Synthesis, characterization of pyrazole derivatives by using benzoyl glycine methyl ester as starting material: DNA binding and biological studies

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Abstract

Four pyrazole derivatives{Methyl-2-benzamido-2-(1,3,5-trimethyl-1H-pyrazol-4-yl)acetate (1A), 2-(benzamido)-2-(1,3,5-trimethyl-1H-pyrazol-4-yl)acetohydrazide (1B), Methyl-2-benzamido-2-(3,5-dimethyl-1H-pyrazol-4-yl)acetate (2A), 2-(benzamido)-2-(3,5-dimethyl-1H-pyrazol-4-yl)acetohydrazide (2B)}were synthesized by the solvent free method and their DNA binding interaction were studied by using UV-Visible spectroscopy and verified by viscometric technique under physiological conditions of p^H (stomach; 4.7, blood; 7.4) and temperature (37 ^oC; (human body temperature). All the compounds exhibited strong binding with the DNA due to the formation of compound–DNA complex via intercalation. Among all the compounds 2B showed greater binding at p^H 7.4 as evident from their greater K_b (5.32×10^4 M). Overall binding data revealed greater binding affinity of all the compounds with DNA at blood p^H. Viscosity studies further verified the intercalation mode of interaction of these compounds with DNA. Standard Gibb's free energy changes (ΔG) were evaluated negative and indicated spontaneous binding of compounds with DNA. Biological studies revealed greater cytotoxicity activity of 1A and 2B with least LD_{50} values of 170 μg/mL and 171μg/mL, respectively, while all the compounds showed broader range of antibacterial activity with MIC values ranging from 150-200 gL^{-1}. None of the compounds showed significant antioxidant activity.

Keywords: *Pyrazole derivatives, spectroscopic DNA binding, binding constant, viscosity measurement, cytotoxic activity*

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Introduction

Scientists are paying attention to the synthesis of pharmacologically active hetero cycles having pyrazoles moiety because pyrazole is an important structural unit known to contribute to various types of medicines (Dias et al., 2012). Pyrazole and their derivatives exhibit wide range of biological activities like anti-inflamamtory (Kumar et al., 2016), analgesic (Eweas et al., 2015), anti-bacterial (Yu et al., 2015), anti-fungal (Sun & Zhou, 2015), anti-tumor (Nitulescu et al., 2013), anti-malarial (Kumar et al., 2018) and anti-convulsant (Aragade et al., 2012). Pyrazole is an important pharmacophore used in drug designing because it can target many diseases (Bhat & Belagali, 2018).

During the past few decades the interaction studies of organic molecules with nucleic acids are much-focused research area. The interaction studies of drug with the DNA are highly important in the area of chemotherapeutic applications. Anticancer drugs show their biological action through their interaction with the DNA molecule. DNA exhibit considerable changes in absorbance spectra after complex formation with drug molecule. Spectroscopic study is an important tool to determine the mode of interaction of DNA with drug and also its therapeutic effects on a particular disease (Lu et al., 2014).

In literature a number of evidences present about the interaction of pyrazole derivatives with DNA. By keeping in mind the tremendous importance of pyrazoles, we have synthesized new pyrazole derivatives and studied their interaction with DNA by using UV-Visible spectroscopy and viscometric studies. These compounds were further investigated for their biological activities.

Experimental

Materials and methods

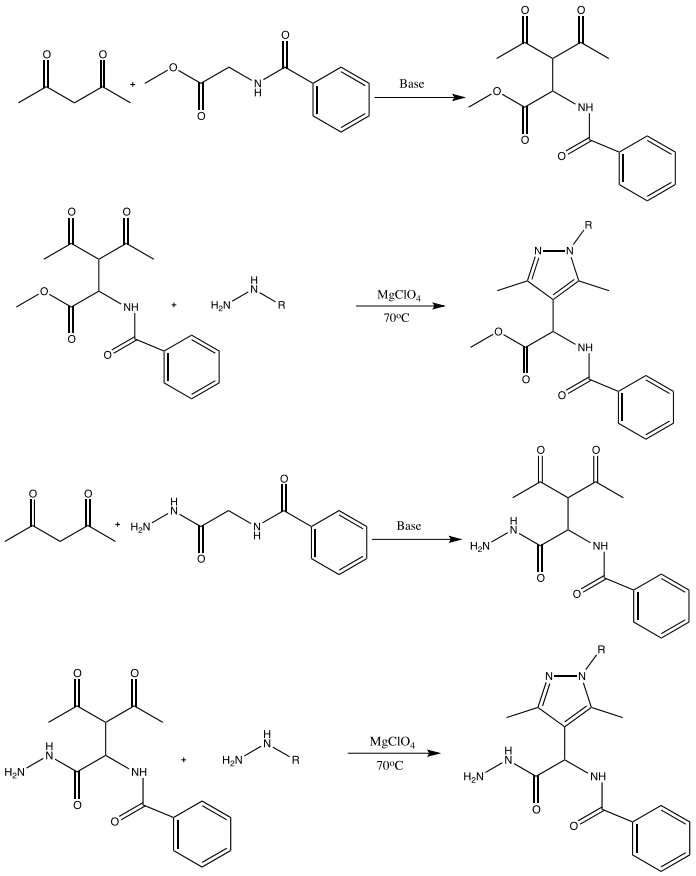
All reagents used for the synthesis of the pyrazole derivatives were purchased from E.Merk. All the solvents were purchased from the Fluka. All the solvents were dried before using by the methods available in the literature. DNA extraction was done in the laboratory from chicken blood by following the Falcon method (Sambrook et al., 1989) and concentration of DNA was determined spectrophotometrically at 260 nm using molar extinction coefficient (ε_{260}= 6600 $cm^{-1}M^{-1}$) (Reichmann et al., 1954). Purity of DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm and A_{260}/A_{280} ratio was found greater than 1.8, indicating that DNA was sufficiently pure and free from protein (Babkina & Ulakhovich, 2005).

Instrumentations

The melting points were determined in capillary tubes using a Sanyo Gallenkamp melting point apparatus. UV- spectra was recorded on UV-Visible spectrophotometer (UV-1700 pharma Spec). FTIR spectra (KBr) were recorded on a Nicolet iS 10 FTIR spectrophotometer in the range of 4000–400 cm^{-1}. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz.

Synthesis

Pyrazole derivatives were synthesized by reacting the mixture of the 1,3-diketones, substituted hydrazines and $Mg(ClO_4)_2$. Few drops of acetic acid were added to this mixture and mixture was heated in round bottom flask at 70 ^{o}C. Reaction was monitored by TLC (chloroform: methanol). After the completion of the reaction the crude product was separated from the mixture and purified by recrystallization in ethanol. The general synthetic route of chemical reactions is given in Scheme 1.



R= CH_3, H

Scheme 1 Synthetic route for the synthesis of pyrazole derivatives

Physical data

Methyl-2-benzamido-2-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)acetate (1A)

Colorless solid. M.P.: 178-180 ^{o}C. FTIR data (KBr, cm^{-1}): (C-O) 1216, (N-H) 3413, (N-N) 1135, (C=N) 1637, (C=C) 1578,1534,1438, (C=O) 1753,1680, (sp^{3}C-H) 2844, (sp^{2}C-H) 2962-2968.^{1}H NMR data (DMSO, ppm): 3.63 (s, H-1), 5.71 (s, H-4), 8.03 (s, H-6), 7.70-8.08 (phenyl protons).^{13}C NMR data (DMSO, ppm): 169.5 (C-8), 167.8 (C-10), 38.3 (C-1), 9.9 (C-2), 12.7 (C-6).

2-(benzamido)-2-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)acetohydrazide (1B)

Colorless solid. M.P.: 150-152 ^{o}C.FTIR data (KBr, cm^{-1}):(C-O) 1225, (N-H) 3480,3413,3309(2^{o}), (N-N) 1122, (C=N) 1630, (C=C) 1529,1458,1438, (C=O) 1745,1685, (sp^{3}C-H) 2930, (sp^{2}C-H) 2995-2965.^{1}H NMR data (DMSO, ppm): 3.63 (s, H-1), 8.02 (s, H-5), 8.0 (s, H-6), 8.03 (s, H-8), 7.70-8.08 (phenyl protons).^{13}C NMR data (DMSO, ppm): 38.3(C-1), 170.3 (C-8), 167.8 (C-9), 127.5(C-11, C-15), 128.8(C-12, C-14), 132.1(C-13).

Methyl-2-benzamido-2-(3,5-dimethyl-1*H*-pyrazol-4-yl)acetate (2A)

Colorless solid. M.P.: 156-158 ^{o}C.FTIR data (KBr, cm^{-1}): (C-O) 1217, (N-H) 3413,3318 (N-N) 1137, (C=N) 1611, (C=C) 1490,1458,1437, (C=O) 1750,1685, (sp^{3}C-H) 2895, (sp^{2}C-H) 2965-2918.^{1}H NMR data (DMSO, ppm)11.98 (s, H-1), 8.03 (s, H-6), 1.93 (s, H-3), 8.03 (d, H-7 and H-11), 7.63 (d, H-8, H-10).^{13}C NMR data (DMSO, ppm): 169.5 (C-7), 167.8 (C-9),127.5(C-11, C-15), 128.8(C-12, C-14), 132.1(C-13).

2-(benzamido)-2-(3,5-dimethyl-1*H*-pyrazol-4-yl)acetohydrazide (2B)

Colorless solid. M.P.: 168-170 ^{o}C.FTIR data (KBr, cm^{-1}): (C-O) 1157, (N-H) 3475, 3414, 3325(2^{o}), (N-N) 1135, (C=N) 1617, (C=C) 1577, 1541, 1491, (C=O) 1701, 1675, (sp^{2}C-H) 2970-2950.^{1}H NMR data (DMSO, ppm) 3.63 (s, H-1), 8.03 (s, H-5), 7.70-8 (H-8, 9, 10, 11, 12).^{13}C NMR data (DMSO, ppm): 170.3 (C-8), 167.8 (C-9), 127.5 (C-11, C-15), 128.8 (C-12, C-14), 132.1(C-13), 38.3(C-1).

Procedure for DNA binding analysis

UV–visible spectroscopy

Spectroscopic titrations were carried out at stomach (4.7) and blood (7.4) p^H and at 37 ^{o}C (human body temperature). Solutions of 1A and 2A were prepared in ethanol-water mixture (7:3), while solutions of 1B and 2B were prepared in distilled water.The absorbance measurements by UV–visible spectrophotometer were recorded by keeping the concentration of each compounds constant (10×10^{-6} M) but varying the concentration of ds-DNA from 10 to 50 μM by standard addition method. In order to achieve the equilibrium between the compound and DNA, solutions were allowed to stay for at least 5 min before each measurement was made. Temperature was maintained at 37 ^{o}C by temperature controller device.

Viscosity measurements

Viscosity of the DNA solution (η) was determined at stomach (4.7) and blood (7.4) p^H under physiological temperature (37 ^{o}C). The specific viscosity contribution (η), due to DNA (10×10^{-6} M), was determined in the presence of increasing concentration of the investigated compounds. The values of the relative specific viscosities $(\eta/\eta_o)^{1/3}$ for the compound and the compound–DNA complex were plotted against [compound/DNA]concentration.

Procedure for biological studies

Brine shrimp cytotoxicity assay

The cytotoxicity was studied by the brine-shrimp lethality assay method (Ahmad et al., 2008). Brine shrimps (Artemiasalina) were hatched using brine shrimp eggs in sterile artificial sea water (prepared using sea salt 38 gL^{-1}) under constant aeration for 48 h at room temperature. After hatching, active shrimps free from eggs were collected

from brighter portion of the hatching chamber and used for the assay. Ten shrimps were drawn through a glass capillary and placed in each vial containing 5 mL of artificial sea water with 200, 66.6, 22.4, 7.4 μg mL^{-1} final concentration of each test compound from their stock solutions. The vials were maintained under illumination at room temperature. After 24 h, the number of surviving shrimp was counted. Experiment was performed in triplicate. Data was analyzed with Finney computer program to determine LD_{50} (Lethal Dose that killed 50% of shrimps) values.

Antibacterial assay

Test compounds were screened to determine their antibacterial activity against six bacterial strains; Three gram positive, *Staphylococcus aureus* (ATCC 6538), *Salmonella typhimurium* (ATCC 14028) and *Bacillus subtilis* (ATCC 6633) and three gram negative, *Echerichia coli* (ATCC 15224), *Enterobacteraerogenes* (ATCC 13048) *Bordetellabronchiseptica* (ATCC 4617) by using "Disc Diffusion Method". The organisms were cultured in nutrient broth at 37ºC for 24 h. One percent both culture containing approximately 10^6 colony forming units (CFU/mL) of test strain was added to nutrient agar medium at 45ºC and poured into sterile petri plates. The medium was allowed to solidify. Five microliters of the test compounds (40 mg/mL in DMSO) was poured into on 4-mm sterile paper discs and placed on nutrient agar plates respectively. In each plate DMSO served as negative control and standard antibacterial drugs Roxithromycin (1mg/mL) and Cefexime (1mg/mL) served as positive control. Triplicate plates of each bacterial strain were prepared. The plates were incubated at 37 ºC for 24 h. The antibacterial activity was determined by measuring the diameter of zones showing complete inhibition

DPPH free radical scavenging assay

The scavenging activity of the DPPH radical by different test samples was determined by according to the method reported in the literature with some modifications.15 μL of each test sample or DMSO (-ve control) was mixed with 2985 μL of freshly prepared 0.1 mM DPPH solution. Each concentration was assayed in triplet and reaction mixture was incubated for 30 min at 37 ºC in dark. After incubation absorbance was measured at 517 nm. Mixture of 2985 μL of methanol and 15μL of DMSO was used as a blank for spectroscopic measurement. The percent scavenging of DPPH free radical for each concentration of each test sample was calculated.

Results and Discussion

Physical description of the data

Four pyrazole derivatives were synthesized by solvent free and environmentally friendly procedure by using $Mg(ClO_4)_2$. Products were obtained with excellent yields and short reaction time under mild reaction condition from 1,3-diketone and substituted hydrazine. Synthesized pyrazole derivatives were characterized by IR spectroscopic methods and NMR spectroscopy as mentioned in the experimental section. Data confirmed the synthesis of pyrazole moiety. Characteristics peak for N-N absorption was appeared in the range of 1122-1135 $cm^{-1}$and for C=N were appeared in the range of 1611-1636^{-1} cm^{-1}.

UV-Visible spectrum of pyrazole derivatives

UV-Visible spectrum of synthesized compound were scanned at both p^H (4.7 & 7.4), Fig. 1. The λ_{max} values at p^H 4.7 for 1A, 2A, 1B and 2B were 20.10 nm, 229.60 nm, 226.40 nm and 229.20 nm respectively. Similarly λ_{max} at p^H 7.4 for 1A, 2A,1B and 2B were 231.20 nm, 224.50 nm, 225.80 nm and 226.60 nm respectively. The spectra of 1A, 1B, 2B and 2A were also recorded at different concentrations in order to determine the extinction coefficient and shown in Fig. 2. Increase in the concentration has increased the absorbance by obeying Beer's law, Fig. 3. The molar extinction coefficients were calculated and their values are displayed in Table 1. These values depicted n- π* transition in 1A and 2A while π- π* transition in 1B and 2B.

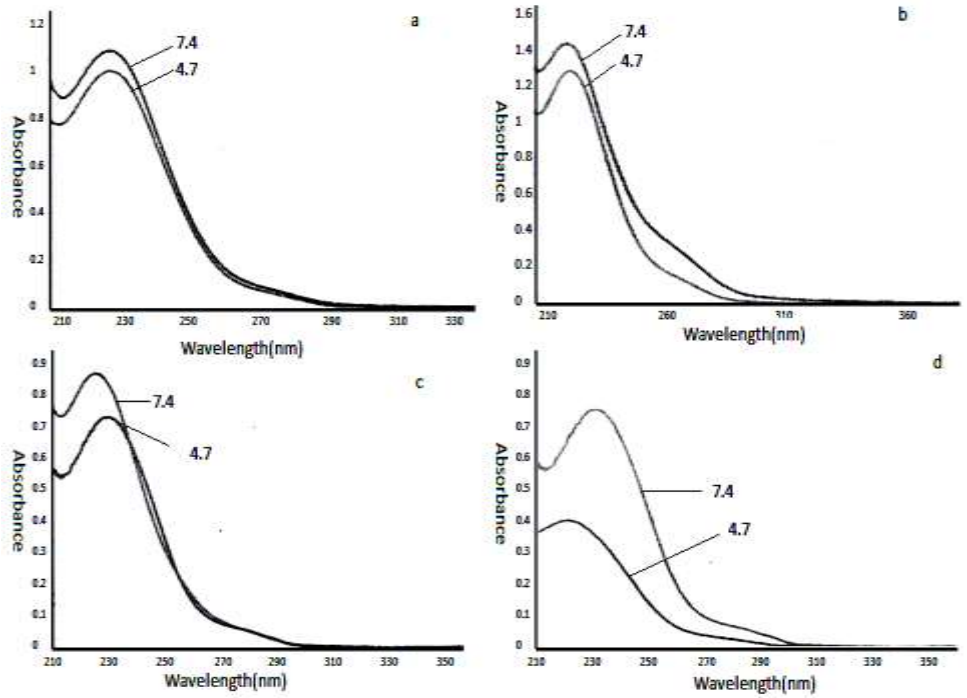


Fig. 1 UV- spectra of the synthesized compounds at p^H 4.7 and 7.4 at concentration of 10×10^{-6} M. (a) 1A, (b) 1B, (c) 2A, (d) 2B

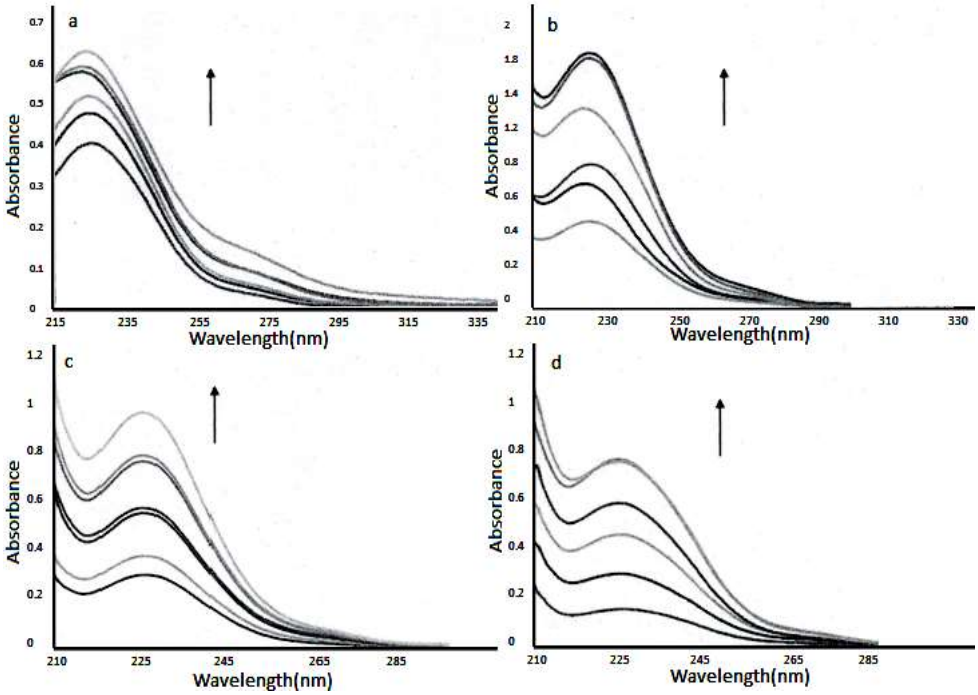


Fig. 2 UV- spectra of the synthesized compounds at different concentrations (µM). (a) 1A, (b) 2A, (c) 1B, (d) 2B

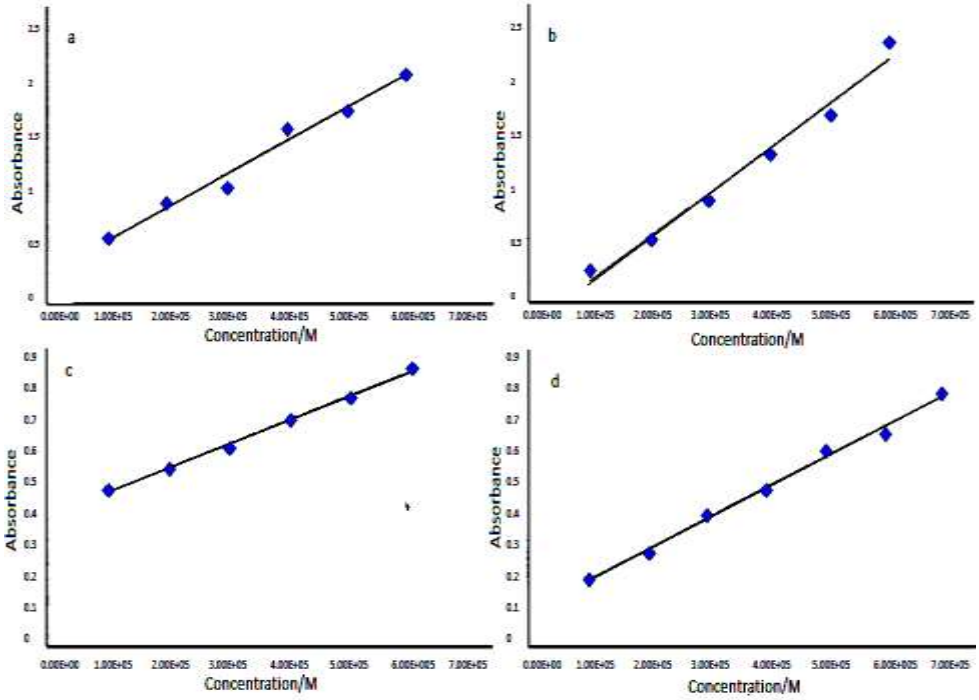


Fig. 3 Concentration profiles of the synthesized pyrazole derivatives (a) 1A, (b) 2b, (c) 2A, (d) 1B

Table 1
Molar extinction coefficients of the synthesized compounds

Compounds	Ɛ ($M^{-1}cm^{-1}$)
1A	8864
2A	7080
1B	26780
2B	36780

DNA binding studies by UV-Visible spectroscopy

Interaction of DNA with synthesized pyrazole derivatives was studied by the UV- visible spectral studies. UV-Visible spectral changes with various concentrations of the compounds with fixed concetration of DNA led to determine interaction and binding mechanism of pyrazole derivatives with the DNA.The UV-Visible spectra of all the compounds were recorded under physiological conditions of pH and temperature.

The effect of varying concentration of DNA (10-50 µM) on the electronic absorption spectra of 10×10^{-6} M of 1A, 2A,1B and 2B at p^H 4.7 (0.1 M acetate buffer) and 7.4 (0.1 M phosphate buffer) at 37 ºC were studied and shown in Fig. 4 & 5. At p^H 4.7, the binding of 1A, 2A, 1B and 2B to DNA caused progressive blue shifts of magnitude 1.7 nm, 1.2 nm, 0.5 nm and 1.2 nm, respectively, upon the addition of 50 µM DNA along with the hypochromic effect (decrease in absorbance peak intensity). A progressive blue shift was also observed at p^H 7.4 with magnitude of 2.5 nm, 1.12 nm, 1.9 nm and 3.4 nm respectively for 1A, 2A, 1B and 2B by the addition of 50 µM DNA along with hypochromic effect.

The hypochromic effect indicated the interaction of the electronic state of the intercalating chromophore of compounds and those of the stacked base pairs of ds-DNA. The blue shift could be directly linked with the improper coupling (due to conformational changes) of π*- orbital of intercalated drug with the π-orbital of the base pairs. Furthermore, the spectral changes at stomach p^H 4.7 and at blood p^H 7.4 were characterized by one isosbestic point for all compounds except 2B at p^H 7.4 on titration with DNA. So we may rule out the presence of the species other than the free and

intercalated compound. The presence of these isosbestic points indicated that there is an equilibrium between bound DNA and the free form of compounds and suggestive of intercalation of the compounds into the DNA base pairs. For compound 2B, though no isosbestic point was observed at blood pH; hypochromic effect along with blue shift may be inferred intercalation between compound and ds-DNA.

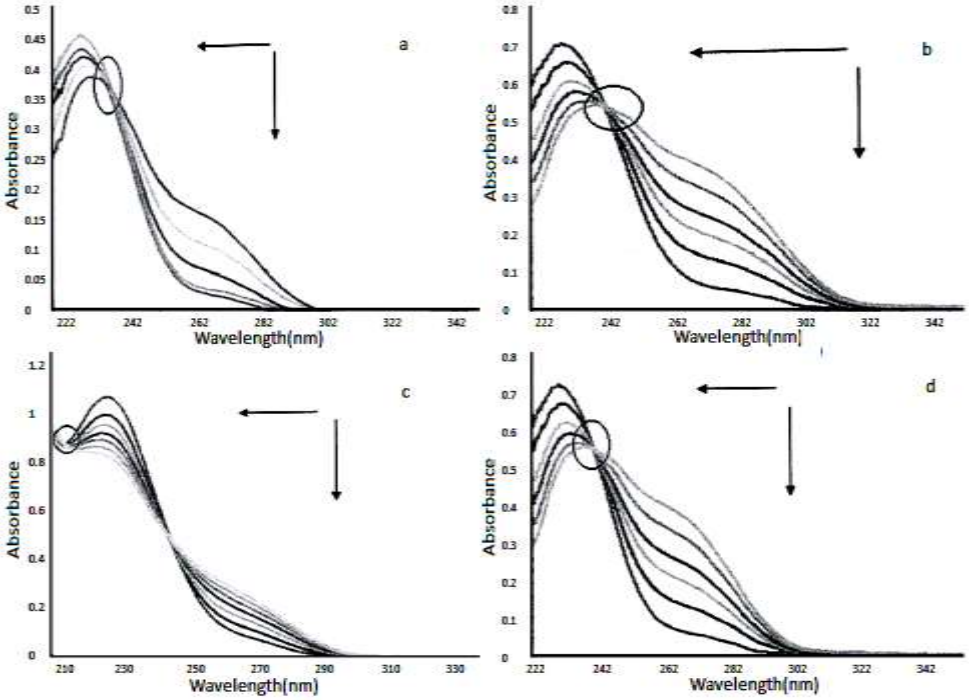


Fig.4 UV- spectra of compounds (10×10^{-6} M) in the absence and presence of different concentrations of DNA at p^H 4.7. (a) 1A, (b) 2A, (c) 1B, (d) 2B

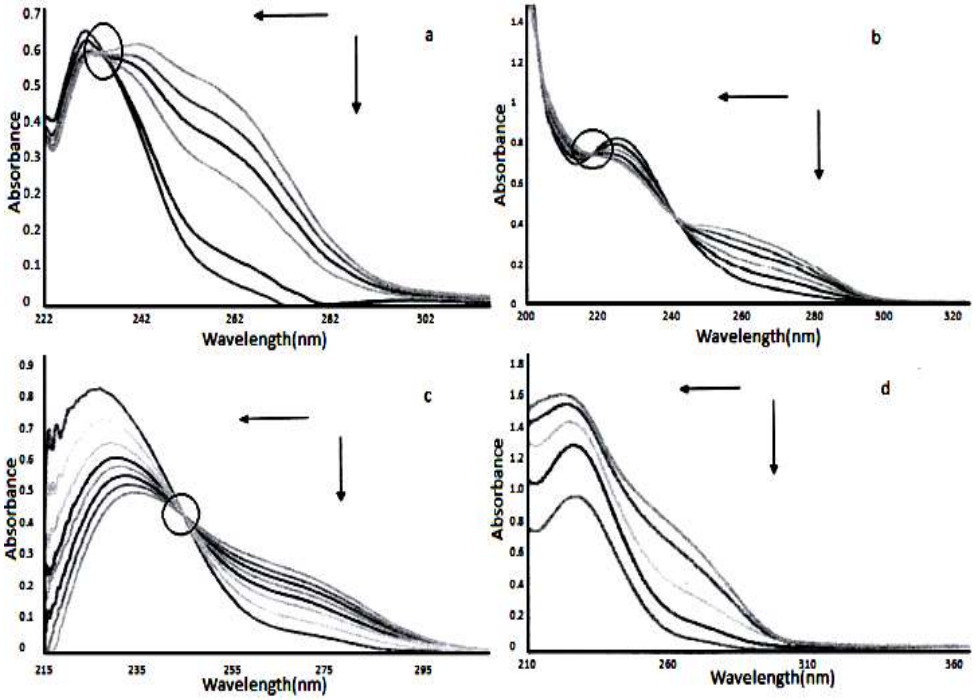


Fig.5 UV- spectra of compounds (10×10^{-6} M) in the absence and presence of different concentrations of DNA at p^H 7.4. (a) 1A, (b) 2A, (c) 1B, (d) 2B

Variation in the absorbance values of the compound measured spectrophotometrically after the addition of ds-DNA could be further used in Benesi-Hildebrand equation to calculate the binding constant "K_b" of compound–DNA complex.

$$\frac{A_o}{A-A_o}=\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}+\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K_b[DNA]}$$

Where A_o and A are the absorbance of free compound and DNA bound complex respectively, ε_G and ε_{H-G} the molar extinction coefficients of free compound and DNA bound complex respectively. From the plot of $A_o/(A- A_o)$ to $1/[DNA]$, the ratio of the intercept to the slope gave the value of binding constant "K_b". Plots for "K_b" calculations are given in Fig. 6 & 7 at p^H 4.7 and 7.4, respectively and binding data is tabulated as Table 2.

Table 2
Binding constants data from UV-Visible spectroscopy for compound – DNA binding at p^H 4.7 and 7.4 and at 37 °C

Compounds	K_b (M^{-1}) p^H 4.7	K_b (M^{-1}) p^H 7.4
1A	1.17×10^4	4.08×10^4
1B	2.38×10^3	1.13×10^4
2A	8.03×10^3	1.15×10^4
2B	2.67×10^4	5.32×10^4

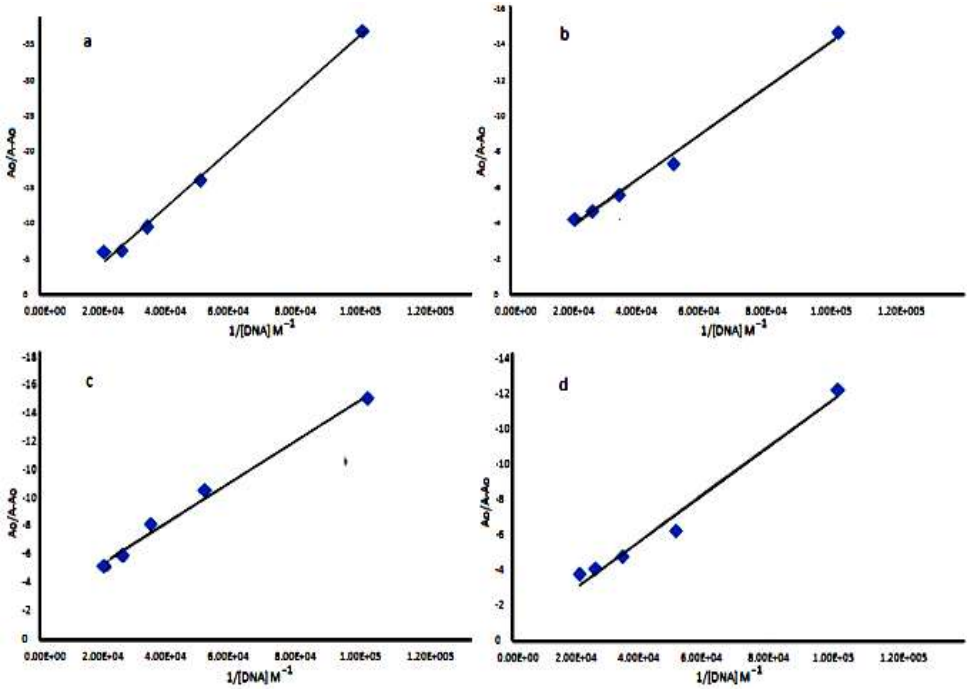


Fig. 6 Plot of $A_o/A\text{-}A_o$ *vs. 1/[DNA]* M^{-1} for the application of Benesi-Hildebrand equation for the calculation of DNA binding constant at p^H 4.7. (a) 1A, (b) 2A, (c) 1B, (d) 2B

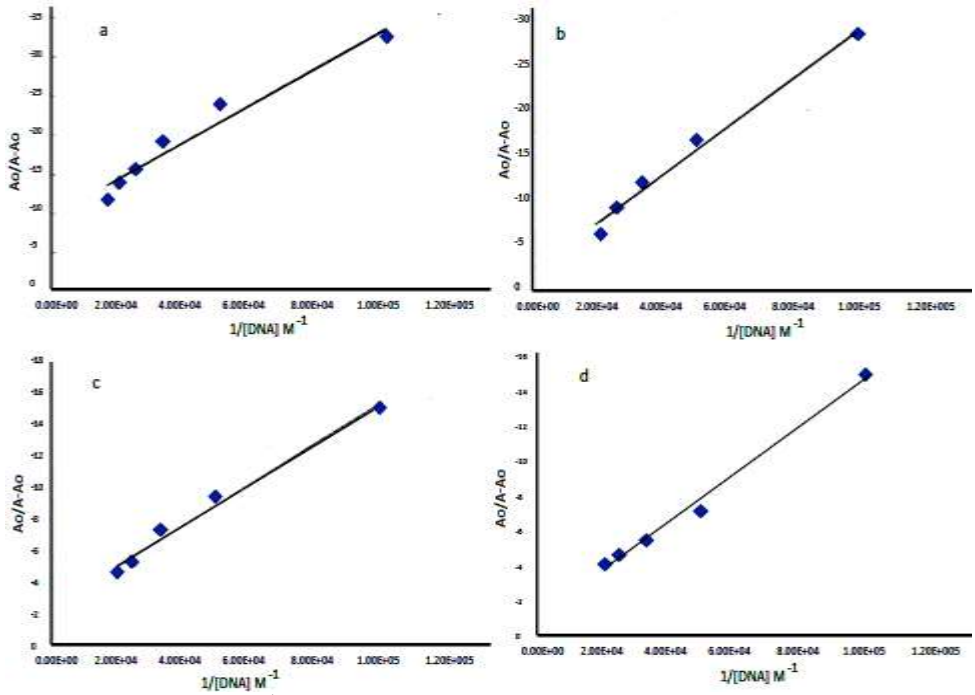


Fig.7 Plot of $A_o/A\text{-}A_o$ *vs. 1/[DNA]* M^{-1} for the application of Benesi-Hildebrand equation for the calculation of DNA binding constant at p^H 7.4. (a) 1A, (b) 2A, (c) 1B, (d) 2B

From the values of binding constant, K_b**,** free energy change (ΔG) of compound–DNA complex was calculated, using the following Van't Hoff's equation. Binding constant describes the compound-DNA complex stability while free energy change indicates the spontaneity/non- spontaneity of compound–DNA binding.

$\Delta G = -RTIn\ K_b$

Free energies of all the compounds at both p^H values were evaluated as negative values showing the spontaneity of compound–DNA interaction, Table 3. However binding constant and free energy changes are affected by the change of p^H. Greater values of *"K_b"* and more negative values of "ΔG" at p^H 7.4 indicated comparatively better binding of the compounds with DNA at blood p^H. Among all the compounds 2B showed greater binding parameters at both p^H.

Table 3
Free energy changes of complexes of 1A, 2A, 1B and 2B with ds-DNA at pH 4.7 and 7.4 and at 37 ^oC

Compounds	ΔG (kJmol^{-1})	ΔG (kJmol^{-1})
	P^H 4.7	P^H 7.4
1A	-24.14	-27.36
2A	-20.03	-24.05
1B	-23.17	-24.09
2B	-26.26	-28.04

Viscometric studies

DNA binding studies of compounds can be verified by viscosity measurement. Usually the viscosity of DNA solutions increases upon the addition of various concentrations of a compound if it accommodates within the DNA base pairs and cause lengthening of DNA helix. This rise in viscosity clearly verifies that a compound either completely or partially intercalated within DNA base pairs. Conversely, if the drop in DNA viscosity is observed upon compound's addition, the factor of thermal denaturation of DNA may arises.

The DNA viscosity at its fixed concentration was measured by before and after the addition of varying compound's concentrations, separately, at p^H 4.7 and 7.4. A graph is then plotted between $(\eta/\eta_o)^{1/3}$ (cube root of relative specific viscosity) {where, η and η_o are DNA viscosity in the absence and presence of the compound, respectively} and compound / DNA concentration ratio, Figs. 7 (p^H 4.7) & 8 (p^H 7.4). A linear rise in relative specific viscosity of the DNA solution in the presence of each compound's concentrations, separately, verified the lengthening of DNA duplex due to insertion of compound's planner portions into the DNA base pairs. This enhancement in viscosity showed that all the investigated compounds have structures that intercalated within DNA base pockets and further supporting the spectroscopic findings. Since the media used in present work are aqueous and viscosity rise was observed in the presence of compound's concentrations; the possibility of DNA denaturation, as usually observed in non-aqueous media, could be ruled out (Farooqi et. al., 2018).

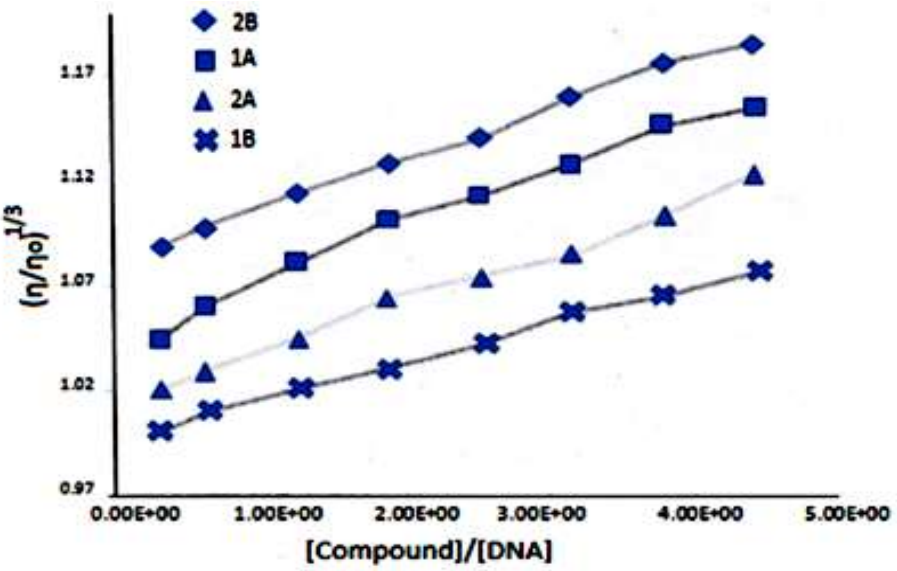


Fig.7 Plot of relative specific viscosity *vs.* [compound]/ [DNA] for 1A, 2A, 1B and 2B at p^H 4.7 and at body temperature 37 °C

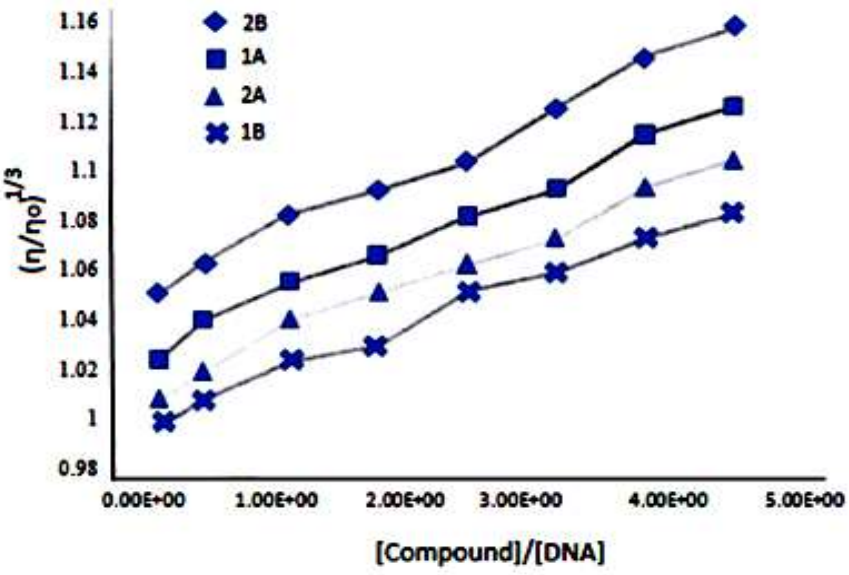


Fig. 8 Plot of relative specific viscosity *vs.* [compound]/ [DNA] for 1A, 2A, 1B and 2B at p^H 7.4 and at body temperature 37 °C

Cytotoxic activity

Brine shrimp cytotoxicity assay was performed to screen out the biologically active compounds. Brine shrimp lethality assay is a pre screen assay to test antitumor potential of any compound. The cytotoxicity was studied by the brine-shrimp lethality method and the results are summarized in Table 4. The LD_{50} data showed that 1A and 2B has least LD_{50} values than that of other two compounds, hence showing greater cytotoxic activity.

Table 4
Cytotoxic activity of compounds

Compound	Number of shrimps killed				LD_{50} (μg/mL)
	200 (μg/mL)	66.6 (μg/mL)	22.2 (μg/mL)	7.4 (μg/mL)	
1A	8	6	3	3	170
1B	5	2	1	1	897
2A	6	4	2	1	371
2B	8	3	2	0	171

Antibacterial activity
The compounds were screened for their antibacterial activity against six bacterial strains by using disc diffusion method. Cefexime and Roxithromycin were used as positive control and each experiment was performed in triplicate. The results of antibacterial activities in the form of MIC (minimum inhibitory concentration) are summarized in Table 5. All of the compounds showed varying degree of antibacterial activity with MIC values of 150 and 200 μgL^{-1} against different bacterial strains

Table 5
Antibacterial activity of compounds

Compounds	Minimum Inhibitory Concentrations (MIC μgL^{-1})					
	S.aureus	E.coli	B.subtilis	S.typhi	B.bronchi	E.aerogenes
1A	200	200	200	-	200	200
1B	150	200	200	200	200	200
2A	150	200	200	200	200	-
2B	200	200	200	200	-	200

DPPH free radical scavenging activity

DPPH free radical scavenging activity of the compounds was evaluated spectrophotometrically at different concentrations i.e., 200 μg/mL, 66.6 μg/mL, 22.2 μg/mL, 7.4 μg/mL and 2.4 μg/mL and the results are summarized in Table 6. Discoloration of DPPH indicates the scavenging potential of the antioxidant activity of a compound. DPPH assay measures lightening of DPPH color after reacting with the synthesized compounds spectrophotmetrically at 517 nm. The absorbance of DPPH at 517 nm decreases as color changes from purple to yellow due to scavenging of the free radical by the antioxidant compound. In this assay none of the synthesized compounds showed significant DPPH free radical scavenging activity at the concentrations tested. All the compounds have IC_{50} value > 200 μg/mL.

Table 6
Percentage scavenging of pyrazole derivatives at different concentrations

	%age scavenging of at different concentrations					
Compounds	200 (μg/mL)	66.6 (μg/mL)	22.2 (μg/mL)	7.4 (μg/mL)	2.4 (μg/mL)	IC_{50} (μg/mL)
1A	25	10	0	0	0	>200

2A	27	15	10	0	0	>200
1B	15	2	0	0	0	>200
2B	20	10	1	0	0	>200

Conclusions

Four pyrazole derivatives 1A, 2A, 1B and 2B were synthesized and investigated for their binding studies with DNA by UV-Visible spectroscopy and viscometric technique at physiological conditions of pH (stomach p^H 4.7 and blood p^H 7.4) and at body temperature (37 ^oC). The results obtained from these studies indicated that all the compounds interacted with DNA through intercalation predominantly. K_b and ΔG values were calculated for all the synthesized compounds – DNA complexes which revealed their comparatively greater binding and spontaneity at blood p^H. Among all the compounds 2B showed comparatively greater binding at both p^H. Cytotoxic activity of 1A and 2B was found significant as compared to 2A and 1B. Varying degree of antibacterial activity (MIC; 150 - 200 μgL^{-1}) against different bacterial strains showed significant antibacterial activity of all the compounds, while these compounds were non- significant for antioxidant activity.

Author(s) Contribution Statement Nasima Arshad conceived the idea and wrote the manuscript. Muhammad Rashid performed experimental studies. Naghmana Rashid provided chemicals for DNA binding studies and their characterization data. Nadia Riaz helped in manuscript writing. Bushra Mirza helped in biological studies.

Conflict of Interest Authors declare that they have no conflict of interest.

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References

Ahmad, M. S., Hussain, M., Hanif, M., Ali, S., Qayyum, M., & Mirza, B. (2008). Di-and Triorganotin (IV) Esters of 3, 4-Methylenedioxyphenylpropenoic Acid: Synthesis, Spectroscopic Characterization and Biological Screening for Antimicrobial, Cytotoxic and Antitumor Activities. *Chemical biology & drug design*, *71*(6), 568-576.

Aragade, P., Kolhe, S. ,Kamble, H. 1.,Baheti, D., &Maddi, V. (2012). Synthesis and Preliminary Evaluation of Some Substituted Pyrazoles as Anticonvulsant Agents. *International Journal of Drug Design and Discovery*, *3*(1), 688-693.

Babkina, S. S.,&Ulakhovich, N. A. (2005). Complexing of heavy metals with DNA and new bioaffinity method of their determination based on amperometric DNA-based biosensor. *Analytical chemistry*, *77*(17), 5678-5685.

Bhat, M., &Belagali, S. L. (2018). Synthesis, characterization and biological screening of pyrazole-conjugated benzothiazole analogs. *Future medicinal chemistry*, *10*(1), 71-87.

Dias, L. R. S., & Salvador, R. R. S. (2012). Pyrazolecarbohydrazide derivatives of pharmaceutical interest. *Pharmaceuticals*, *5*(3), 317-324.

Eweas, A. F., El-Nezhawy, A. O. H., Abdel-Rahman, R. F.,& Baiuomy, A. R. (2015). Design, Synthesis.In Vivo Anti-inflammatory, Analgesic Activities and Molecular Docking of Some Novel Pyrazolone Derivatives. *Medicinal chemistry*, *5*(10), 458-466.

Kumar, G. Tanwar, O. Kumar, J. Akhter, M. Sharma, S. Pillai, S.R. Alam, M.M. &Zama, M.S.(2018).Pyrazole-pyrazoline as promising novel antimalarial agents: A mechanistic study. European journal of medicinal chemistry, 149,139-147.

Kumar, R. S., Arif, I. A., Ahmad, A., & Idhayadhulla, A. (2016). Anti-inflammatory and antimicrobial activities of novel pyrazole analogues. *Saudi journal of biological scienes. 23*(05), 614-620.

Lu, Y. Ran, T. Lin, G. Jin, Q. Jin, J. Li, H. Guo, H. Lu, T. & Wang, Y. (2014). Novel 1H-pyrazole-3-carboxamide derivatives: synthesis, anticancer evaluation and identification of their DNA-binding interaction. *Chemical and Pharmaceutical Bulletin*, *62*(3), 238-246.

Nitulescu, G. M., Draghici, C., & Olaru, O.T. (2013). New Potential Antitumor Pyrazole Derivatives: Synthesis and Cytotoxic Evaluation. *International journal of molecular science, 14* (11), 21805-21818.

Reichmann, M. E., Rice, S. A., Thomas, C. A., & Doty, P. (1954). A further examination of the molecular weight and size of desoxypentose nucleic acid. Journal of the American Chemical Society, 76(11), 3047-3053.

Sambrook, J., Fritsch, E.F., &Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Nova York.

Sun, J. & Zhou Y.(2015). Synthesis and Antifungal Activity of the Derivatives of Novel PyrazoleCarboxamide and IsoxazololPyrazole Carboxylate. *Molecules, 20*(3), 4383-4394.

Yu, L.G., Ni, T.F., Gao, W., He, Y., Wang, Y.Y., Cui, H.W., Yang, C.G., & Qiu, W.W. (2015). The synthesis and antibacterial activity of pyrazole-fused tricyclic diterpene derivatives, *Eurpean Journal of Medicinal Chemistry*, *90*, 10-20.

Farooqi, S.I., Arshad, N., Channar, P.A., Perveen, F., Saeed, A., Larik, F.A.,& Javeed, A.(2018) Synthesis, theoretical, spectroscopic and electrochemical DNA binding investigations of 1, 3, 4-thiadiazole derivatives of ibuprofen and ciprofloxacin: Cancer cell line studies, *Journal of Photochemistry and Photobiology B: Biology, 189*, 104-118.