0}c): 214-218, IR (cm^{-1}): 3000-3400(NH$_2$), 2976(-CH aromatic), 1590(C=N), 1506(C=C), 1000(C=F), 829(C-S-C); ^{1}H-NMR: 7.26-7.36(d, 4H, aromatic), 7.57(s, 2H, NH$_2$); ^{13}C-NMR: 116ppm, 128ppm, 155ppm, 161ppm, 164ppm, & 169ppm, LCMS(m/z) for C$_8$H$_6$N$_3$SF: 195.213, Found: 196.05.

4.3.5. 5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-amine(5)

MP (^{0}c): 206-210, IR(cm^{-1}): 3072-3243(NH$_2$), 1591(C=N), 1508(C=Car), 829(C-S-Cstr), 681(C-Cl); ^{1}H NMR: 7.75(d, 2H,aromatic, J=8Hz), 7.51(d, 2H,aromatic, J=7.6Hz), 7.45(s, 2H, NH$_2$);^{13}C NMR: 128.3ppm, 129.6ppm, 130.3ppm, 134.4ppm, 155.6ppm &169.3ppm; LCMS(m/z) for C$_8$H$_6$N$_3$SCl: 211.663, Found: 212.50.

4.3.6. 5-(4-Chloro-1-methyl-1H-pyrazol3-yl)-1,3,4-thiadiazol-2-amine(6)

MP (^{0}c): 265-270, IR(cm^{-1}): 3092-3256(NH$_2$), 1636(C=N), 1498 (C=Car), 2936(-CH aromatic), 829(C-S-Cstr), 681(C-Cl); ^{1}H-NMR: 3.82(s, 3H, CH$_3$), 7.37(s, 1H, aromatic), 8.04(s, 2H,NH$_2$);^{13}C-NMR: 106ppm, 132ppm, 139ppm, 148ppm &168ppm, LCMS(m/z) for C$_6$H$_6$N$_5$SCl: 215.657, Found 216.

4.3.7. 5-(3-Nitrophenyl)-1,3,4-thiadiazol-2-amine (7)

MP (^{0}c): 206-208,IR(cm^{-1}) : 3137-3268(NH$_2$), 1622 (C=N), 1470 (C=Cstr), 774 (C-S); ^{1}H NMR: 8.293 (s, 1H NH$_2$), 8.06(d, 1H,7.2Hz);7,95.(d, 1H,7.2Hz) 7.58(1H(t), 8Hz,7.6 Hz);^{13}C NMR: 120.4 ppm, 124.2 ppm, 131.3 ppm, 132.8 ppm, 132.9 ppm, 148.6 ppm, 154.5 ppm &169.8 ppm; LCMS (m/z) Caculated for C$_8$H$_6$N$_4$O$_2$S: 222.22 Found 223.

4.4. DNA binding studies

4.4.1.DNA studies by absorption titrations

The electronic spectroscopy is commonly used technique to study the DNA binding activity studies. A solution of CT-DNA in 50mM Tris-HCl/50mM NaCl buffer solution was prepared at pH 6.9-7.01 gives a ratio of UV absorbance at 260 and 280 nm of 1.8-1.9 indicating that DNA was free of proteins[51]. Then a concentrated stock solution of DNA was prepared in 50mM Tris HCl/50mM NaCl in double distilled water at pH 6.9-7.01 and the concentration of CT-DNA was determined per nucleotide by taking the absorption coefficient (6600 dm^3 mol^{-1} cm^{-1}) at 260 nm[52] Stock solutions were stored at 4^{0}C and were used after no more than 4 days. A 2mL solution in 1cm quartz containing fixed concentration of the compounds, except **1** and **5** (1.36-6.65mM) was titrated by successive addition of 25µl to 350µl DNA whose stock CT-DNA concentration 2.273x10^{-6} molL^{-1}. The spectra were recorded against blank solution containing same concentration of DNA. Then the intrinsic binding constant K$_b$[53]

$$\frac{[DNA]}{(\epsilon_A - \epsilon_B)} = \frac{[DNA]}{(\epsilon_B - \epsilon_F)} + \frac{1}{K_b(\epsilon_B - \epsilon_F)}$$

where, ϵ_A, ϵ_B and ϵ_F corresponds to the apparent, bound and free compound extinction coefficients, respectively. A plot of $\frac{[DNA]}{(\epsilon_A - \epsilon_F)}$ versus [DNA] gave a slope of $\frac{1}{(\epsilon_B - \epsilon_F)}$ and Y-intercept equal to $\frac{1}{K_b(\epsilon_B - \epsilon_F)}$, Hence K$_b$ was obtained from the ratio of the intercept to the slope[37]. The percentage of hyperchromicity or hypochrocicity for the CT-DNA/[Ligand] was obtained from ($\epsilon_a - \epsilon_f$)/ ϵ_f x100.

4.4.2. DNA studies by Fluorescence Studies

Ethidium bromide, cationic dye, which interacts strongly and specifically with DNA, is widely used in spectrofluorimetric studies due to increase in fluorescence upon binding that indicates intercalation of dye with DNA. Hence ethidium bromide-DNA complex quenching technique becomes a routine to

compare the DNA binding mode of the prepared compounds. The fluorescence spectra of the compounds were recorded by using the excitation wavelength of 510 nm, the emission wavelength was around 600nm. Before measurements, the mixture was mixed well. In the ethidium bromide (EB) fluorescence displacement experiment, 10 µL of the EB Tris solution (50 µM) was added to 10 µL of DNA solution (10 µL at saturated binding level)[52]. The compound was then titrated into the EB/DNA mixture. Before measurements, the solution was well mixed at room temperature for 5 min. Fluorescence spectra of EB bound to DNA were obtained at an excitation wavelength of 540 nm and an emission wavelength of 592 nm.

4.5. DNA cleavagestudies

The DNA Cleavage studies of the prepared compounds were studied using Gel electrophoresis technique, which is based on the migration of DNA under the influence of an electric potential. DNA cleavage was monitored by pUC18 DNA using tris–acetic acid-EDTA (TAE) buffer (pH 8.0). The samples were incubated for 1 h at 37 ^{0}C. After incubation, 2 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 at 100 V in Tris-acetic acid- EDTA (TAE) buffer till the bromophenol blue reached 3/4th of the gel. Bands were visualized by using UV transilluminator and photographed. For comparison purposes, the cleavage reaction for compounds was carried out in the absence and presence of H_2O_2. The ability of DNA cleavage was determining based on the capacity of thiadiazole moieties in conversion of open circular (OC) or nicked circular (NC) nucleic acid from its super coiled (SC) structure.

Conflicts of Interest

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

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