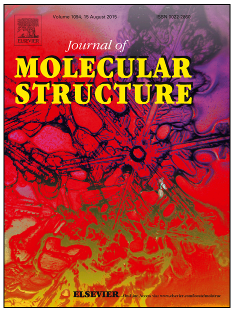

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PII: S0022-2860(18)30733-6

DOI: 10.1016/j.molstruc.2018.06.032

Reference: MOLSTR 25319

To appear in: *Journal of Molecular Structure*

Received Date: 14 March 2018

Revised Date: 8 June 2018

Accepted Date: 8 June 2018

Please cite this article as: Çğ. Karabacak Atay, F. Duman, M. Gökalp, T. Tiliki, S. Ozdemir Kart, A rapid synthesis of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid: Experimental, DFT study and DNA cleavage activity, *Journal of Molecular Structure* (2018), doi: 10.1016/j.molstruc.2018.06.032.

A rapid synthesis of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid: Experimental, DFT study and DNA cleavage activity

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Abstract

The newly synthesized 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid has been prepared by diazotization of anthranilic acid and coupling with 2-amino-4,6-dimethylpyrimidine. Its structure has been characterized by spectroscopic measurements (^{1}H-NMR spectra, FT-IR spectra, mass spectra and UV-visible spectra) and thermal analysis technique. The DNA cleavage activity of compound is evaluated by agarose gel electrophoresis with a series of concentrations. Our measurements show that neither a disruptive effect created by 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid on pBR 322 DNA are observed, nor the dependence of the concentration on the activity of newly synthetized chemical on pBR 322 plasmid DNA such as cleavage or break DNA double helix structure. Moreover, computational chemistry method based on Density Functional Theory (DFT) employing B3LYP level with 6-31G(d) basis set has been used to study geometry and spectroscopic properties such as FT-IR and UV-vis spectra of the titled compound considered in this work. The computations of the chemical shifts for ^{1}H-NMR of the title compound have been carried out via Gauge-Invariant Atomic Orbital (GIAO) method utilizing the same basis set. It is observed that DFT results are compatible with the experimental results.

Keywords: Anthranilic acid, Heterocyclic dye, Spectroscopic property, pBR322 DNA cleavage, Density Functional Theory.

1. Introduction

Anthranilic acid contains carboxyl (-COOH) and amino (-NH_2) groups which is a precursor to the amino acid tryptophan. Because of its medicinal and biological properties, researchers have motivated to study anthranilic acid and its derivatives [1-4]. Moreover, biological–medical studies of azo dyes such as anticancer [5], antitumor [6], antifungal [7], antioxidant [8] and antibacterial [9] has been extensively studied. Therefore, the synthesis of azo dyes with anthranilic acid takes important notice of not only scientifically but also technologically.

High yielding and clean synthesis of azo dyes make them to be significant compounds. Although, several properties of azo dyes have been widely investigated, there is limited study about DNA cleavage properties of azo dyes. S.M. Pradeepa et. al. [10] have synthesized Cu(II) and Co(II) complexes of azo-containing Schiff base and investigated their DNA photo cleavage abilities by agarose gel electrophoresis. The presence of azo and carboxylic acid group complexes in the compound reveals the efficient DNA photo cleavage activity. Carla T. Mapp et al. [11] have synthesized the symmetrical carbocyanine dyes and evaluated their DNA photo cleavage activities. They have reported that the irradiation at 575, 588, 623, or 700 nm produces good photo cleavage of plasmid DNA.

Because of the biological importance of azo dyes and anthranilic acid, we have carried out the synthesis of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid with anthranilic acid and 2-amino-4,6-dimethylpyrimidine and evaluated their DNA cleavage activity for a series of concentrations. The results obtained from this study may be useful for the usage of azo dyes and further cancer studies.

The computational chemistry methods based on DFT are useful tools to determine some characteristic properties of the chemical and biological molecules [12-14]. Computational chemistry methods can be used to investigate the molecular structure, thermodynamic properties, frontier molecular orbitals, molecular electrostatic potential, non-linear optical properties, fundamental vibrational modes and NMR spectra for small and large sized chemical molecules [15-29]. They are used to investigate structural, vibrational and NMR properties of the dithiophosphonates [28,29], calix[4]arenes [15,30], conduction polymer [31] and azo dyes [32-34].

Experimental method for the synthesis and characterization of the molecule being taken into account in this work are presented in Section **2**. The computational procedure followed in this work is given in Section **3**. Both experimental and theoretical results for the structural and vibrational properties of the compound considered in this study are given in Section **4**. The results obtained from DFT method are compared with the experimental data in the same section. The summary and conclusion arising from this work are given in the last section.

2. Experimental

2.1. Synthesis of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid

20 mmol of anthranilic acid is dissolved in hydrochloric acid:water (1:1). The solution is then cooled to 0-5^{o}C by stirring. While stirring, sodium nitrite (2 mmol) in water (10 ml) is gradually added to this solution. The reaction mixture is stirred for 2h at 0-5 ^{o}C. The resulting diazonium salt solution is then added dropwise to a cooled and stirred solution of 2-amino-4,6-dimethylpyrimidine (20 mmol) in sodium acetate (4 g) dissolved in 20 mL ethanol:water (1:1). Stirring is continued for 4h at 0-5 ^{o}C. The precipitated products diluted with cold water, filtered off, washed with water several times, and dried. The obtained product is recrystalized from DMF-H_2O mixture (Orange crystal, melting point: 240 ^{o}C decomposition).

The general route for the synthesis of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid is shown in **Figure 1**.

2.2. DNA cleavage experiments

pBR322 plasmid DNA is purchased from commercially (Thermo Fischer Scientific, SD0041). Cleavage effect of newly synthetized chemical 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid on pBR 322 plasmid DNA is monitored performing gel electrophoresis experiment. Cleavage studies are conducted by following the procedure given in the study of Duman et al. [35] with some modifications. Briefly, 3 µL of plasmid DNA (0.5 µg/mL) are incubated with 30 µL aliquots of decreasing concentrations of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid from 100 µM to 20 µM at 37 °C during overnight in a buffer solution containing DMSO. After that, 10 µL aliquots of chemical/DNA mixtures are loaded onto the 1% agarose gel with loading buffer (2 µL bromophenol blue dye). The gel is run at 80 V for 4 h in a TAE buffer (40 mM Tris acetate/1 mM EDTA, pH 8). After electrophoresis, the gel is subsequently stained by ethidium bromide (0.5µg/mL). At last, the bands observed are visualized under UV light and photographed with a video camera.

3. Computational Method

DFT calculation utilizing B3LYP level with 6-31G(d) basis set has been carried out by using Gaussian 09W [36] program to predict the molecular structure and vibrational frequencies of the title compound. B3LYP level is the three-parameter hybrid method, which is the Lee–Yang–Parr gradient-corrected correlation functional (LYP) [37] and coupled with Becke's three-parameter gradient exchange correlation functional (B3) [38]. The three dimensional optimized structure of the title compound obtained from GaussView program [39] is shown in **Figure 2**. The equilibrium structural parameters of the title molecule are used to calculate the vibrational frequencies, the chemical shifts and UV-vis spectrum. The vibrational wavenumbers of the molecule have been assigned by combining the results of the GaussView 5.0.8 program and the potential energy distribution (PED) obtained from VEDA4 program [40]. Theoretical ^{1}H and ^{13}C NMR isotropic shielding calculations of title molecule are obtained via the Gauge-Invariant Atomic Orbital (GIAO) method [41]. The ^{1}H and ^{13}C NMR chemical shift calculations are investigated by considering Tetramethylsilane (TMS) as a reference. UV-vis spectra of the title compound are also studied via Time Dependent Density Functional Theory (TD-DFT) utilizing B3LYP level with the 6-31G(d) basis set in the different solvents.

4. Results and Discussion

4.1. Molecular Geometry

The geometric structure of compound, synthesized in this work, predicted from DFT/B3LYP/6-31G(d) level are presented in **Figure 2** along with the atom numbering scheme. The geometry of the compound possesses C_1 point group symmetry. This compound has 33 atoms and has got 93 fundamental vibrational modes.

4.2. Structural Properties

Some important structural parameters such as bond lengths, bond angles and dihedral angles, obtained by using the DFT/B3LYP/6-31G(d) method, are given in **Table 1**.The bond lengths of O20-H33, N17-H31, O19-C18 and N8-N7 for the compound are calculated as 0.9757 Å, 1.0079 Å, 1.2139 Å and 1.2661 Å, respectively. The bond angles of C10-N11-C12 and N8-N7-C4 for the title compound are found as 117.28° and 112.95°. The dihedral angles of N8-N7-C4-C5 and N13-C14-C9-N8 are predicted as -30.04° and -180.89°. All data describing the compound studied in this work are given in **Table S1** as a supplementary data in **Appendix A**. 33 bond lengths, 53 bond angles and 67 dihedral angles are necessary in order to define the molecular structure of the compound. These bond lengths, bond angles and dihedral angles are given in the **Table S1** as shown in **Figure 2**. To the best of our knowledge, there is no experimental data on the geometric structure of the compound in the literature.

4.3. FT-IR spectra, ^{1}H-NMR spectra, mass spectra and UV-Vis Spectra

The FT-IR spectra of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid shows aromatic (Ar–H) band at 3200 cm^{-1}, aliphatic (Alip–H) band at 2640 cm^{-1} and azo (N=N) band at 1640 cm^{-1}. FT-IR spectra of compound and transmittance versus wavenumber of compound computed from DFT/B3LYP method with 6-31G(d) basis set are illustrated in **Figure 3**. It is observed that the title molecule has 93 normal modes; 32, 31 and 30 modes of vibrational modes are stretching vibrations, bending vibrations and 30 torsional vibrations, respectively. This molecule has also 30 modes of CH vibrations. The vibrational frequencies computed from DFT/B3LYP/6-31G(d) level are multiplied by a scale factor of 0.9613 to compare the experimental frequencies, because the DFT method tends to overestimate the vibrational modes [42]. Deficiencies of DFT method are due to the insufficiencies of the theoretical approximations used in the calculations. Vibrational frequencies evaluated from DFT/B3LYP level with 6-31G(d) basis set are given in **Table 2** along with the experimental data. All of the wavenumbers of the title compound are calculated to be positive and this case supports that title molecule is a true minimum on the potential surface. The theoretical data are in agreement with the experimental data except for some wavenumbers. GaussView 5.0.8 and VEDA4 programs are used to assign the vibrational normal modes of the molecule synthesized in this work. **Figure 4** shows the linear regression between theoretical and experimental data. Linear equation of $y = ax + b$ is used to perform the linear regression. Here, a and b in the equation are fit constants. The linear regression equality of $y = 0.9792x - 16.221$, (R =0.988), is obtained. It is shown that the theoretical frequencies computed from DFT/B3LYP level with 6-31G(d) basis set are consistent with those of the experiment, since the slope of the linear regression value (R =0.988) goes to unity as shown in **Figure 4.**

^{1}H-NMR spectra of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid shows broad peaks at 8.01 ppm (NH_2, pyrimidine) and 11.97 ppm (-COOH) of anthranilic acid. The other δ values of 7.92–7.05 ppm (aromatic H), 2.47 ppm (CH_3) of pyrimidine are recorded. ^{1}H-NMR spectra of compound is shown in **Figure 5**. ^{1}H-NMR values calculated by DFT/B3LYP/6-31G(d) for the compound show broad peaks at 4.25 and 4.28 ppm (NH_2, pyrimidine) and 5.54 ppm (-COOH) of anthranilic acid. The other δ values of 7.44–7.17 ppm (aromatic H), 2.62-1.62 ppm (CH_3) of pyrimidine are predicted. ^{1}H-NMR experimental data and theoretical values calculated from DFT/B3LYP/6-31G(d) method are given in the **Table 3**. As shown in the **Table 3**, except for X-H, the experimental data of aromatic and aliphatic protons are consistent with the results of the theoretical results. Different results seen in X-H can be explained as tautomerization and resonate of X-H protons at different regions.

Theoretical ^{13}C-NMR resonances at 141.74, 117.83, 115.93, 114.81 and 108.42 ppm are obtained and assigned to phenyl carbons (C4, C2 and C6, C3, C1 and C5). Moreover, the resonances of 161.49, 146.45, 146.26 and 124.78 ppm are computed and defined to pyrimidine carbons (C14, C10, C12 and C9). 155.79 ppm is also identified to carboxylic acid carbon (C18) and 20.42 and 15.36 ppm are dedicated to methyl carbons (C16 and C15). The ^{13}C-NMR values calculated from DFT/B3LYP/6-31G(d) method are given in **Table 4**.

Mass spectra are recorded as MS: (m/z, 100 eV): 272 $[M+1]^{+}$. A mass spectrum of compound is illustrated in **Figure 6.**

UV-spectra analysis of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid have been investigated in eight different solvents of dimethyl formamide, methanol, acetic acid, chloroform, acetonitrile, tetrahydrofurane, dichloromethane and dimethyl sulphoxide. Absorption spectra are determined for a serious of concentrations (10^{-6}–10^{-8} M) between 300-700 nm. A single maximum with a shoulder is observed in all solvents except for acetic acid and acetonitrile. The UV–vis spectra show that the bathochromic shift of λ_{max} values is greater for chloroform than that for the other solvents. The shoulders values predicted in all the solvents are very similar. Experimental absorption spectra of the compound are compared with the corresponding DFT Uv-vis results in the **Figure 7**. Upon examined the UV results obtained from DFT method, λ_{max} values of compound do not show remarkable change for all solvents used. The solvent effects on λ_{max} (nm) obtained by both the experimental measurement and theoretical methods are given in **Table 5**. Experimental and theoretical λ_{max} (nm) values for the different solvents are compatible with each other, as shown in **Table 5**.

To analyze the thermal stability of the compound, thermogravimetric analysis (TGA) is performed. Thermal stability of compound is illustrated in **Figure 8**. The absence of weight loss up to 100 ^{o}C indicates that the solids have humidity and water molecule. The curve of TGA analysis shows approximately 24% weight loss in the temperature range of 100-316 ^{o}C, which represents the presence of adsorptive solvent molecules and the initial decomposition temperatures (T_d) is 316^{o}C. Consequently, we can say that the compound exhibits good thermal stabilities up to initial decomposition temperature T_d of 316 ^{o}C.

4.4. Interaction with pBR322 plasmid DNA and DNA cleavage

DNA cleavage studies have been conducted extensively to examine the potential effect of newly synthesized compound [43-45]. When the electrophoresis behavior given in **Figure 9** is examined, two different bands can be seen clearly. First band, namely supercoiled Form I, has faster migration rate than the second band called as open circular Form II. That is, bands can be divided by each other under electrical charge. Each column in **Figure 9** refers the behavior of electrophoresis as a function of concentration of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid matter. Any change in the electrophoresis has not observed as the concentration increases. On the other hand, it has been reported in the work conducted by Redy et. al. [46] that some compounds of benzocoumarin-pyrimidine hybrids show DNA cleavage activity and they can be used as antibacterial agent. In this study, we have applied 20 µM, 40 µM, 60 µM, 80 µM and 100 µM concentrations but we do not observe any significant effect on pBR 322 DNA even if we have applied highest concentration (100 µM). In toxicological studies, it is not possible to observe any change without reaching a certain threshold. So, no change was observed as we were not able to reach the threshold value for the conditions we have set for our work.

5. Conclusion

We have synthesized 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid in this work. It is characterized by some spectroscopic studies such as ^{1}H-NMR spectra, FT-IR spectra, mass spectra, UV-visible spectra and thermal analysis technique. DNA cleavage activity of compound is also evaluated by means of agarose gel electrophoresis technique for a series of concentrations. Our results show that the 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid interaction with DNA is weakly for the concentrations applied. When using this synthesized compound for health applications, this trait should be taken into consideration. Additionally, DFT calculations are used to prove the molecular structure and some spectroscopic properties of the title compound. The chemical shift calculations of ^{1}H-NMR and ^{13}C-NMR spectra are performed via Gauge-Invariant Atomic Orbital (GIAO) method. The positions of hydrogen and carbon atoms of molecule are verified by means of the ^{1}H and ^{13}C-NMR chemical shifts computed. UV-vis spectrum analysis of the title compound has been investigated by both experimental technique and TD-DFT method. To our knowledge, spectroscopic and structural investigations of the title compound are performed experimentally as well as theoretically for the first time in this work. It is concluded that the theoretical results are in good agreement with the experimental data when the comparison between the observed and corresponding the calculated values. Hence, the theoretical data have approved that the

DFT theory with B3LYP/6-31G(d) level calculations is powerful tool to investigate the structural and vibrational properties of title compound.

Acknowledgments

The authors are grateful to SDU-BAP (Project No. 4575-YL2-16) for their financial support.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://

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Figure Captions

Figure 1. General route for the synthesis of compound.

Figure 2. The structure of compound optimized via DFT/B3LYP level with the basis set of 6-31G(d).

Figure 3. a) Measurement of FT-IR spectra of compound. b) Transmittance versus wavenumber of compound computed from DFT/B3LYP method with 6-31G(d) basis set.

Figure 4. The linear regression between the experimental and theoretical frequencies of compound.

Figure 5. ^{1}H-NMR spectra of compound.

Figure 6. Mass spectra of compound (experimentally).

Figure 7. Absorption spectra of compound a) Experimental, b) Theoretical computed from TD-DFT/B3LYP level with 6-31G(d) basis set.

Figure 8. Thermal analysis of compound (experimentally).

Figure 9. Cleavage of pBR322 DNA by different concentrations of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid matter. K. Control, 1. 20 µM, 2. 40 µM, 3. 60 µM, 4. 80 µM and 5. 100 µM.

Figure 1.

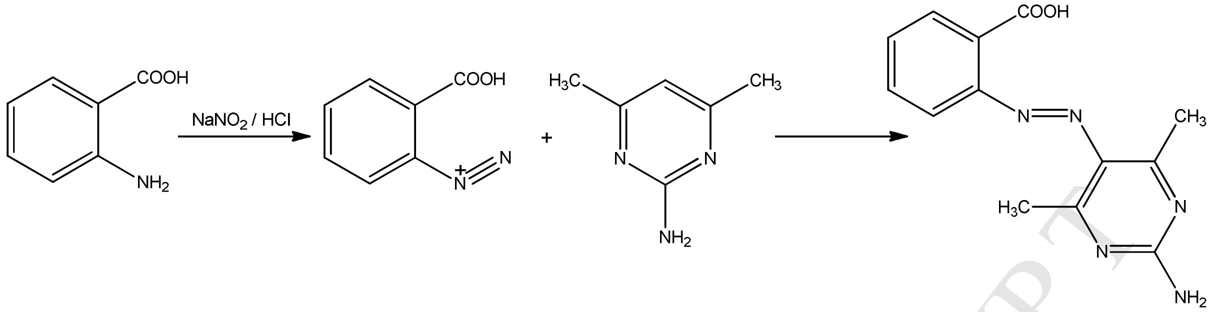


Figure 2.

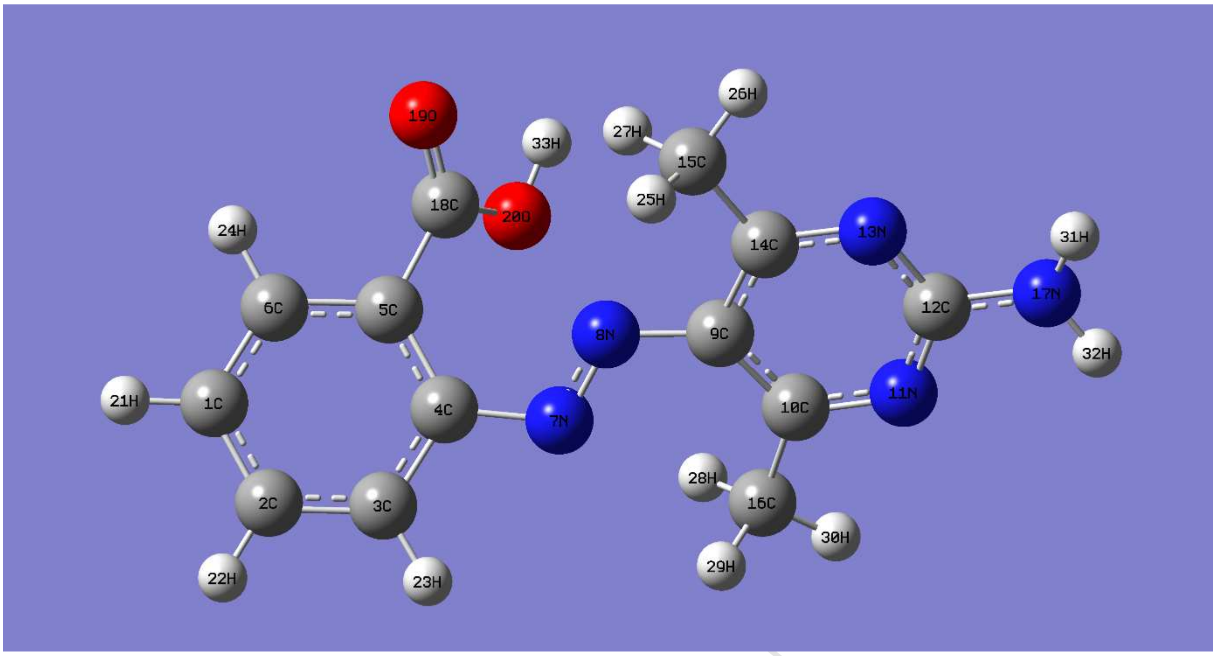
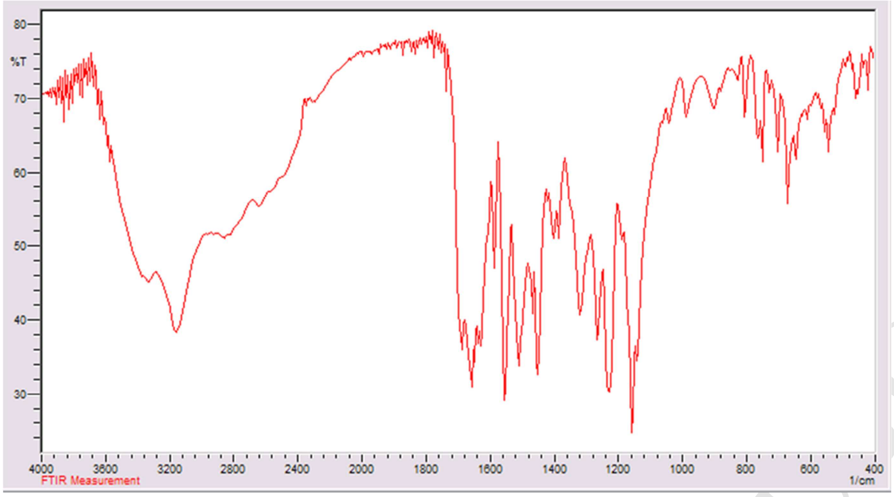

19O
33H
26H
27H
15C
25H
18C
20O
24H
14C
13N
31H
6C
5C
12C
17N
8N
9C
32H
21H
1C
4C
7N
10C
11N
28H
16C
30H
2C
3C
29H
22H
23H

Figure 3.

a)


80
%T
70
60
50
40
30
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
FTIR Measurement

b)

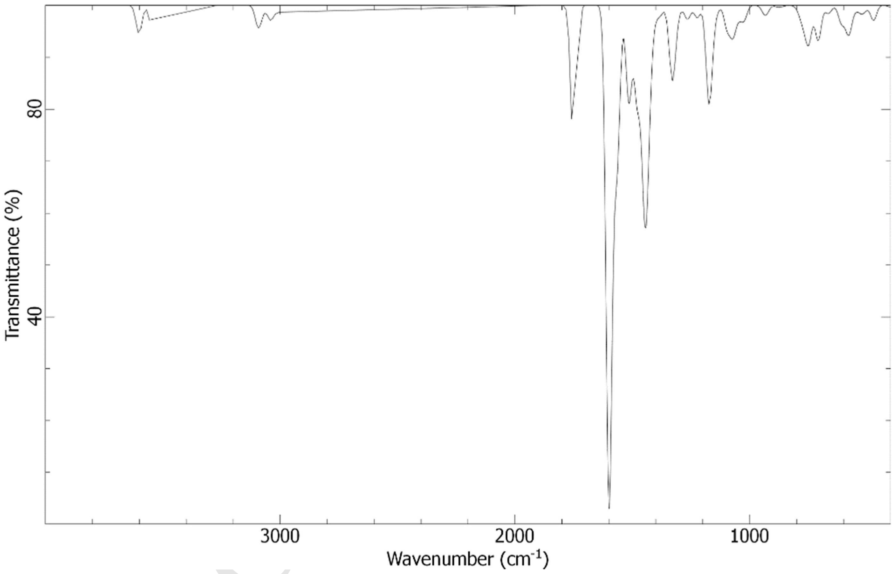

80
40
Transmittance (%)
3000
2000
1000
Wavenumber (cm-1)

Figure 4.

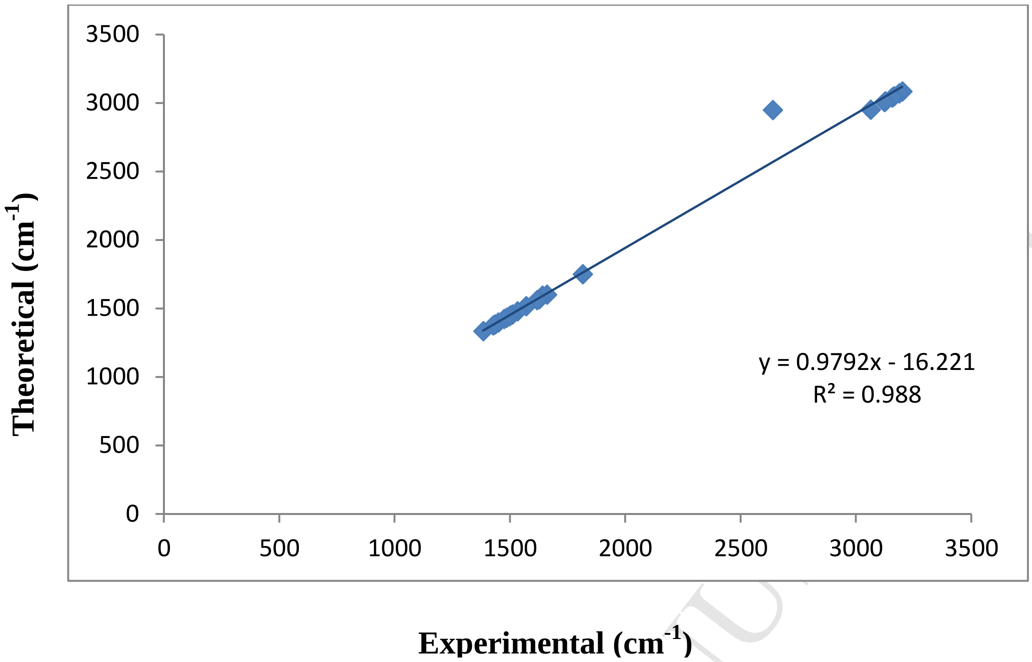

3500
3000
2500
2000
1500
1000
500
0
0
500
1000
1500
2000
2500
3000
3500
y = 0.9792x - 16.221
R² = 0.988
Theoretical (cm-1)
Experimental (cm-1)

Figure 5.

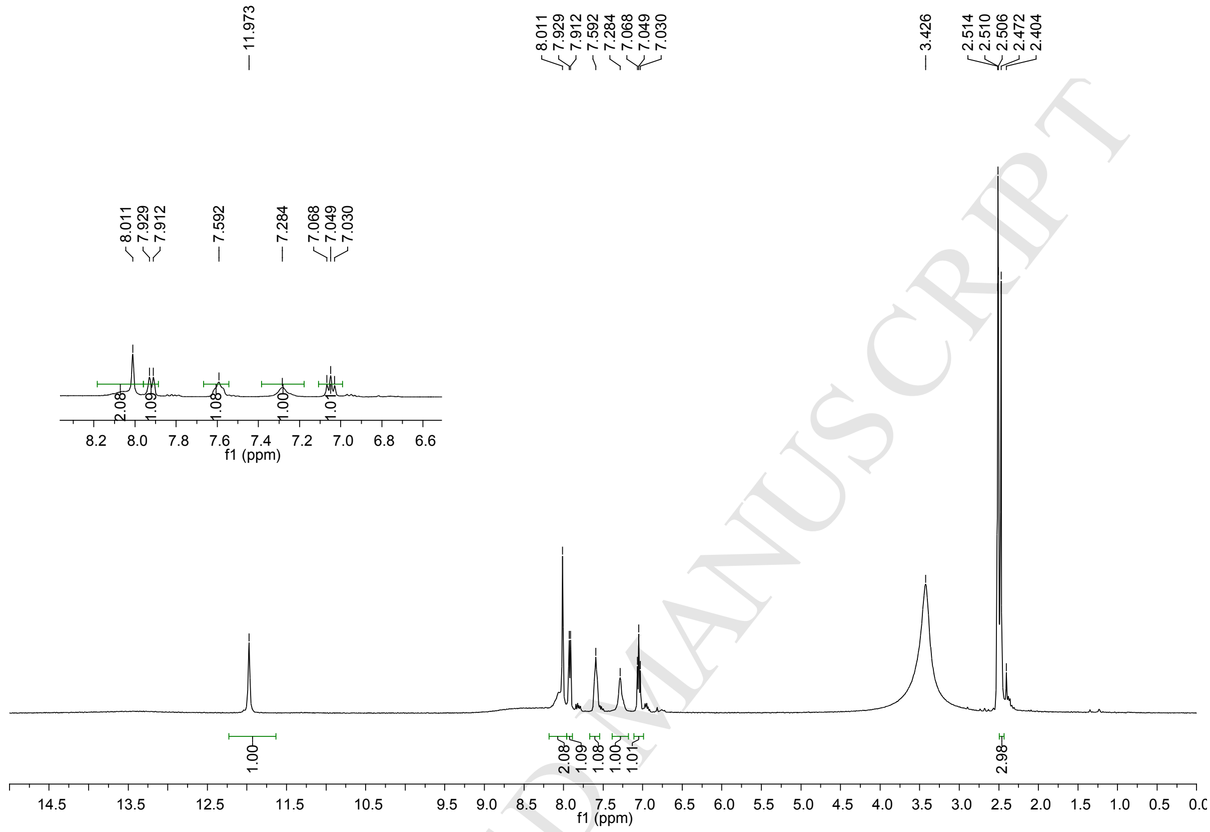

11.973
8.011
7.929
7.912
7.592
7.284
7.068
7.049
7.030
3.426
2.514
2.510
2.506
2.472
2.404
2.08
1.09
1.08
1.00
1.01
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6
f1 (ppm)
1.00
2.08
1.09
1.08
1.00
1.01
2.98
14.5 13.5 12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

Figure 6.

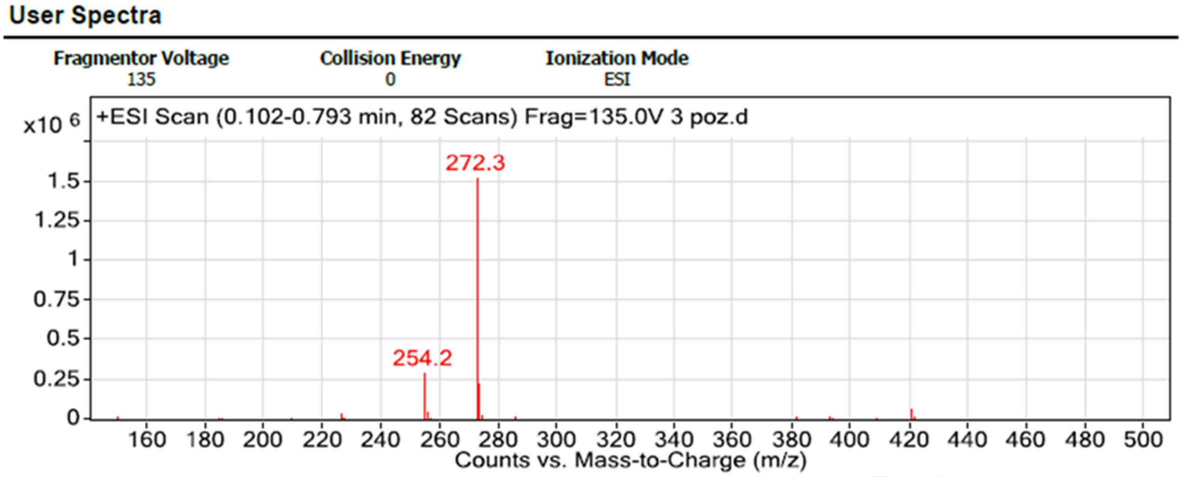

User Spectra
Fragmentor Voltage
135
Collision Energy
0
Ionization Mode
ESI
x10 6
+ESI Scan (0.102-0.793 min, 82 Scans) Frag=135.0V 3 poz.d
1.5
1.25
1
0.75
0.5
0.25
0
272.3
254.2
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
Counts vs. Mass-to-Charge (m/z)

Figure 7.

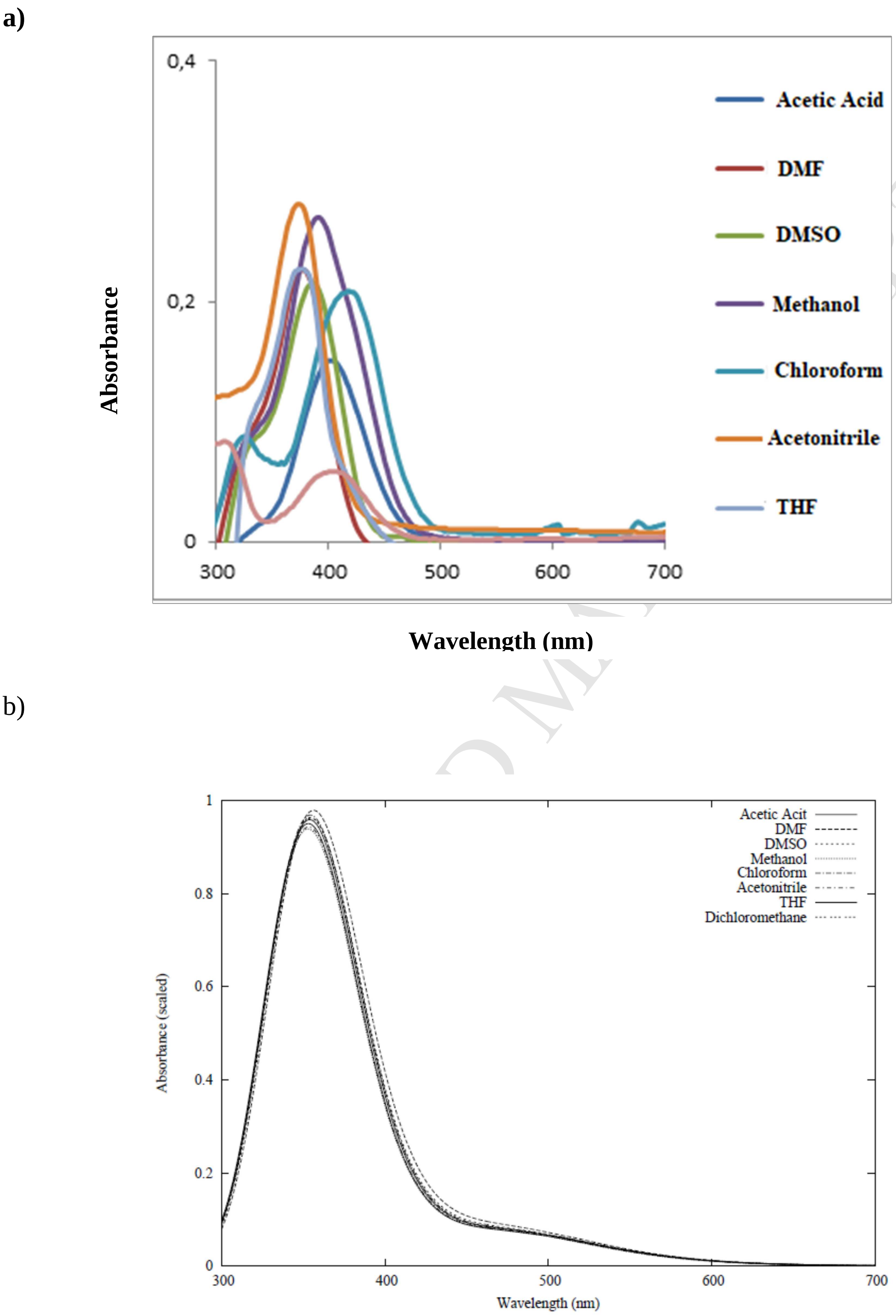

a)
0,4
0,2
0
300
400
500
600
700
Absorbance
Wavelength (nm)
Acetic Acid
DMF
DMSO
Methanol
Chloroform
Acetonitrile
THF
b)
1
0.8
0.6
0.4
0.2
0
300
400
500
600
700
Absorbance (scaled)
Wavelength (nm)
Acetic Acit
DMF
DMSO
Methanol
Chloroform
Acetonitrile
THF
Dichloromethane

Figure 8.

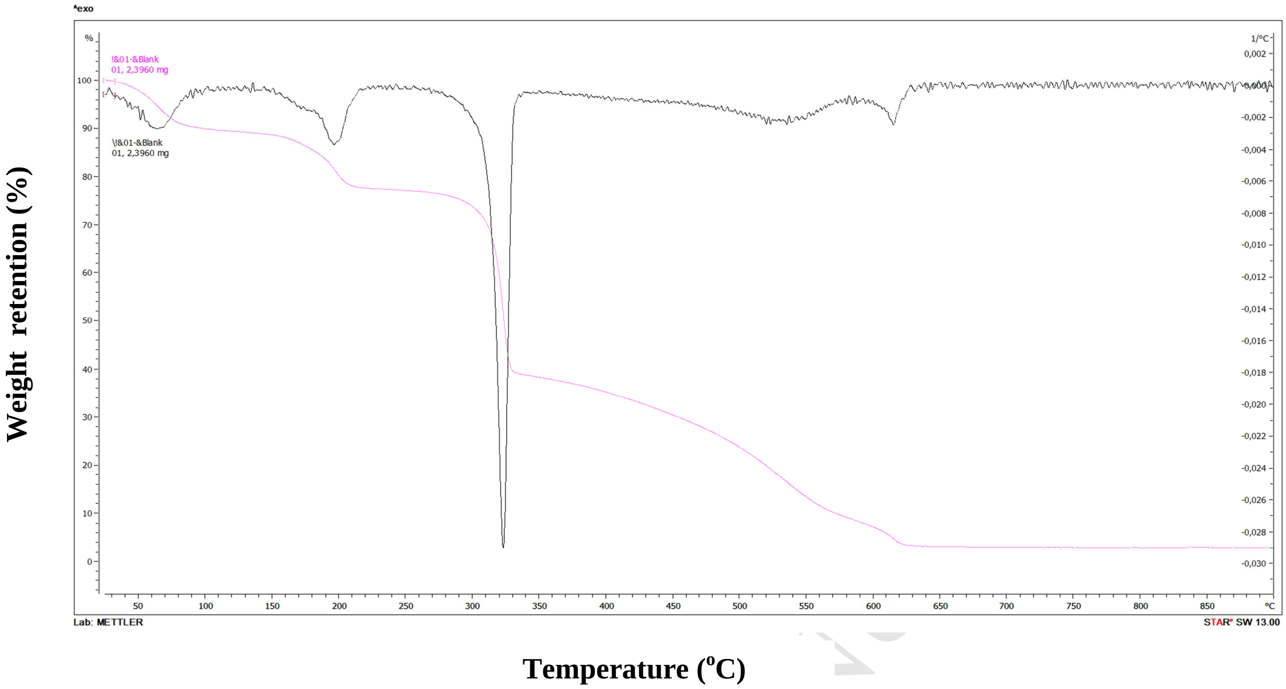

^exo
%
!&01-&Blank
01, 2,3960 mg
\!&01-&Blank
01, 2,3960 mg
100
90
80
70
60
50
40
30
20
10
0
1/°C
0,002
0,000
-0,002
-0,004
-0,006
-0,008
-0,010
-0,012
-0,014
-0,016
-0,018
-0,020
-0,022
-0,024
-0,026
-0,028
-0,030
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
°C
Lab: METTLER
STAR° SW 13.00
Weight retention (%)
Temperature (°C)

Figure 9.

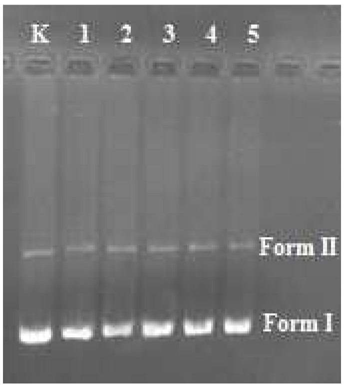


Table Captions

Table 1. The structural parameters computed from DFT/B3LYP/6-31G(d) level in the optimized ground state structure of compound; Bond length (Å), bond angle (^{o}) and dihedral angle (^{o}).

Table 2. FT-IR experimental data and theoretical values obtained from DFT/B3LYP/6-31G(d) method.

Table 3. ^{1}H-NMR experimental data and theoretical values calculated from DFT/B3LYP/6-31G(d) method.

Table 4. Theoretical ^{13}C-NMR values calculated from DFT/B3LYP/6-31G(d) method.

Table 5. The solvent effects on λ_{max} (nm) obtained by both experimental measurement and theoretical methods.

Table 1.

Parameters via Gaussian	Bond Length (Å)	DFT/ B3LYP/6-31G(d)
R (20,33)	O20-H33	0.9757
R (17,31)	N17-H31	1.0079
R (17,32)	N17-H32	1.0079
R (19,18)	O19-C18	1.2139
R (8,7)	N8-N7	1.2661
R (10,11)	N11-C10	1.3334
R (13,14)	N13-C14	1.3306
R (11,12)	N11-C12	1.3479
R (13,12)	N13-C12	1.3482
R (17,12)	N17-C12	1.3560
R (8,9)	N8-C9	1.3930
R (20,18)	O20-C18	1.3572
R (7,4)	N7-C4	1.4136
	Bond Angles (º)	
A(10,11,12)	C10-N11-C12	117.28
A(33,20,18)	H33-O20-C18	105.52
A(31,17,12)	H31-N17-C12	118.75
A(32,17,31)	H32-N17-H31	120.13
A(19,18,20)	019-C18-O20	122.30
A(8,7,4)	N8-N7-C4	112.95
A(13,14,9)	N13-C14-C9	121.75
A(11,12,13)	N11-C12-N13	127.07
A(12,13,14)	C12-N13-C14	116.25
A(17,12,11)	N17-C12-N11	116.41
	Dihedral Angles (º)	
D(8,7,4,5)	N8-N7-C4-C5	-30.04
D(13,14,9,8)	N13-C14-C9-N8	-180.89
D(11,12,13,14)	N11-C12-N13-C14	-0.60
D(14,9,8,7)	C14-C9-N8-N7	-192.38
D(20,18,5,4)	O20-C18-C5-C4	-51.40

Table 2.

Vibrational frequencies (cm^{-1})					
Experimental			DFT/B3LYP*		
ν_{Ar-H}	ν_{Alip-H}	$\nu_{N=N}$	ν_{Ar-H}	ν_{Alip-H}	$\nu_{N=N}$
3200	2640	1640	3080 (C6-H24) 3069 (C1-H21) 3069 (C2-H22) 3080 (C3-H23)	2946 (C15-H25,26,27) 2949 (C16-H28,29,30)	1442

*basis set 6-31G(d), scale factor 0.9613 [42]

Table 3.

Experimental			DFT/B3LYP		
Aro-H	**Alip-H**	**X-H**	**Aro-H**	**Alip-H**	**X-H**
7.05-7.92 (m. 4H)	2.47 (pyrimidine,CH_3)	8.01 (pyrimidine, NH_2)	7.17-7.44 (m. 4H)	2.62 (H27)	4.25 (N17-H31)
		11.97 (-COOH)		2.47 (H29)	4.28 (N17-H32)
				2.19 (H28)	5.54 (H33)
				2.11 (H25)	
				1.79 (H30)	
				1.62 (H26)	

Table 4.

DFT/B3LYP/6-31G(d) Chemical shift values of ^{13}C-NMR* (ppm)
161.49 (C14)
155.79 (C18)
146.45 (C10)
146.26 (C12)
141.74(C4)
124.78 (C9)
117.83 (C6)
117.83(C2)
115.93(C3)
114.81(C1)
108.42(C5)
20.42(C16)
15.36(C15)

*Reference: TMS B3LYP/6-311+G(2d,p) GIAO.

Table 5.

Experimental								B3LYP/B3LYP/6-31G(d)							
DMSO	**DMF**	**Methanol**	**Acetic Acid**	**Chloroform**	**Dichloro methane**	**Acetonitrile**	**THF**	**DMSO**	**DMF**	**Methanol**	**Acetic Acid**	**Chloroform**	**Dichloromethane**	**Acetonitrile**	**THF**
334-388	332-380	330-396	406	328-416	314-404	376	336-374	355	357	354	354	354	355	354	355

Appendix A

Supplementary data for

A rapid synthesis of 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid: Experimental, DFT study and DNA cleavage activity

Çiğdem KARABACAK ATAY[1], Fatih DUMAN[2], Merve GÖKALP[3], Tahir TİLKİ[3*], S. OZDEMIR KART[4]

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[3]*Süleyman Demirel University, Faculty of Science & Arts, Chemistry Department, 32260, Isparta, Turkey*

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tahirtilki@sdu.edu.tr

Table S1: Structural parameters for title compound in the ground state.

Parameters via Gaussian	Bond Length (Å)	DFT/ B3LYP/6-31G(d)
R(1,2)	C1-C2	1.3968
R(1,6)	C1-C6	1.3947
R(1,21)	C1-H21	1.0861
R(2,3)	C2-C3	1.3920
R(2,22)	C2-H22	1.0864
R(3,4)	C3-C4	1.4012
R(3,23)	C3-H23	1.0856
R(4,5)	C4-C5	1.4134
R(4,7)	C4-N7	1.4136
R(5,6)	C5-C6	1.3984
R(5,18)	C5-C18	1.4947
R(6,24)	C6-H24	1.0857
R(7,8)	N7-N8	1.2661
R(8,9)	N8-C9	1.3930
R(9,10)	C9-C10	1.4242
R(9,14)	C9-C14	1.4214
R(10,11)	C10-N11	1.3334
R(10,16)	C10-C16	1.5028
R(11,12)	N11-C12	1.3479
R(12,13)	C12-N13	1.3482
R(12,17)	C12-N17	1.3561
R(13,14)	N13-C14	1.3307
R(14,15)	C14-C15	1.5060
R(15,25)	C15-H25	1.0967
R(15,26)	C15-H26	1.0919
R(15,27)	C15-H27	1.0930

R(16,28)	C16-H28	1.0947
R(16,29)	C16-H29	1.0945
R(16,30)	C16-H30	1.0924
R(17,31)	N17-H31	1.0080
R(17,32)	N17-H32	1.0080
R(18,19)	C18-O19	1.2139
R(18,20)	C18-O20	1.3572
R(20,33)	O20-H33	0.9758
	Bond Angles (º)	
A(2,1,6)	C2-C-C6	119.7889
A(2,1,21)	C2-C1-H21	120.3552
A(6,1,21)	C6-C1-H21	119.8509
A(1,2,3)	C1-C2-C3	119.8822
A(1,2,22)	C1-C2-H22	120.2594
A(3,2,22)	C3-C2-H22	119.8567
A(2,3,4)	C2-C3-C4	120.7086
A(2,3,23)	C2-C3-H23	121.3321
A(4,3,23)	C4-C3-H23	117.9276
A(3,4,5)	C3-C4-C5	119.4763
A(3,4,7)	C3-C4-N7	115.9606
A(5,4,7)	C5-C4-N7	124.5544
A(4,5,6)	C4-C5-C6	119.0494
A(4,5,18)	C4-C5-C18	124.2160
A(6,5,18)	C6-C5-C18	116.6963
A(1,6,5)	C1-C6-C5	120.9821
A(1,6,24)	C1-C6-H24	120.4774
A(5,6,24)	C5-C6-H24	118.5295
A(4,7,8)	C4-N7-N8	112.9540

A(7,8,9)	N7-N8-C9	118.3174
A(8,9,10)	N8-C9-C10	127.9575
A(8,9,14)	N8-C9-C14	114.8943
A(10,9,14)	C10-C9-C14	117.1084
A(9,10,11)	C9-C10-N11	120.5298
A(9,10,16)	C9-C10-C16	124.0221
A(11,10,16)	N11-C10-C16	115.4302
A(10,11,12)	C10-N11-C12	117.2809
A(11,12,13)	N11-C12-N13	127.0687
A(11,12,17)	N11-C12-N17	116.4135
A(13,12,17)	N13-C12-N17	116.5053
A(12,13,14)	C12-N13-C14	116.2503
A(9,14,13)	C9-C14-N13	121.7479
A(9,14,15)	C9-C14-C15	121.6340
A(13,14,15)	N13-C14-C15	116.6034
A(14,15,25)	C14-C15-H25	110.3658
A(14,15,26)	C14-C15-H26	109.1017
A(14,15,27)	C14-C15-H27	111.9538
A(25,15,26)	H25-C15-H26	108.6438
A(25,15,27)	H25-C15-H27	106.3258
A(26,15,27)	H26-C15-H27	110.3779
A(10,16,28)	C10-C16-H28	111.2373
A(10,16,29)	C10-C16-H29	111.0122
A(10,16,30)	C10-C16-H30	108.6418
A(28,16,29)	H28-C16-H29	105.7029
A(28,16,30)	H28-C16-H30	110.4709
A(29,16,30)	H29-C16-H30	109.7595
A(12,17,31)	C12-N17-H31	118.7540

A(12,17,32)	C12-N17-H32	118.7864
A(31,17,32)	H31-N17-H32	120.1262
A(5,18,19)	C5-C18-O19	124.5698
A(5,18,20)	C5-C18-O20	112.8178
A(19,18,20)	O19-C18-O20	122.3028
A(18,20,33)	C18-O20-H33	105.5161
	Dihedral Angles (º)	
D(6,1,2,3)	C6-C1-C2-C3	1.0528
D(6,1,2,22)	C6-C1-C2-H22	-179.4115
D(21,1,2,3)	H21-C1-C2-C3	-179.7559
D(21,1,2,22)	H21-C1-C2-H22	-0.2202
D(2,1,6,5)	C2-C1-C6-C5	0.0304
D(2,1,6,24)	C2-C1-C6-H24	178.8086
D(21,1,6,5)	H21-C1-C6-C5	-179.1649
D(21,1,6,24)	C21-C1-C6-H24	-0.3867
D(1,2,3,4)	C1-C2-C3-C4	0.4835
D(1,2,3,23)	C1-C2-C3-H23	178.3918
D(22,2,3,4)	H22-C2-C3-C4	-179.0541
D(22,2,3,23)	H22-C2-C3-H23	-1.1458
D(2,3,4,5)	C2-C3-C4-C5	-3.0657
D(2,3,4,7)	C2-C3-C4-N7	175.9129
D(23,3,4,5)	H23-C3-C4-C5	178.9564
D(23,3,4,7)	H23-C3-C4-N7	-2.0650
D(3,4,5,6)	C3-C4-C5-C6	4.0819
D(3,4,5,18)	C3-C4-C5-C18	-173.5864
D(7,4,5,6)	N7-C4-C5-C6	-174.8030
D(7,4,5,18)	N7-C4-C5-C18	7.5287
D(3,4,7,8)	C3-C4-N7-N8	-148.8798

D(5,4,7,8)	C5-C4-N7-N8	30.0405
D(4,5,6,1)	C4-C5-C6-C1	-2.6001
D(4,5,6,24)	C4-C5-C6-H24	178.5984
D(18,5,6,1)	C18-C5-C6-C1	175.2418
D(18,5,6,24)	C18-C5-C6-H24	-3.5597
D(4,5,18,19)	C4-C5-C18-O19	-134.8834
D(4,5,18,20)	C4-C5-C18-O20	51.4010
D(6,5,18,19)	C6-C5-C18-O19	47.3981
D(6,5,18,20)	C6-C5-C18-O20	-126.3174
D(4,7,8,9)	C4-N7-N8-C9	-176.6114
D(7,8,9,10)	N7-N8-C9-C10	14.7643
D(7,8,9,14)	N7-N8-C9-C14	-167.6174
D(8,9,10,11)	N8-C9-C10-N11	178.3930
D(8,9,10,16)	N8-C9-C10-C16	0.0045
D(14,9,10,11)	C14-C9-C10-N11	0.8200
D(14,9,10,16)	C14-C9-C10-C16	-177.5685
D(8,9,14,13)	N8-C9-C14-N13	-179.1072
D(8,9,14,15)	N8-C9-C14-C15	2.3317
D(10,9,14,13)	C10-C9-C14-N13	-1.2167
D(10,9,14,15)	C10-C9-C14-C15	-179.7778
D(9,10,11,12)	C9-C10-N11-C12	0.1907
D(16,10,11,12)	C16-C10-N11-C12	178.7117
D(9,10,16,28)	C9-C10-C16-H28	48.7624
D(9,10,16,29)	C9-C10-C16-H29	-68.6457
D(9,10,16,30)	C9-C10-C16-H30	170.5715
D(11,10,16,28)	N11-C10-C16-H28	-129.7005
D(11,10,16,29)	N11-C10-C16-H29	112.8913
D(11,10,16,30)	N11-C10-C16-H30	-7.8915

D(10,11,12,13)	C10-N11-C12-N13	-0.9806
D(10,11,12,17)	C10-N11-C12-N17	-179.6417
D(11,12,13,14)	N11-C12-N13-C14	0.6037
D(17,12,13,14)	N17-C12-N13-C14	179.2638
D(11,12,17,31)	N11-C12-N17-H31	-171.8043
D(11,12,17,32)	N11-C12-N17-H32	-9.1136
D(13,12,17,31)	N13-C12-N17-H31	9.3895
D(13,12,17,32)	N13-C12-N17-H31	172.0802
D(12,13,14,9)	C12-N13-C14-C9	0.5476
D(12,13,14,15)	C12-N13-C14-C15	179.1775
D(9,14,15,25)	C9-C14-C15-H25	70.6814
D(9,14,15,26)	C9-C14-C15-H26	-170.0157
D(9,14,15,27)	C9-C14-C15-H27	-47.5424
D(13,14,15,25)	N13-C14-C15-H25	-107.9502
D(13,14,15,26)	N13-C14-C15-H26	11.3527
D(13,14,15,27)	N13-C14-C15-H27	133.8260
D(5,18,20,33)	C5-C18-O20-H33	178.4932
D(19,18,20,33)	O19-C18-O20-H33	4.6150

Highlights

- The newly synthesized 2-((2-amino-4,6-dimethylpyrimidine-5yl)diazenyl)benzoic acid was prepared by diazotization of anthranilic acid and coupling with 2-amino-4,6-dimethylpyrimidine.
- The DNA cleavage activity of compound was evaluated by agarose gel electrophoresis with a series of concentrations.
- DNA cleavage studies have been conducted extensively to examine the potential effect of newly synthesized compound.
- The computations of the chemical shifts for 1H-NMR of the title compound have been carried out via Gauge-Invariant Atomic Orbital (GIAO) method utilizing the same basis set.
- DFT results are compatible with the experimental results.