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STRUCTURE OF MATTER AND QUANTUM CHEMISTRY

Physicochemical Study of Some Thiobarbiturate Derivatives and Their Interaction with DNA in Aqueous Media[1]

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Abstract—The aim of this study is to investigate the physicochemical behavior of some selected thiobarbituartes and explore their interaction with ds-DNA. To the best of our knowledge limited work has been reported on physicochemical investigation of these medicinal compounds and their interaction with ds-DNA. Therefore, this lack of information on the fundamental physicochemical studies of such medicinal compounds needs to be addressed. For this purpose five thiobarbiturate derivatives, denoted as: MKA 25, MKA26, MKA27, MKA28, MKA29, have been synthesized and characterized. Various physicochemical investigations of these thiobarbiturates are carried out. The thermal decomposition of these medicinal compounds was also studied by TGA technique. Further the mode of interaction and binding of these newly synthesized compounds with ds-DNA was carried by using UV–Vis spectrometry. It was seen from the overall results that the physicochemical properties as well as the extent of their interaction with biopolymer not only depend on the side alkyl groups but also on varying the functional groups of the samples.

Keywords: thiobarbiturates, physicochemical study, TGA, DNA-binding, interaction, UV–Vis spectrometry
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INTRODUCTION

The physicochemical investigation and binding study of small molecules to DNA is important from both the academic and applied point of view. As the binding of conventional molecules to DNA affects various functions of living organisms, such small molecules have potential as therapeutic agents that function by controlling gene expression. Hence such investigation can provide a key in the chemical and biological drug discovery research [1]. The main cause of many genetic diseases are DNA mutation. DNA is genetic and heredity material which is responsible for the coding of proteins. The medicinal properties of thiobarbiturates can be traced from its specificity, potency and interaction with DNA. Those compounds which containing pyrimidine ring play an important role in many medicinal fields. These rings have binding sites for many metal ions, so barbiturates are medicinal compounds and used for the treatment of many such like diseases [2]. Recently barbiturates are mainly replaced by benzodiazepines [3]. The general preparation method of barbituric acid is given in Fig. 1.

Barbiturates have different solubility in various solvents, which depends on their structure, which can also affect their in vivo properties. 2,4,6-Pyrimidinetrione ring is hydrophilic, and 5,5-substituents are lipophilic [5]. Barbiturates are the medicinal compounds that generally depress muscular function [6]. Phenobarbital (5-ethyl-5-phenylbarbituric acid) is an example of a widely used barbiturate drug. Barbiturates may also possess some other types of biological activity [7].

To enhance the biomedical applications of barbiturates, some derivatives of barbiturates are synthesized. Of the various derivatives, thiobariturates are very important from potential biomedical point of view. Thiobarbiturates have a sulfur atom in the place of the carbonyl oxygen at C2 position. The replacement of the carbonyl oxygen by sulfur at the C2 position favors fat solubility and decrease duration of action. Thiobarbiturates are more efficient in their toxicity to micro organism as compared to oxobarbiturates. The thiobarbiturates may be used as antiviral, antifertility, and antimalarial agents. The thiobarbiturates have antibacterial, antifungal and anti-inflammatory applications, therefore their characterization is very important. Some thiobarbiturates can be used for the treatment of malaria and many other diseases. They

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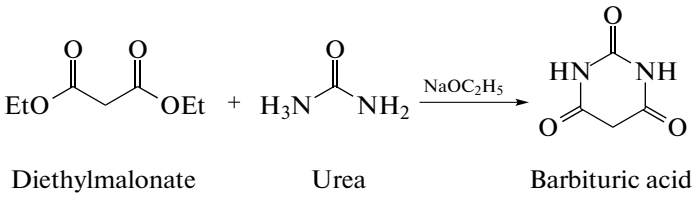


Fig. 1. General preparation method barbituric acid.

may also posess antiallergic, antiviral, and anti tumor activity [8]. Thiobarbiturates have sedative and antibiotic applications. DNA is a macromolecule and active for interaction with thiobarbiturates and other medicinal compounds. DNA-drug interaction involves non covalent bonds and this non covalent bond can be divided into three categories i.e., (i) groove binding, (ii) intercalation, (iii) non specific electrostatic surface binding. The interaction of DNA with potential medicinal compounds can be affected by the concentration, pH, temperature, and the presence of electrolytes.

The physicochemical investigation of barbiturates, which is expected to have medicinal properties, is very important to understand their physicochemical behavior under the influence of various factors, such as temperature, pH, and their kinetic studies. The interaction of such compounds with DNA can be governed by Van der Waals, hydrophobic, and electrostatic forces, from which the interaction mechanism can be traced out. The binding strength between dsDNA and medicinal compounds can be affected by replacing the different alkyl group. The use of computational approach can be used to understand the binding mechanism. The steric effects has also important role in the binding [9]. The thiobarbiturates and their derivatives have great sites for hydrogen bonding, and lastly binding between thiobarbiturates and DNA supposed to be inhibited. Different mode of interactions, such as surface binding, groove binding and intercalation between dsDNA and small molecules can occur [10].

From the biochemical and clinical point of view, the binding of DNA with small molecules is very important. Cancer is one of the main diseases which can be prevented by understanding such type interactions. Thiobarbiturates can be used for anesthesia and brain disease, they also effect muscular system [11]. Thiobarbiturates are biologically active substances and they interact with double stranded DNA and this interaction is mostly of physical type [12]. The interaction between DNA and thiobarbiturates is important because it give us the response of thiobarbiturates and its applications. By screening the available literature, it was seen that less work has been reported on physicochemical study of the thiobarbiturates derivatives and their interactions with DNA. Therefore, by keeping all these facts in mind, five new thiobarbiturate derivatives denoted as MKA20, MKA21, MKA22, MKA23, and MKA24, have been synthesized, physicochemically characterized and their interaction with DNA are studied in detail. This research work will help to understand the physicochemical behavior of thiobarbiturates derivatives and explore their interaction with DNA.

EXPERIMENTAL

Materials

The reagents used in this research study were of analytical grade and obtained from Sigma Aldrich. These reagents include ethanol, ammonium chloride, potassium hydrogen carbonate, EDTA, and hydrochloric acid, which were purified before any experimental use. Thiobarbiturates and their derivatives were soluble in organic solvents but insoluble in water [13, 14]. Thiobarbiturates and their derivatives showed good solubility in the ethanol–water mixture (95% ethanol and 5% water), so this mixed solvent was used throughout in this project. The double distilled autoclaved water was also used for DNA experiments. The thiobarbiturates were prepared by already established method. The thiobarbiturates used were stable powders of different colors. All the DNA related stock solutions were stored below 5°C and used within 5 days.

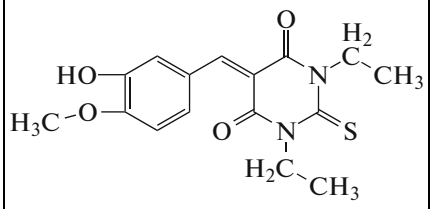
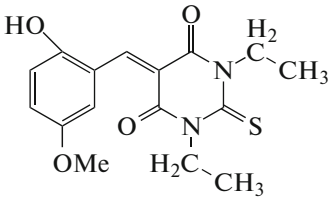
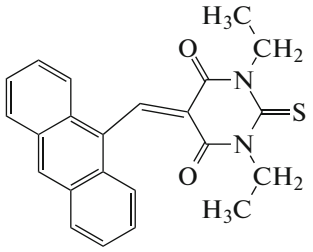
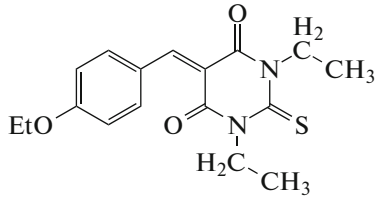
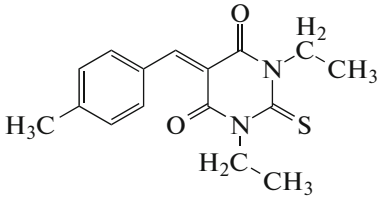
Preparation of Selected Thiobarbiturates Derivatives

All the selected thiobarbiturates and their derivatives were synthesized by the reaction of diethylthiobarbituric acid with different aromatic aldehydes in water. A typical reaction for a representative sample is given in Fig. 2. Further the diethylthiobarbituric acid (1 mmol) was mixed with the aromatic aldehydes (1 mmol) in the presence of 10 mL distilled water, and refluxed for about 30 min. Deuterated DMSO was used as solvent in NMR studies [14]. The structures and melting points of the prepared compounds are summarized in Table 1.

Physicochemical Characterization

The UV–Vis spectra were recorded on Shimazdu 1800 UV probe 2.43 version. The melting points of the selected thiobarbiturate derivatives were determined

Table 1. Main characteristics of the thiobarbiturates derivatives used in this study

Sample code	Chemical formula/molecular mass	Melting point, °C
MKA25	 $C_{16}H_{18}N_2O_4S/M = 334.1$	182
MKA26	 $C_{16}H_{18}N_2O_4S/M = 334.1$	172
MKA27	 $C_{23}H_{20}N_2O_2S/M = 388.12$	170
MKA28	 $C_{17}H_{20}N_2O_3S/M = 332.12$	150
MKA29	 $C_{16}H_{18}N_2O_2S/M = 302.11$	150

by using Stuart melting point apparatus SMP 10 (Bibbay Scientific). FT-IR spectra were recorded on IRAffinity-1S instrument (Shimadzu). Bomb calorimeter AC 600, Analysis Software v 1.21 (Leco, USA) was used to investigate the calorific values. Thermogravimetric analyzer (TGA) Shimadzu TGA 50 H was used to investigate the thermal properties of the samples. NMR spectra were recorded on Advance Bruker AM 300 and AM 400 instruments; deuterated DMSO was used as solvent.

RESULTS AND DISCUSSIONS

Melting Points of the Compounds Selected

The melting points of all samples and their possible structures are shown in Table 1. The melting points of these compounds lay in the range of 150–182°C. The variation in melting points for different sample is due to the presence of different alkyl groups on one hand and the functional groups on the other hand. It can be seen that varying side groups of the basic skeleton affect the melting points of these medicinal compounds.

Calorific Values Investigation of Thiobarbiturates Derivatives

The calorific values of the studied compounds are listed in Table 2. As seen from the table, the amount of sample taken for analysis was fixed for all samples and also the amount taken was less than a gram. For all samples the average the calorific values are almost six thousands calorific value per gram while the amount of sample used is quite less. From calorific values of these thiobarbiturate derivatives, we concluded that the sample having more the number of carbons and bonds showed greater calorific values. This change in heat energy in terms of calorimetric values with change in the molecular and structural nature of the samples is a step forward toward their better understanding from biomedical point of view.

Thermogravimetric Analysis

In order to study the thermo-kinetic behavior of a solid sample, thermogravimetric analysis (TGA) is one of the important technique which gives valuable information. There are two different ways to perform a TGA experiment. The thiobarbiturates are stable at room temperature and can be used and stored for many months without any physical changes [15]. TGA can be applied in quality controls and new developments in pharmaceutical industry [16]. Thermogravimetric analysis provides valuable information about the phase transition, stability, polymorphism and kinetic parameters of thiobarbiturates and their derivatives The aim of TGA analysis was to study the stability and decomposition pattern of selected thiobarbituratic compounds [17]. A representative thermogram

Table 2. Calorific values of thiobarbiturates and their derivatives

No.	Sample code	CV, cal/g
1	MKA25	5564.4
2	MKA26	5751.8
3	MKA27	6071.3
4	MKA28	5677.5
5	MKA29	5471.5

Mass is equal 0.60 g, fuse length is equal 10.0 cm.

Table 3. Thermogravimetric analysis results for selected thiobarbiturates and their derivatives

No.	Sample	T_0, °C	T_1, °C	Δm, mg	Δm, %
1	MKA25	205.03	346.38	23.470	100.6
2	MKA26	88.63	252.82	48.385	1.273
3	MKA27	32.76	401.16	88.073	0.576
4	MKA28	40.15	350.65	8.00	95.20
5	MKA29	190.44	319.81	4.406	98.397

T_0 is starting point, T_1 is finishing point, Δm is weight loss.

for sample MKA29 is shown in Fig. 3, similar thermograms were also obtained for other samples (which are not shown here), while the resultant TGA data are given in Table 3.

In summary, the MKA25 started the weight loss at 205.03°C and finished at 346.38°C. For this analysis we took an amount of about 25 mg of the concerned sample, and 100 wt % was noted. MKA25 showed decomposition and loss of various species at different heating stages. The thermal pattern of MKA26 was also studied in term of stability, this sample started weight loss at 88°C, and the weight loss before 100°C is occurred due to the removal of water molecules. The weight loss of MKA26 finished at 252.82°C and about 48.385 wt % loss was recorded. So this data show that the concerned compound is less stable as compared to other samples, however 50% of this compound is decomposes at final temperature. In case of sample MKA27 the weight loss started at 32.76°C and completed at 401.09°C. The loss of weight from 32.76–100°C is the indication of loss of water molecules associated with this sample. It can also be concluded that this sample is more hydrophilic as compared to others. From the thermogram of MKA27, we can conclude that this sample is more stable till higher temperature compared to other selected samples. Likewise, the thermal pattern and decomposition of MKA28 is almost similar to that of MKA25. The thermal result for MKA29 obtained showed that the this sample is thermally stable in the temperature range of 190.44 to 319.81°C. For this purpose we took about 5 mg of sample, after completion of analysis 4.604 mg of weight loss occurred, and 98.397% weight loss was noted. The decomposition pattern of MKA29 at different stages is shown in Table 3 and Fig. 3.

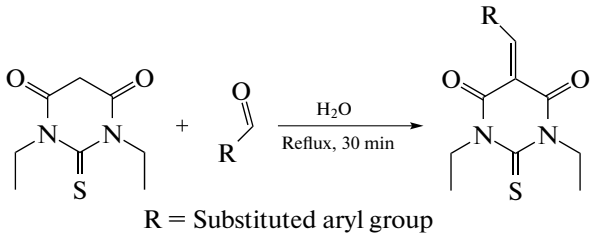


Fig. 2. Synthesis of 5-Arylidene-*N, N*-diethylthiobarbituric acid derivatives.

DNA-Thiobarbiturates Interaction and Their Binding Study

Another important part of this project is to study the interaction of dsDNA with thiobarbiturates derivatives. Looking to the chemical nature of these compounds, it was expected that UV–Vis spectroscopy may be the more valuable and easier technique to investigate the DNA–thiobarbiturates interactions. The maximum absorbance (λ_{max}, nm) values of selected thiobarbiturates have been studied by UV–Vis spectrophotometry (Shimadzu 1800 UV probe 2.43 version). All the selected samples showed good solubility in the ethanol water mixture (95% ethanol and 5% water), so this solvent mixture was used as reference. UV–Vis spectra shows that different samples of thiobarbiturates showed different maxima (peaks) in the spectra, the maximum absorption wave length (λ_{max}) is very important for this study, as it is used as spectral tracer of thiobarbiturates–DNA interactions. Representative plots for maximum absorbance (λ_{max}) and a typical calibration curve of absorbance for MKA25 are shown in Fig. 4. Similarly, UV–Vis spectra of all thiobarbiturate samples in the presence of different concentration of DNA are given in Figs. 5–9. The important spectroscopic parameters extracted from these measurements are summarized in Table 4.

The selected compounds showed different response and behavior in spectroscopic study. A shift of an absorption maximum to a longer wave length is called red shift or bathochromic effect, while a shift to shorter wavelength is called hypsochromic shift [18]. The present thiobarbiturate derivatives showed both or one of them upon the addition of solution of DNA, samples, MKA25, MKA26, MKA27, and MKA29 showed decrease in wave length (λ_{max}) of 2, 6, 2, and 5 nm, respectively, while MKA28 show increase in wavelength of 6 nm. Further, MKA27 and MKA28

Table 4. UV–Vis spectroscopy data for free thiobarbiturates and DNA–thiobarbiturates complexes (A_{max} is maximum absorbance, ε is molar absorptivity coefficient)

Sample	Free thiobarbiturates			Thiobarbiturates–DNA complex			
	λ_{max}, nm	A_{max}	ε, $(mol\ L^{-1})^{-1}\ cm^{-1}$	λ_{max}, nm	A_{max}	ε, $(mol\ L^{-1})^{-1}\ cm^{-1}$	K_b, $(mol\ L^{-1})^{-1}$
MKA25	432	0.012	6.47×10^1	430	0.016	8.63×10^1	1.11×10^2
MKA26	363	0.341	1.84×10^3	369	0.225	1.21×10^3	5.1×10^2
MKA27	362	0.193	1.042×10^3	364	0.239	1.29×10^3	4.1×10^2
MKA28	411	0.136	7.34×10^2	417	0.071	3.83×10^2	1.7×10^2
MKA29	366	0.088	4.75×10^2	361	0.156	8.42×10^2	2.6×10^2

showed decrease in maximum absorption (A_{max}). So this blue shift and hypochromic effect is the indication of penetration of benzyl group occurred, which results in the formation of H–π bond with the bases of DNA and finally the damage of the stands of DNA occurred [19]. MKA26 and MKA29 showed an increase in the absorbance after the addition of DNA, and the increase in the absorbance is known as hyperchromosim. Hence the hyperchromisim indicated that the concerned thiobarbiturates during intercalating caused the conformational changes in their respective rings of DNA. Further the addition of DNA to thiobarbiturates solutions, caused reduction in face to face base stacking and extended the DNA strand, and finally hyperchromisim occurred. The literature study suggested that when the unstaking of base pairs of DNA occurs, it results hyperchromisim [20]. The addition of DNA to thiobarbiturates solutions showed different response and behavior which give us the evidence of interactions. This is systematically and subsequently discussed here.

The UV–Visible spectroscopic response of MKA25 showed the maximum absorbance at λ_{max} = 432 nm. When we added the known amount of DNA, the wave length shifted to 430 nm. The absorbance increased from 0.012 to 0.016. So the free MKA25 has λ_{max} = 432 and absorbance at this point was 0.012. When we added the DNA, the blue shift of 2 nm occurred and the absorbance increased in this case. The compound MKA26 showed the maximum absorbance at 363 nm. The concerned compound has maximum absorbance of 0.341 at this wave length. When mixed with a known concentration of DNA, the wave length (λ_{max}) shifted to 369 nm and the absorbance at this point was recorded 0.225, which showed the decrease in absorbance. Thus finally this compound showed the red shift of 5 nm and decrease in its corresponding absorbance. Similarly, according to the spectroscopic response of MKA27, it has a maximum absorbance of 362 nm and the absorbance at this point was recorded 0.193. After addition of DNA an increase in both the λ_{max} = 2 nm and A_{max} = 0.239 was noted.

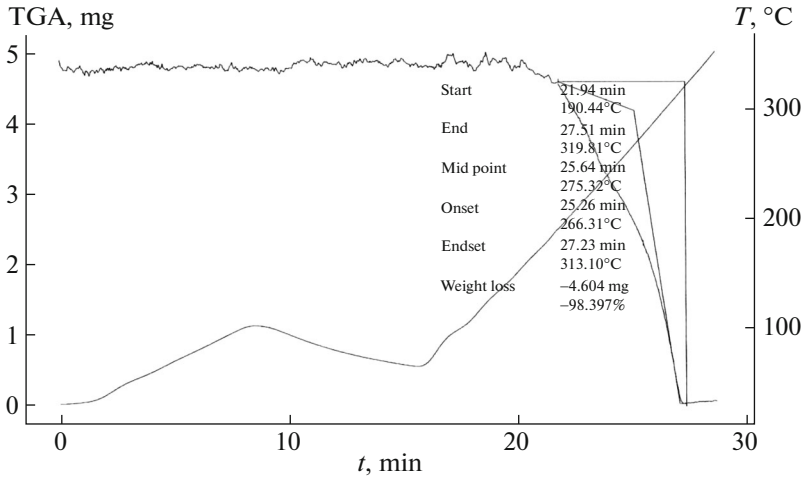


Fig. 3. Representative thermogram for sample MKA29.

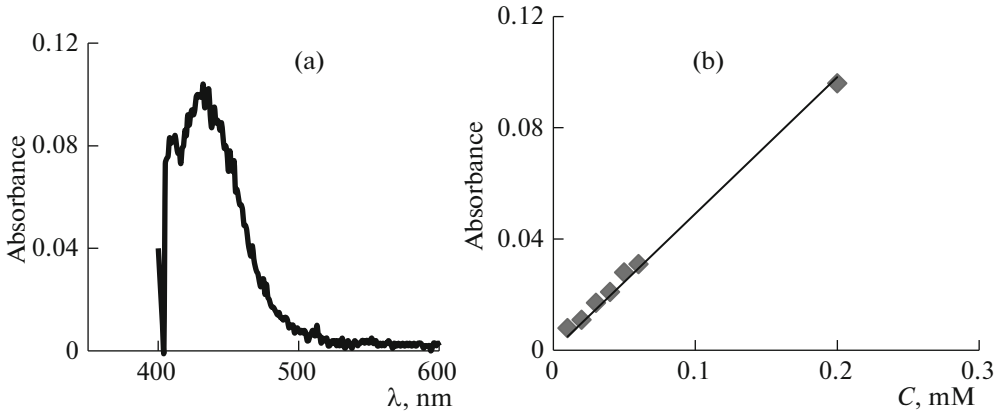


Fig. 4. Representative plots of maximum absorbance, λ_{max} (a) and calibration curves of absorbance (b) for MKA25.

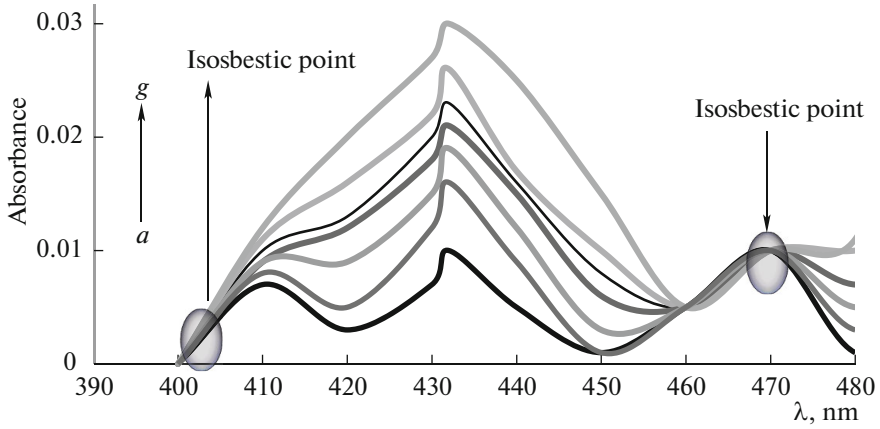


Fig. 5. UV-spectra of MKA 25 (0.01 mM dm^{-3}) in the absence and in the presence of different concentration of DNA. Concentration of DNA (1×10^{-6} mol dm^{-3}) added in mL: (*a*) 0, (*b*) 2, (*c*) 4, (*d*) 6, (*e*) 8, (*f*) 10, (*g*) 12.

This special response is again a prominent indication of the binding of this molecule with DNA. Likewise, the DNA free MKA28 compound has a maximum absorbance at 411nm and the absorbance at this wave length was 0.136. When DNA was added the λ_{max} shifted to 417 nm, which indicated the red shift of 6 nm. However the absorbance decreased to 0.071. So in this compound the bathochromic shift and hypochromism was observed in DNA–MKA28 mixed system. Also the spectroscopic results for DNA free MKA29 showed that this compound has λ_{max} = 366 nm and the absorbance at this point was 0.088. Upon the addition of DNA the compound showed the blue shift of 5 nm because the λ_{max} shifted to 361 nm. The absorbance at this point was 0.156, which reflects hyperchromic effect or increase in the absorbance.

From the overall results it is seen that the interaction of these thiobarbiturate derivatives with DNA showed different response and this is evidence of the interactions of thiobarbiturates with DNA. Also clear isosbestic points were observed in Figs. 5–9, where an isosbestic point is the wavelength at which all the absorbance become/remains constant for the whole reactions [21]. Further, the numbers and position of isosbestic points in the compounds-DNA mixed systems are changing, which is another physicochemical evidence of the fruitful interactions. All these results show that the compound where a red shifting was observed is the evidence of partial interaction between DNA and the compound. Due to these interaction the transition energy of π–π^* may be decreased and hence bathochromic shift occurred. The compounds where decrease in absorption (hypochromic effect) and blue shift in λ_{max} (decrease in λ_{max}) was observed, reflects stronger interaction with DNA. Subsequently this blue shift and hypochromic effect is the indication of penetration of benzyl group occurred, which results the formation of H–π bond with the bases of DNA and finally the damage of the stands of DNA may be occurred.

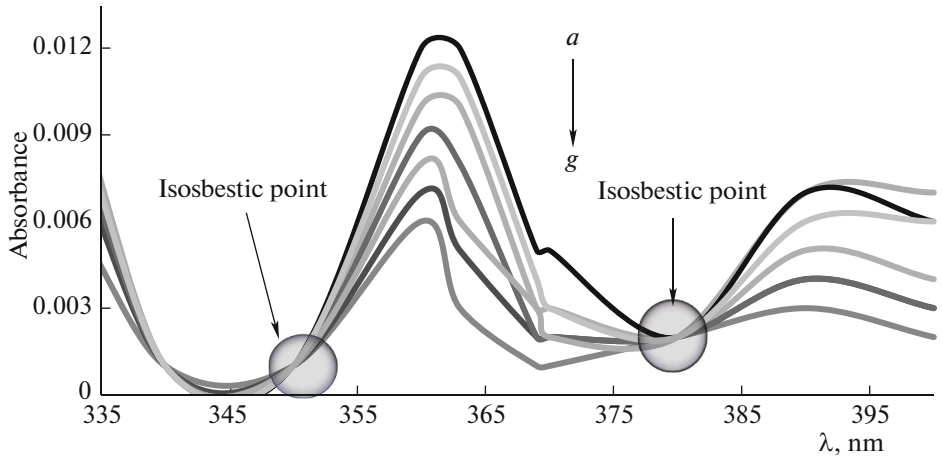


Fig. 6. UV-spectra of MKA26 (0.01 mM dm^{-3}) in the absence and in the presence of different concentration of DNA. Concentration of DNA (1×10^{-6} mol dm^{-3}) added in mL: (*a*–*g*) see Fig. 5.

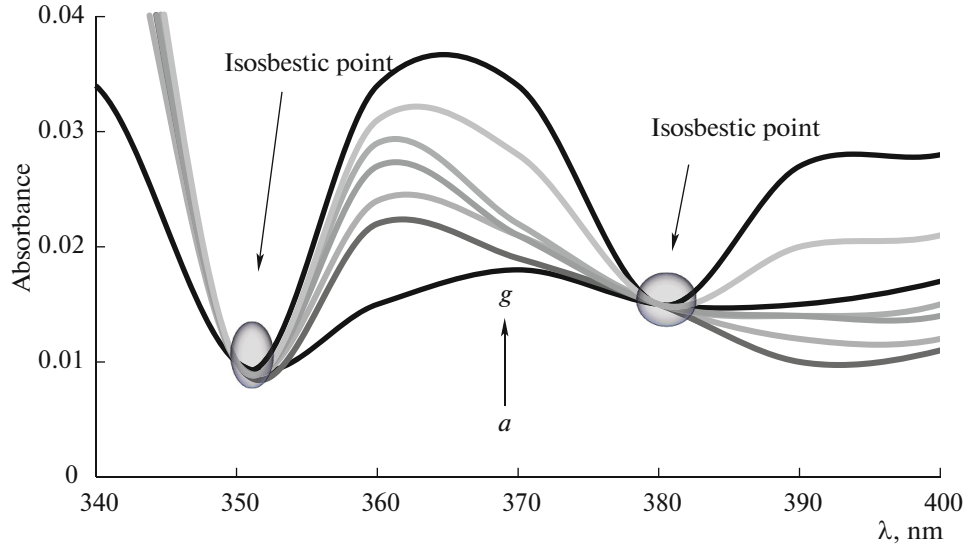


Fig. 7. UV-spectra of MKA27 (0.01 mM dm^{-3}) in the absence and in the presence of different concentration of DNA. Concentration of DNA (1×10^{-6} mol dm^{-3}) added in mL: (*a*–*g*) see Fig. 5.

It is expected that the interaction of small molecules such as thiobarbiturate derivatives with a host (macromolecule/DNA) results in a bigger complex, so, the formation of this complex can be assessed by the knowledge of binding or formation constant K_f/K_b. Therefore, the strength and type of interaction of DNA with thiobarbiturate derivatives was traced by calculating the binding constants (K_b) using the following Benesi–Hildebrand equation:

$$\frac{A_0}{A - A_0} = \frac{\epsilon_G}{\epsilon_{H-G} - \epsilon_G} + \frac{\epsilon_G}{\epsilon_{H-G} - \epsilon_G} \frac{1}{K[\text{DNA}]}, \qquad (1)$$

where K is binding constant, A_0 and A are the absorbances of the free thiobarbiturates and its complex with DNA respectively, ϵ_G and ϵ_{H-G} are the molar absorption coefficients of the thiobarbiturates and thiobarbiturates–DNA complex, respectively. The binding constant of selected thiobarbiturates–DNA was obtained from the slope of the plot by plotting the variables of above straight-line equation and the K_b values obtained are given in Table 4. The values of K_b for all thiobarbiturates–DNA complexes are positive which is an indication of the positive interaction. The sample having lower K_b value means lesser interaction with DNA while for higher K_b values the interactions are supposed to be stronger. Like other parameters, the K_b values are also changing from sample to sample which is attributed to the structure effect.

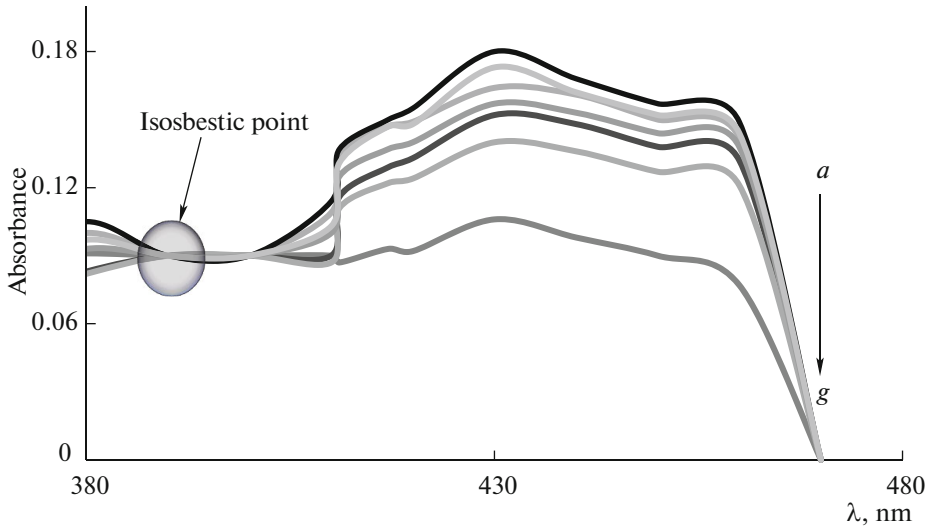


Fig. 8. UV-spectra of MKA28 (0.01 mM dm^{-3}) in the absence and in the presence of different concentration of DNA; (*a*–*g*) see Fig. 5.

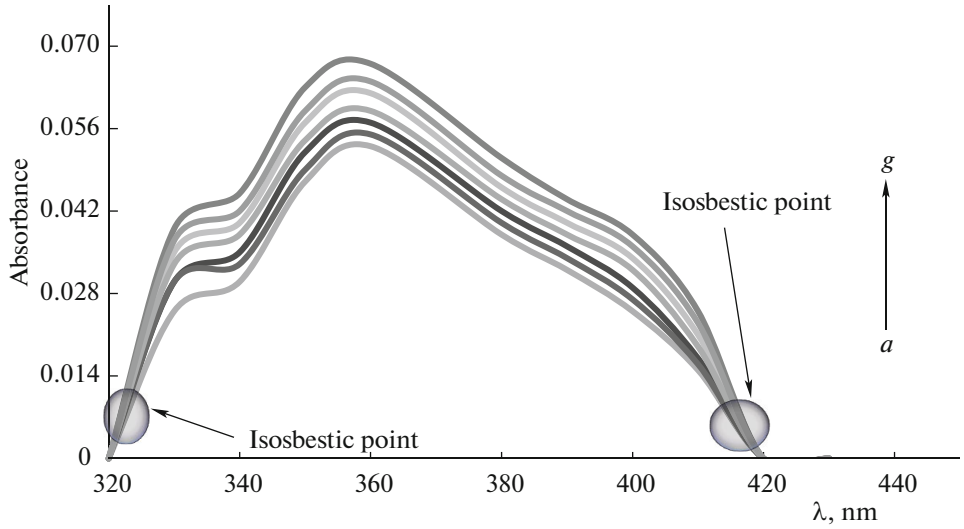


Fig. 9. UV-spectra of MKA29 (0.01 mM dm^{-3}) in the absence and in the presence of different concentrations of DNA; (*a*–*g*) see Fig. 7.

CONCLUSION

In this study we have synthesized several thiobarbiturates derivatives by the reaction of diethylthiobarbituric acid with different aromatic aldehydes in distilled water. The physicochemical properties of these newly synthesized samples were studied. The prapared thiobarbiturates derivatives have the melting points in the range of 150–182°C. The melting points decrease in the order MKA25 > MKA29 > MKA26 > MKA27 > MKA28. The calorific values were in the range of 5471.5–6071.3 cal/g for these samples. From the calorific values it can be concluded that sample having greater number of carbons and bonds showed the higher calorific values. It can be seen that varying side groups of the basic skeleton affect both the melting points and calorific values. TGA results show that the thiobarbiturates derivatives are stable in the range of 32.76–401°C. The stability of selected thiobarbiturates derivatives in decreasing order is MKA27 > MKA25 > MKA29 > MKA26.

The interaction of thiobarbiturates with dsDNA was the main part of this research work. Before studying thiobarbiturates–DNA interaction, maximum absorbance (λ_{max}, nm) values for selected samples were calculated, and were found in the range of 363–432 nm. The maximum absorbance (λ_{max}, nm) values

in decreasing order are given as MKA25 > MKA28 > MKA29 > MKA27 > MKA26. Molar absorptivity coefficient for selected free thiobarbiturates and thiobarbiturates–DNA complex were calculated. For free thiobarbiturates molar absorptivity coefficient occurs in the range of 6.47×10^1–1.84×10^3 [(mol $L^{-1})^{-1}$ cm^{-1}], while for thiobarbiturates–DNA complex it was in the range of 8.63×10^1–1.290×10^3. The change in molar absorptivity values are the indication of thiobarbiturates–DNA interactions. When we added the DNA solution of different concentration to solutions of compound MKA29, it showed decrease in absorbance as well as in maximum wavelength. Samples MKA27 and MKA28 showed decrease in absorption maximum. Similarly, MKA25, MKA26, and MKA29 showed hyperchromisim which reflect that the conformational changes occurred in rings of DNA. All this spectroscopic response and behavior reflects that interactions of selected thiobariturates occurred with dsDNA. The binding constants and strength of DNA with thiobarbiturates was find out by using Benesi–Hildebrand equation. The binding constants K_b was calculated, which was in the range of 8.63×10^1–1.290×10^3 (mol $L^{-1})^{-1}$. It is concluded that change in various physicochemical properties and strength of interaction with DNA of different sample is attributed to the change in the chemical structure of the compounds.

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