

Research Article

DFT Study and Antiparasitic Activity of Some Azo Dyes Containing Uracil

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In this study, for the first time, molecular modeling and antiparasitic activity studies were carried out on some azo dyes containing uracil, 6-amino-5-[(4-nitrophenyl) diazenyl] pyrimidine-2,4 (1H, 3H)-dione (dye I) and 6-amino-5-[(4-bromophenyl) diazenyl] pyrimidin-2,4 (1H, 3H)-dione (dye II), which were resynthesized using the same method in the literature and whose molecular structures were confirmed using FTIR and ¹H-NMR methods. In molecular modeling study, all calculations were performed using DFT/B3LYP/6-311++G(d,p) method. The molecular structures of the possible tautomeric forms of dyes I and II were optimized, and their molecular total energies were calculated in the gas phase and DMSO solvent. IR and ¹H-NMR spectral data of the possible tautomeric forms of dyes were obtained, and theoretical spectral data were compared with experimental ones. The evaluations show that, for both dyes, the spectral data of the imine-diketo-hydrazone form, which has the lowest energy and is hence determined to be the most stable form, are in agreement with the experimental ones. In antiparasitic activity study, dyes I and II were tested for the first time against parasites *Leishmania infantum*, *Leishmania major*, *Leishmania tropica* promastigotes, and *Trichomonas vaginalis* trophozoites. *In vitro* antileishmanial activities against *Leishmania* promastigotes were tested by microdilution broth assay with Alamar Blue in RPMI 1640 medium, and *in vitro* trichomonacidal activities against *Trichomonas vaginalis* parasite were tested using TYM medium. In tests, antileishmanial and trichomonacidal effects were determined by comparing the obtained minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values with those obtained for standard drugs (amphotericin B and metronidazole, respectively).

1. Introduction

Azo dyes are important colorants with chromosome azo group (-N=N-). Due to their special chemical and biological properties, they have potential applications in medicine and pharmacology, such as antibacterial, antifungal, antitumor, and antioxidant activities [1–3]. In addition, they are easy to use and offer strong colors in textile applications as well as being relatively inexpensive. With these aspects, azo dyes attracted considerable attention in scientific studies [4, 5]. Uracil derivatives belonging to the pyrimidines group, which have an important role in the binding of ribosomes and phosphate in the synthesis of enzymes required in basic biological processes, show important pharmacological

activities [6, 7]. Studies on synthetic pyrimidines reveal the presence of remarkable biological and pharmacological properties [8]. Therefore, uracil derivatives are used in drugs with antitumor, antibacterial, and antiviral properties [7].

Leishmaniasis is a disease caused by *Leishmania* parasites and transmitted by the bites of some sandflies, which differs clinically and epidemiologically, and is an important health problem worldwide [9, 10]. Visceral leishmaniasis (VL, Kala-Azar), cutaneous leishmaniasis (CL, Oriental Boil), and mucocutaneous leishmaniasis (MCL) are three different clinical forms of leishmaniasis [11]. In the Mediterranean basin, types of *Leishmania* causing leishmaniasis are as follows: generally *Leishmania infantum* (*L. infantum*) for VL and *Leishmania major* (*L. major*) and *Leishmania*

tropica (*L. tropica*) for CL [12]. About 1 billion people are at risk in five continents, and it is estimated that the number of new patients every year is 30 000 for VL and 1 million for CL [13]. The treatment alternatives of the disease are limited, and many of the drugs used are expensive and have side effects that cause significant health problems. Therefore, in recent years, many studies have been carried out to develop a drug that is cheap and safe and has a very low side effect, and antileishmanial tests have been applied on a variety of natural and synthetic compounds [14–17].

Trichomonas vaginalis (*T. vaginalis*) is a protozoan parasite with flagellates and only human host, found in trophozoite form [18]. It causes infection in the urogenital system of both women and men. Trichomoniasis causes vaginitis, cervicitis, and urethritis, especially in women [19]. 5-Nitroimidazole derivatives (metronidazole, ornidazole, and tinidazole) are currently used in the treatment of trichomoniasis, and these drugs cause DNA damage and cause cell death of the parasite. Although metronidazole is the first choice drug, it has mutagenic and carcinogenic effects. Therefore, alternative treatment methods in the treatment of trichomoniasis are being investigated [20].

In this study, in order to contribute to pharmacological studies, it was aimed to investigate azo dyes containing uracil, which have remarkable pharmacological properties but have not been previously tested for antiparasitic activity. For this reason, some azo dyes given in literature were resynthesized by the same method, and their structures were confirmed by FTIR and ¹H-NMR methods. For the first time, theoretical study and antiparasitic activity study were carried out on the azo dyes containing uracil called as 6-amino-5-[(4-nitrophenyl) diazenyl] pyrimidine-2,4 (1H, 3H)-dione (dye I) and 6-amino-5-[(4-bromophenyl) diazenyl] pyrimidin-2,4 (1H, 3H)-dione (dye II). In the theoretical study, the molecular structures of the possible tautomeric forms of dyes I and II were optimized, and the structural and some spectral parameters of dyes were determined by density functional theory (DFT) method with 6-311++G(d,p) basis set. Theoretical IR and ¹H-NMR data of the possible tautomeric forms of dyes I and II were compared with experimental ones. Thus, molecular structures of dyes I and II were firstly investigated by DFT methods. *In vitro* antileishmanial and trichomonacidal activities of these dyes were tested for the first time against parasites *L. infantum*, *L. major*, *L. tropica*, and *T. vaginalis*, respectively. *In vitro* antileishmanial activities were tested by microdilution broth assay with Alamar Blue in Roswell Park Memorial Institute (RPMI) 1640 medium, and *in vitro* trichomonacidal activity was tested using Trypticase Yeast extract Maltose (TYM) medium. As standard control drugs, amphotericin B for *Leishmania* and metronidazole for *Trichomonas* were used. Investigation of *in vitro* antileishmanial and trichomonacidal activities of azo dyes containing uracil, dyes I and II, can make an important contribution to drug design and development studies and can guide the determination of chemicals to be considered in these studies in terms of increasing the variety of compounds that can be used in pharmacological research.

2. Experimental and Computational Method

2.1. Instrumentation. ¹H-NMR spectra of dyes I and II were recorded on a Varian-Mercury 400 MHz spectrometer. These measurements used TMS as an internal standard and DMSO-*d*₆ as solvent. The FTIR spectra of dyes I and II were recorded on a Perkin-Elmer Spectrum One Fourier-transform IR spectrometer (resolution 4) in KBr pellets.

2.2. Synthesis. In this study, azo dyes containing uracil given in the literature, dye I and dye II, were resynthesized by the same method [21, 22]. The synthesis reaction of azo dyes containing uracil is shown in Scheme 1. *N,N*-Dimethylaniline, KOH, 4-bromine, concentrated HCl acid, NaNO₂, and 6-amino uracil used for the synthesis of dyes I and II were purchased from MERCK and used without further purification. Pure water was obtained in the laboratory.

2.3. Computational Procedures. Gaussian09 [23] program was used to obtain the optimized structures, IR vibration frequencies, and ¹H-NMR chemical shifts of azo dyes containing uracil by using DFT/B3LYP [24, 25] method and 6-311++G(d,p) basis set. The geometries were visualized by GaussView 5.0 [26] software package. The calculated vibration frequencies were scaled with scale factors 0.983 and 0.958 applied to frequencies smaller than 1700 cm⁻¹ and greater than 1700 cm⁻¹, respectively [27, 28]. By GIAO method, ¹H-NMR chemical shift values in DMSO solvent of dyes I and II were calculated at B3LYP/6-311++G(d,p) level [29, 30]. Chemical shift values were scaled by scale factor of 31.9681 ppm [31, 32].

2.4. In Vitro Antiparasitic Activity. The antiparasitic properties of dyes I and II were determined by testing their antileishmanial and trichomonacidal activities against *L. infantum*, *L. tropica*, *L. major* promastigotes, and *T. vaginalis* trophozoites, respectively. The study was carried out *in vitro* using microdilution broth assay. The results of antileishmanial activity against *Leishmania* promastigotes and trichomonacidal activity against *T. vaginalis* were evaluated by taking into account minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values, respectively.

2.4.1. Antileishmanial Activity. The axenic standard *L. infantum*, *L. tropica*, and *L. major* promastigotes were used in the study. The promastigotes were propagated on RPMI-1640 medium and maintained by passaging [33, 34]. Promastigotes were washed with phosphate buffered saline (PBS). Each of azo dyes containing uracil was dissolved in DMSO/H₂O (10%) and sterilized by membrane filter with a diameter of 0.45 μm. Final concentrations were prepared by adding RPMI-1640 medium. The stock solution was diluted in 96-well microplates, and 2.5 × 10⁷ cells/mL *Leishmania* promastigotes were added onto the wells. After incubating the microplates at 27°C for 20 hours in the incubator, Alamar Blue was added to the wells and incubated again at 27°C for 4

hours. Microplates were evaluated at 24, 48, and 72 hours, and the results were recorded. In the study, amphotericin B was used as a control drug, and all tests were repeated twice. As expected, the color will change from blue to pink in the positive control well and no color change was observed in the negative control well [35, 36].

2.4.2. Trichomonacidal Activity. *T. vaginalis* was produced in TYM medium. 1 mL inactivated human serum was added onto the medium under sterile conditions. Penicillin, streptomycin, and triflax were added under sterile conditions and then incubated in a 37°C incubator. Two days later, whether there was reproduction was observed. To test the viability of the trophozoites, 0.4% trypan blue was used and counted in the hemocytometer slide [37].

Azo dyes containing uracil, dyes I and II, were prepared in concentrations of 32, 16, 8, 4, 2, and 1 mg/mL in saline and distributed into sterile Eppendorf tubes. *T. vaginalis* were incubated at room temperature with 5×10^6 trophozoites/mL. The viability of the parasites was checked at specific times and noted. Recontrol seedlings were performed from the tubes in which the live cells were not detected, and the reproduction was evaluated. Parasites not added to dyes I and II were kept in the same environment as control [38].

3. Results and Discussion

3.1. Optimized Geometries. In theoretical study of azo dyes containing uracil called 6-amino-5-[(4-nitrophenyl) diazenyl] pyrimidine-2,4 (1H, 3H)-dione (dye I) and 6-amino-5-[(4-bromophenyl) diazenyl] pyrimidin-2,4 (1H, 3H)-dione (dye II), firstly, the molecular structures of dyes I and II in possible tautomeric forms given in Scheme 2 were optimized by DFT/B3LYP/6-311++G(d,p) method in the gas phase and in DMSO solvent, and their molecular total energies, in kcal/mol, were calculated. The most stable forms for dyes I and II in gas phase and in DMSO solvent are the imine-diketo hydrazone forms with the lowest energy. As in the gas phase/in DMSO solvent, molecular total energies of the imine-diketo hydrazone forms are obtained as -637226.06/-637240.19 kcal/mol for dye I and as -2123760.46/-2123771.10 kcal/mol for dye II, respectively. The optimized molecular structures of dyes I and II in imine-diketo-hydrazone forms are shown in Figure 1, and their structural parameters are presented in Table 1.

The structural parameters obtained by geometry optimization were compared with those obtained from X-ray method for similar single-crystalline compounds given in the literature; thus, the suitability of the method used and that of the selected basis set were discussed.

As can be seen from Table 1, the C=O bond lengths in the uracil rings of dyes I and II were calculated as 1.209, 1.231/1.211, 1.232 Å for the imine-diketo-hydrazone forms, respectively. In the literature, this length is between 1.2042 (19) and 1.2143 (19) Å [39, 40]. The C1-N17 and C1-N18 bond lengths were calculated as 1.373-1.403/1.373-1.401 Å, respectively. In literature, this length is 1.373 (2) and 1.376 (2) Å [22]. The N17-C1-N18 bond angles in the uracil rings of

dyes I and II were calculated as 114.3/114.3° for the imine-diketo-hydrazone forms, respectively. In the literature, this angle was given as 117.11 (14)° [41]. The C10-C5-N15-N16 torsion angle was calculated as 180.0/179.9° for the imine-diketo-hydrazone forms of dyes I and II, respectively. In the literature, this angle is given as 177.8 (3)° [42].

Since dyes I and II have values compatible with the structural parameters of similar single crystalline compounds in the literature, it can be concluded that the appropriate method and basis set were chosen. This is also an indication that spectral parameters can also be determined correctly.

3.2. Vibrational Spectra and ¹H-NMR Spectra. The IR and ¹H-NMR calculations of dyes I and II were carried out at DFT/B3LYP/6-311++G(d,p) level by considering the possible tautomeric forms to T-VIII from T-I (Scheme 2). Theoretical IR and ¹H-NMR data were compared with experimental data, and the compatibility of theoretically obtained values for dyes I and II with experimental data was discussed.

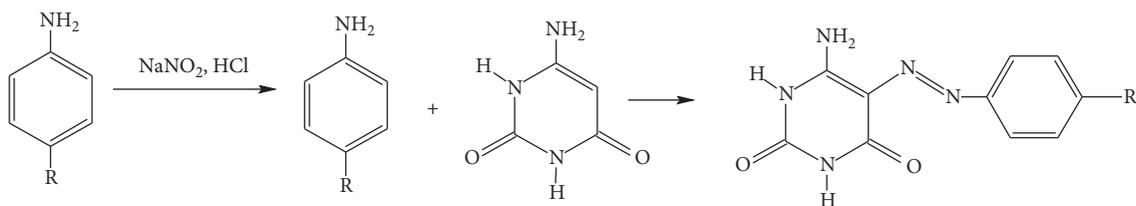
Considering Scheme 2, for T-I, NH₂, C1=O/C4=O, and N=N stretching bands are calculated as 3535_{as}/3405_s cm⁻¹, 1733/1696 cm⁻¹, and 1468 cm⁻¹ for dye I and as 3530_{as}/3405_s cm⁻¹, 1728/1692 cm⁻¹, and 1493 cm⁻¹ for dye II. For T-II, NH₂, C1=O/C4O-H, and N=N stretching bands are calculated as 3561_{as}/3439_s cm⁻¹, 1713/3012 cm⁻¹, and 1408 cm⁻¹ for dye I and as 3558_{as}/3435_s cm⁻¹, 1707/3007 cm⁻¹, and 1488 cm⁻¹ for dye II. For T-III, NH₂, C1O-H/C4=O, and N=N stretching bands are calculated as 3573_{as}/3440_s cm⁻¹, 3620/1695 cm⁻¹, and 1438 cm⁻¹ for dye I and as 3578_{as}/3443_s cm⁻¹, 3622/1691 cm⁻¹, and 1504 cm⁻¹ for dye II. For T-IV, NH₂, C1O-H/C4O-H, and N=N stretching bands are calculated as 3581_{as}/3453_s cm⁻¹, 3627/2969 cm⁻¹, and 1439 cm⁻¹ for dye I and as 3584_{as}/3456_s cm⁻¹, 3630/2973 cm⁻¹, and 1440 cm⁻¹ for dye II.

Accordingly, since the NH₂ and CO-H bands given above are not observed in the FTIR spectra of dyes I and II given in Figure 2, the possible tautomeric forms to T-IV from T-I can be eliminated.

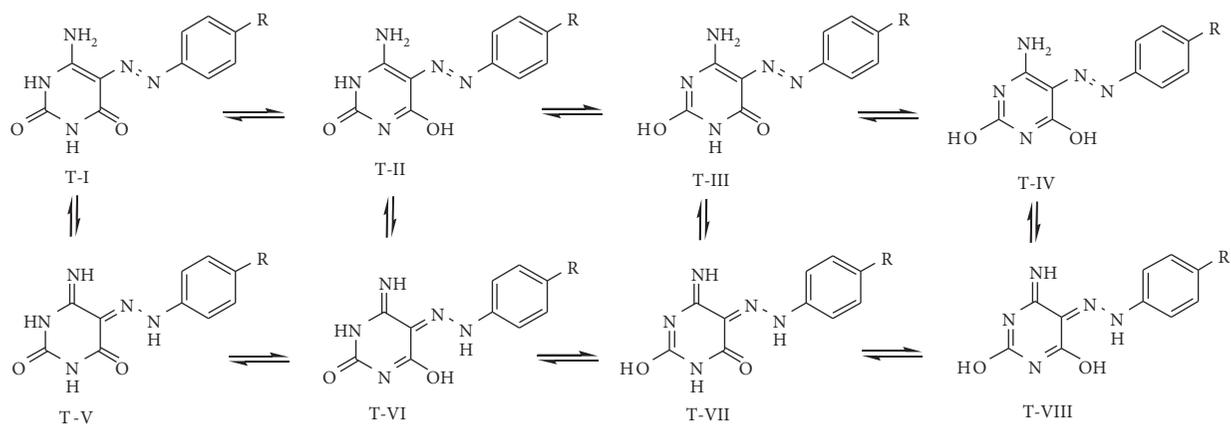
Similarly, the bands calculated as dye I/dye II are as follows: for T-VI, C4O-H bands at 3569/3571 cm⁻¹, respectively; for T-VII, C1O-H band at 3614/3615 cm⁻¹, respectively; for T-VIII, C1O-H and C4O-H bands at 3612/3615 cm⁻¹ and 3568/3570 cm⁻¹ which are absent in FTIR spectra of dyes I and II (Figure 2), and thus possible tautomeric forms to T-VIII from T-VI can be eliminated.

As a result, when the FTIR spectra of dyes I and II are compared with the theoretical ones, it can be said that, in the solid state, dyes I and II are in the imine-diketo-hydrazone form (T-V in Scheme 2). Some vibration bands are considered to confirm the molecular structures of dyes I and II, and theoretical ones obtained in the gas state for imine-diketo-hydrazone forms are given in Table 2.

As evaluated in Table 2, N17-H/N18-H stretching bands in the FTIR spectra of dyes I and II (Figures 2(a) and 2(b)) were observed at 3116/3116 cm⁻¹ and 3144/3144 cm⁻¹, respectively, and they were calculated as 3452/3444 cm⁻¹ for



SCHEME 1: Synthesis path of azo dyes containing uracil: dye I ($R=NO_2$) and dye II ($R=Br$).



SCHEME 2: Possible tautomeric forms of azo dyes containing uracil: dye I ($R=NO_2$) and dye II ($R=Br$).

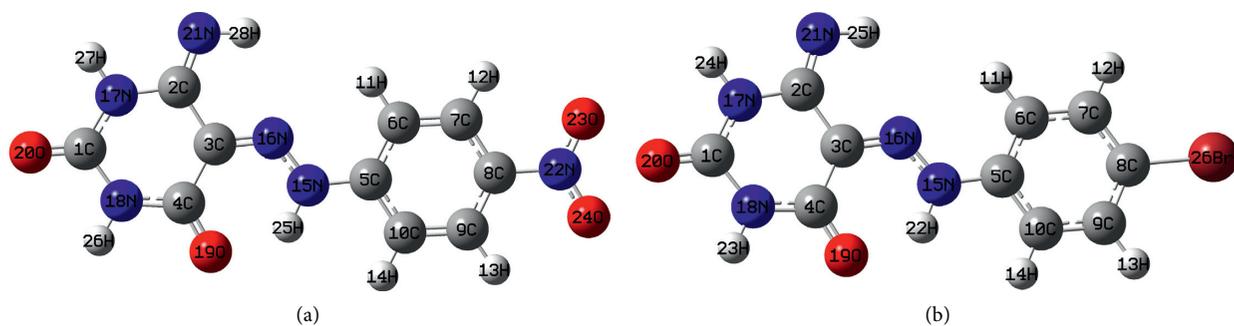


FIGURE 1: The molecular structures in the imine-diketo-hydrazone form of (a) dye I and (b) dye II.

dye I and $3455/3448\text{ cm}^{-1}$ for dye II. The $N21-H$ imine band was observed as $3116/3099\text{ cm}^{-1}$ for dyes I/II and was calculated as $3369/3369\text{ cm}^{-1}$, respectively. $N15-H$ hydrazone band calculated as $3198/3195\text{ cm}^{-1}$ for dyes I/II was observed at $3168/3216\text{ cm}^{-1}$. The experimental and calculated values of other stretching bands given in Table 2 are quite compatible.

As can be seen from Figure 1, the optimized molecular structures of dyes I and II were obtained in the most stable structure revealing the presence of $N15-H\cdots O19$ type intramolecular hydrogen bonds. Therefore, deviations between experimental and calculated ones are small, and values obtained as $30/21\text{ cm}^{-1}$ for dyes I/II, respectively, can be considered compatible. As observed for $N17-H/N18-N17$ stretching bands and $N21-H$ imine bands, the considerable deviations calculated as $336/328\text{ cm}^{-1}$ and 253 cm^{-1} for dye

I, $311/304\text{ cm}^{-1}$ and 270 cm^{-1} for dye II, respectively, can be explained by the presence of $N-H\cdots O$ type strong intermolecular hydrogen bonds.

^1H-NMR spectra of dyes I and II were recorded in $DMSO-d_6$ (Figures 3(a) and 3(b)). For this reason, primarily the molecular structures of possible tautomeric forms of the dyes were optimized in $DMSO$ solvent by DFT/B3LYP/6-311++G(d,p) method and, for all, ^1H-NMR calculations in $DMSO$ solvent were performed using DFT/B3LYP/6-311++G(d,p) method.

Considering Scheme 2, to T-IV from T-I and as dye I/dye II, NH_2 proton signals are calculated as 5.03, 5.20, 5.32, 5.33 and 7.05, 6.79, 7.07, 6.73 ppm/4.89, 5.04, 5.18, 5.19 and 6.94, 6.64, 6.95, 6.59 ppm, respectively. These signals are not observed in ^1H-NMR spectra of dyes I and II given in Figure 3. For this reason, possible tautomeric forms to T-IV

TABLE 1: Some structural parameters obtained by DFT/B3LYP/6-311++G(d,p) method for imine-diketo-hydrazone (T-V) forms of dyes I and II.

Dye I		Dye II	
Bond lengths (Å)		Bond lengths (Å)	
C1–O20	1.209	C1–O20	1.211
C1–N17	1.373	C1–N17	1.373
C1–N18	1.403	C1–N18	1.401
C2–N17	1.401	C2–N17	1.402
C2–N21	1.272	C2–N21	1.273
C4–N18	1.376	C4–N18	1.379
C4–O19	1.231	C4–O19	1.232
C2–C3	1.487	C2–C3	1.483
C3–C4	1.474	C3–C4	1.468
C3–N16	1.307	C3–N16	1.311
N15–N16	1.309	N15–N16	1.303
C5–N15	1.398	C5–N15	1.405
N22–O23	1.225	C8–Br26	1.913
N22–O24	1.225	—	—
Bond angles (°)		Bond angles (°)	
N17–C1–O20	124.2	N17–C1–O20	124.1
N18–C1–O20	121.5	N18–C1–O20	121.6
N17–C2–N21	117.8	N17–C2–N21	117.6
N17–C1–N18	114.3	N17–C1–N18	114.3
C3–C2–N21	127.8	C3–C2–N21	128.0
C2–C3–N16	116.3	C2–C3–N16	116.2
C3–N16–N15	121.4	C3–N16–N15	121.5
C5–N15–N16	121.6	C5–N15–N16	121.8
C6–C5–N15	122.2	C6–C5–N15	122.3
C3–C4–O19	123.3	C3–C4–O19	123.5
C7–C8–N22	119.3	C7–C8–Br26	119.6
O23–N22–O24	124.7	—	—
Torsion angles (°)		Torsion angles (°)	
N18–C1–N17–C2	–0.16	N18–C1–N17–C2	–0.1
O20–C1–N17–C2	179.8	O20–C1–N17–C2	179.9
O20–C1–N18–C4	179.9	O20–C1–N18–C4	179.9
N17–C2–C3–C4	–0.2	N17–C2–C3–C4	0.0
N21–C2–C3–N16	–0.14	N21–C2–C3–N16	0.0
C4–C3–N16–N15	0.0	C4–C3–N16–N15	0.0
C2–C3–N16–N15	179.9	C2–C3–N16–N15	–179.9
C6–C5–N15–N16	0.0	C6–C5–N15–N16	0.0
C6–C5–C10–C9	0.0	C6–C5–C10–C9	0.0
C10–C5–N15–N16	180.0	C10–C5–N15–N16	179.9
C5–C6–C7–C8	0.0	C5–C6–C7–C8	0.0
C7–C8–N22–O24	–179.9	C6–C7–C8–Br26	–180.0

from T-I can be eliminated. Similarly, CO–H proton signals, as dye I/dye II, are obtained at 7.05/6.88 ppm for T-VI, at 6.00/5.91 ppm for T-VII, and 6.08 and 7.18/5.97 and 7.02 ppm for T-VIII which are absent in ¹H-NMR spectra of dyes I and II. Therefore, T-VI, T-VII, and T-VIII are eliminated.

Thus, the ¹H-NMR spectra of dyes I and II are consistent with the theoretical data of dyes I and II in the imine-diketo-hydrazone form (T-V). Theoretical ¹H-NMR spectral data obtained for the imine-diketo-hydrazone form are given in Table 3 together with the experimental values.

As seen in Table 3, N15–H proton signals were observed at 14.03/14.02 ppm for dyes I/II, respectively, and were calculated as 13.76/13.65 ppm. These results are consistent with the presence of N15–H···O19 type intramolecular

hydrogen bonds shown by the optimized molecular structures of dyes I and II. N21–H, N17–H, and N18–H protons were observed at 11.06/10.80/10.89 ppm for dye I and 10.96/10.53/10.71 ppm for dye II, respectively. The deviation of these values from the calculated chemical shift values is 2.18/3.58/3.75 ppm for dye I and 2.36/3.37/3.68 ppm for dye II, respectively. Significant deviations of the experimental chemical shift values from the calculated values reveal the existence of N–H···O type intermolecular hydrogen bonds.

As evaluated in the FTIR and ¹H-NMR experimental data (Figures 2 and 3) supporting each other of dyes I and II together with the theoretical data in Tables 2 and 3, the presence of strong intramolecular and intermolecular hydrogen bonds suggested for both dyes is consistent with the literature [22, 43].

3.3. The Results of Antiparasitic Activity Test

3.3.1. The Results of Antileishmanial Activity Test. Antileishmanial activities of dyes I and II against *L. infantum*, *L. major*, and *L. tropica* promastigotes were determined *in vitro* by microdilution broth assay with Alamar Blue, and MIC values were obtained. In the evaluation of antileishmanial activity, it is expected that Alamar Blue does not change color in the negative control. In addition, in promastigotes added as positive control, the blue color should turn pink, since inhibitory dye is added, and it is expected to continue vitality. In the case of negative and positive controls working, results can be evaluated. In other words, if the blue color does not change in the wells, the absence of vitality and turning pink determine that the vitality continues.

The results are shown in Figures 4–6. MIC values obtained in the tests are given in Table 4. According to the data obtained, it was determined that dye I was ineffective against all *Leishmania* species at the concentrations studied. Dye II was observed to be effective only against *L. major* promastigotes (MIC: 2500 µg/mL), while it was found that it did not have antileishmanial activity against other *Leishmania* species. It was determined that amphotericin B, which is used as a control drug, is effective against all *Leishmania* species even the lowest concentration studied (MIC: 19 µg/mL).

3.3.2. Trichomonacidal Activity Results. For trichomonacidal activities of dyes I and II against *T. vaginalis* trophozoites, MLC values were evaluated as *in vitro* with trypan blue of 0.5% at 16, 24, and 48 hours under invert microscope. For the lethal percent, formula 100 – (number of living cells/total number of cells × 100) was used. The MLC values obtained at the 16th, 24th, and 48th hours in the tests are given in Table 5. The microscopic images of the results of dyes I and II obtained against *T. vaginalis* trophozoites at the end of 48 hours are shown in Figures 7 and 8, respectively.

In *in vitro* trichomonacidal activity tests, at the initial evaluation at 16th hours, mobile *T. vaginalis* trophozoites were detected in all wells to which dyes I and II were added, including the positive control. At the second evaluation at

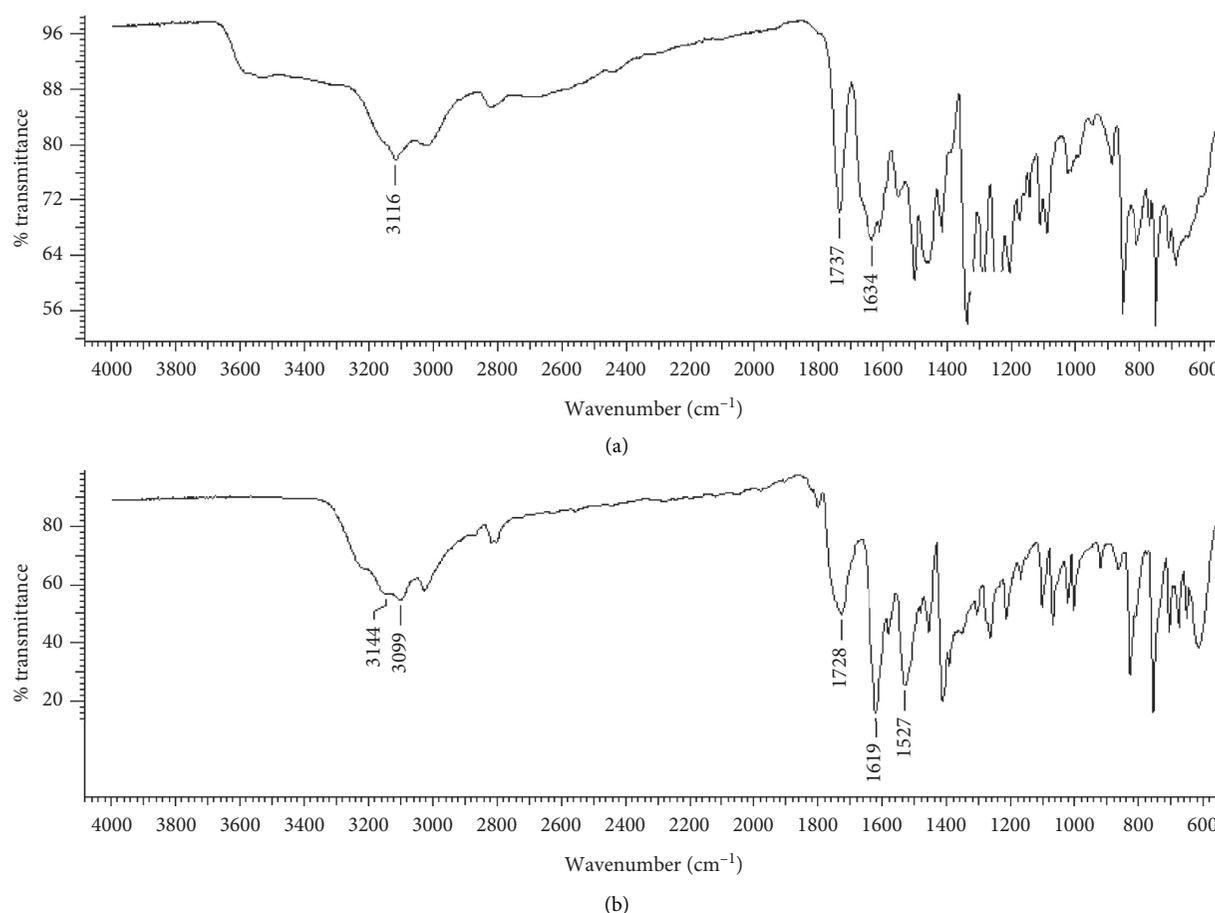


FIGURE 2: FTIR spectra of (a) dye I and (b) dye II.

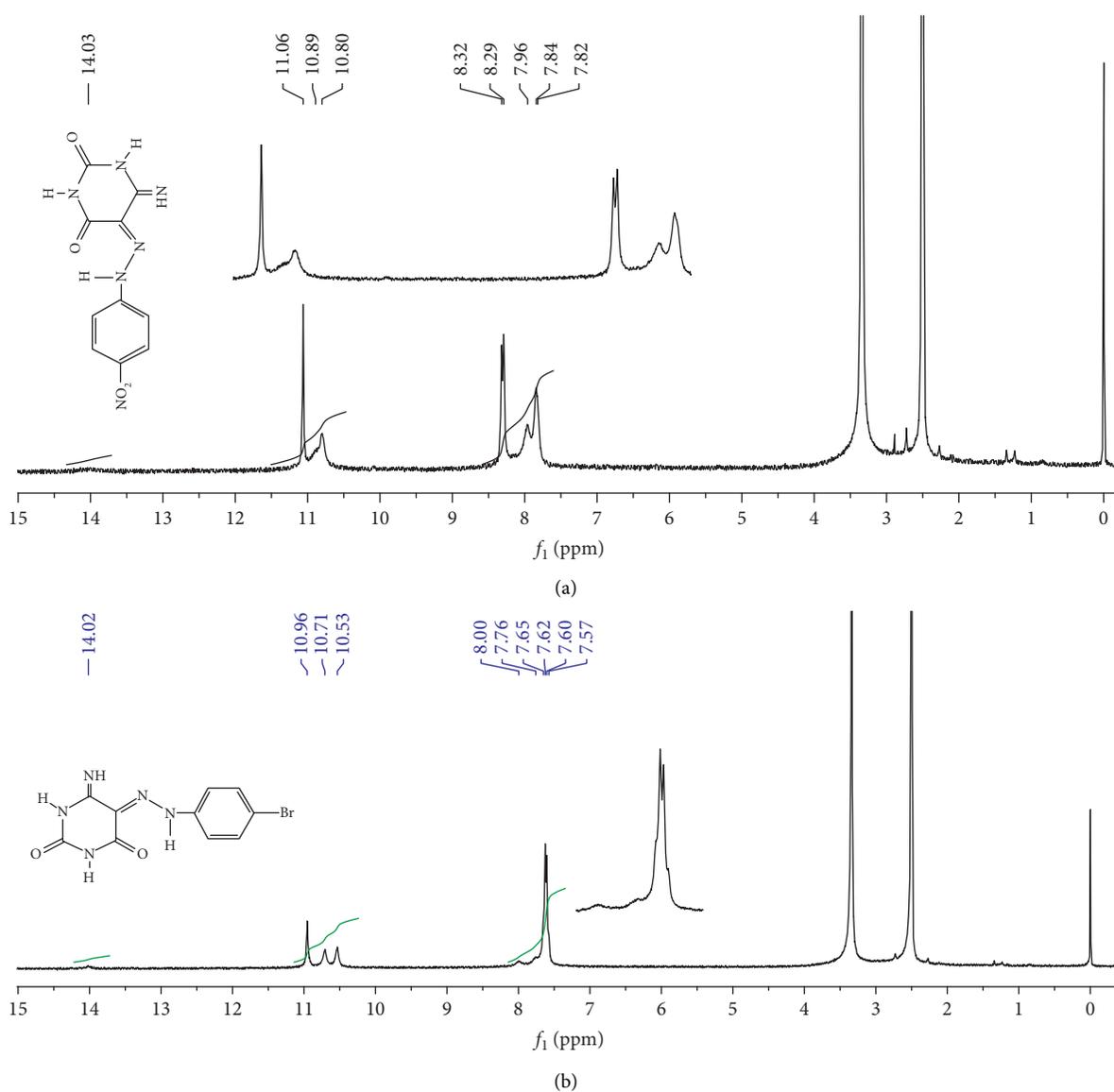
TABLE 2: Some vibration frequencies (cm^{-1}): experimental and calculated by DFT/B3LYP/6-311G++(d, p) for imine-diketo-hydrazone forms of dyes I and II.

Assignments ^a	Experimental (cm^{-1})		Calculated (cm^{-1})	
	Dye I	Dye II	Dye I	Dye II
ν (N17-H)	3116	3144	3452	3455
ν (N18-H)	3116	3144	3444	3448
ν (N21-H) imine	3116*	3099**	3369	3369
ν (N15-H) hydrazone	3116*	3144***	3198	3195
ν (C1=O20)	1737	1728	1730	1724
ν (C4=O19)	1634	1619	1650, 1664	1643, 1662
ν (C2=N21)	1634	1619	1650, 1664	1643, 1662
ν (C=C)	1610	1619	1611	1600
ν (C3=N16)	1552, 1502, 1458	1580, 1455	1561, 1545, 1472	1554, 1459

^a ν , stretching. *In the range of 3140–3092 cm^{-1} , centered at 3116 cm^{-1} ; **in the range of 3130–3068 cm^{-1} , centered at 3099 cm^{-1} ; ***in the range of 3158–3130 cm^{-1} , centered at 3144 cm^{-1} .

the end of the 24th hour, dyes I and II did not show any lethal effects. In the positive control, it was determined that the metronidazole used showed 75%, 59%, 43%, 25%, and 10% lethal effects in other dilutions (400, 200, 100, and 50 $\mu\text{g}/\text{mL}$) starting from the highest concentration (800 $\mu\text{g}/\text{mL}$), respectively. In the 48th hour evaluation, it was determined that dye II had 79% lethal effect at 10,000 $\mu\text{g}/\text{mL}$ dilution and 35% at 5000 $\mu\text{g}/\text{mL}$ dilution.

It was determined that the concentration value of dye I, which has 100% lethal effect against *T. vaginalis* trophozoites *in vitro*, is 10,000 $\mu\text{g}/\text{mL}$. At the end of test, the parasites in the well were taken into TYM medium again to determine whether there was growth at this concentration or not. Growth of the parasite was not detected in the medium, and 100% lethal effect was confirmed. In addition, it was determined that dye I was more effective than dye II and

FIGURE 3: ^1H -NMR spectra of (a) dye I and (b) dye II.TABLE 3: ^1H isotropic chemical shifts of dyes I and II: experimental in $\text{DMSO}-d_6$ and for imine-diketo-hydrazone forms (T-V) calculated in DMSO by DFT/B3LYP/6-311++G(d,p) method (all values in ppm).

Atom	Experimental		Calculated	
	Dye I	Dye II	Dye I	Dye II
N15-H	14.03	14.02	13.76	13.65
N21-H	11.06	10.96	8.88	8.60
N17-H	10.80	10.53	7.22	7.16
N18-H	10.89	10.71	7.14	7.03
H11	7.96–7.82	7.60–7.57	8.26	8.16
H12	8.32–8.29	7.62–7.60	8.66	7.75
H13	8.32–8.29	7.62–7.60	8.68	7.70
H14	7.96–7.82	7.60–7.57	7.32	7.29

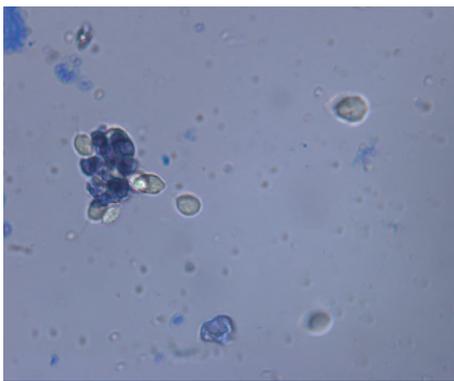


FIGURE 7: Microscopic image of the trichomonocidal activity of dye I against *T. vaginalis* trophozoites after 48 hours.

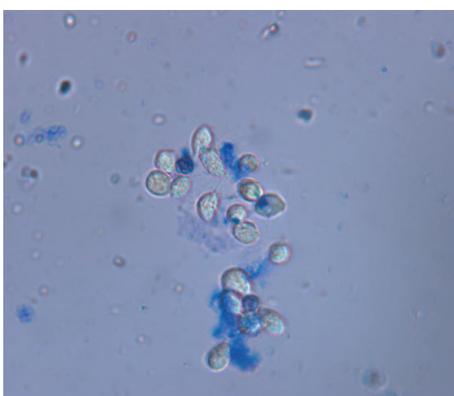


FIGURE 8: Microscopic image of the trichomonocidal activity of dye II against *T. vaginalis* trophozoites after 48 hours.

showed 96%, 74%, 61%, and 33% lethal effects at 5000, 2500, 1250, and 625 $\mu\text{g}/\text{mL}$ dilutions, respectively.

4. Conclusion

In this study, for the first time, molecular modeling and antiparasitic activity studies were carried out on 6-amino-5-[(4-nitrophenyl) diazenyl] pyrimidine-2,4 (1H, 3H)-dione (dye I) and 6-amino-5-[(4-bromophenyl) diazenyl] pyrimidin-2,4 (1H, 3H)-dione (dye II) given in the literature, which were resynthesized by the same method and whose molecular structures were confirmed using FTIR, $^1\text{H-NMR}$ spectroscopic methods. In molecular modeling study, the molecular structures of the possible tautomeric forms of dyes I and II in the gas state and in DMSO solvent were optimized using the DFT/B3LYP/6-311++G(d,p) method, and their molecular total energies, optimized structures, IR, and $^1\text{H-NMR}$ spectral data were obtained. IR vibration frequencies were calculated in gas state, and $^1\text{H-NMR}$ chemical shift values were calculated in DMSO solvent. Theoretical spectral data of the possible tautomeric forms of dyes were compared with experimental data obtained from FTIR and $^1\text{H-NMR}$ spectra of dyes. The results show that, for both dyes, the spectral data of the imine-diketo-hydrazone form, which has the lowest energy and hence is determined as the most stable

tautomeric form, are consistent with the experimental ones. The optimized structures of dyes in imine-diketo-hydrazone form were obtained in the most stable form that reveals $\text{N-H}\cdots\text{O}$ type intramolecular bonds. Therefore, the relevant IR and $^1\text{H-NMR}$ spectral data are compatible with the experimental ones. However, significant deviations from experimental values observed in IR and $^1\text{H-NMR}$ spectral data of dyes I and II in imine-diketo-hydrazone forms indicate the presence of $\text{N-H}\cdots\text{O}$ type strong intermolecular hydrogen bonds in the molecular structure of the dyes.

Antiparasitic activities of the dyes were tested as *in vitro* against *L. infantum*, *L. major*, *L. tropica* promastigotes, and *T. vaginalis* trophozoites by microdilution broth assay. Antileishmanial and trichomonocidal activities were evaluated considering MIC and MLC values, respectively. Antileishmanial activities of dyes were evaluated by comparing the MIC values obtained in the tests with those obtained for the standard control drug amphotericin B. Results show that dye I has no antileishmanial activity at the concentrations studied against *Leishmania* species tested, and dye II is effective against *L. major* promastigotes, but not against other *Leishmania* species studied. If higher concentrations are studied, the dyes may be found to be effective as antiparasitic. In the trichomonocidal activity study, as a standard control drug, metronidazole was used. According to the data obtained, dyes I and II have trichomonocidal activity at some concentrations studied. In tests, it is determined that azo dyes containing uracil show a lethal effect of 33% to 100% and also that dyes tested against *T. vaginalis* trophozoites show a dose-dependent effect *in vitro*.

As a result, the antiparasitic activity results obtained in the study are promising, and due to *in vitro* antileishmanial and trichomonocidal activities, azo dyes containing uracil may be considered in pharmacological research, and thus the diversity of compounds used may increase. In order for dyes I and II to be good drug candidates, *in vivo* control studies and toxicity tests should be performed in experimental animal models.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The numerical calculations reported in this paper were fully performed at TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources). An oral presentation of a part of this article is available (Turkish Physical Society 35th International Physics Congress, Bodrum Municipality-Herodot Culture Center, Konacık-Bodrum/Turkey, September 4–8, 2019). This article is prepared from Pınar Kubaşık's master's thesis (Graduate School of Natural and Applied Sciences, Advanced Technologies, Gazi University, Turkey).

Conflicts of Interest

The authors declare no conflicts of interest.

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