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TETRAHEDRON

On the Tautomerism of 2,1,3-Benzothiadiazinone *S,S*-Dioxide and Related Compounds

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Dedicated to Prof. Dr. J. Elguero on his 65 anniversary

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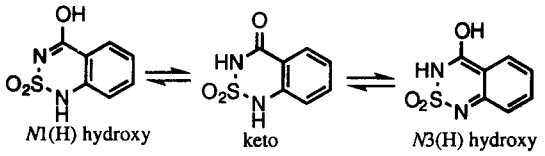
Abstract.- Tautomerism of 2,1,3-benzothiadiazinone 2,2-dioxide and other related fused heterocyclic amides has been studied by means of experimental (^{1}H- and ^{13}C-NMR) and theoretical (*ab initio* calculations) techniques. The synthesis and spectroscopic characterization of mono- and di-blocked derivatives was carried out. The results predict that the keto form is the most abundant tautomer in the gas phase, while the N1(H) hydroxy is the preferred one in solution and the solid state.

Keywords: Benzothiazines; Tautomerism; Theoretical studies; NMR

Tautomerism involves a large modification in the reactive characteristics of molecules, including their biological and pharmacological properties.[1] Experimental and theoretical studies have demonstrated that the tautomer population can be modified by the presence of substituents and also by the influence of environment.[2] Assuming that, typically, only one of the tautomeric forms is the bioactive species, the tautomerism can be considered *a priori* as a possible mechanism to control the activity of the molecules with pharmacological potential, as is the case of purine and pyrimidine derivatives.[3]

Recently, we have discovered the potent antiviral activity of 2,1,3-benzothiadiazinone *S,S*-dioxide derivatives.[4] They show activity against both cytomegalovirus and human inmunodeficiency virus,[5] and can be considered as new leads among the antiviral compounds for the treatment of the most common opportunistic infections in patients suffering from AIDS or any organ transplant.[6] In the 2,1,3-benzothiadiazinone dioxide fused ring system, in addition to the annular prototropy, a ring-substituent tautomerism is also present as in all heterocyclic amides. In this case the tautomerism is the result of two opposing effects: (i) the tendency of the amide function to exist as such (and not in the imidate lactim form) and (ii) the aromaticity of the hydroxy tautomer.[7]



*N*1(H) hydroxy keto *N*3(H) hydroxy

Continuing our interest into this problem,[8-10] here we present an experimental and theoretical study of the tautomerism of 2,1,3-benzothiadiazine *S,S*-dioxide and related substituted or unsubstituted compounds in different environments which leads to a deeper knowledge of these biological active heterocycles. The synthesis of three different monosubstituted derivatives and two of the three possible disubstituted ones has been achieved to provide blocked models for this study.

RESULTS AND DISCUSSION

SYNTHESIS

The benzothiadiazine dioxide ring **1** was obtained in a two-step synthesis following the Cohen and Klarberg procedure[11] starting from methyl anthranilate and sulfamoyl chloride. Alkylation of this heterocycle in a polar, non-protic solvent (DMF) in the presence of a strong, non-nucleophilic base (NaH) with 4-biphenylylmethyl chloride afforded a complex mixture of several compounds (Scheme 1). From this reaction, five pure biphenylylmethyl derivatives **2-6** could be isolated by chromatography. The monoalkylation products (**2-4**) were obtained in a 2:1 ratio over the two disubstituted derivatives **5** and **6**. Moreover, the three different monosubstituted derivatives were isolated in a ratio 5:2:1 for derivatives **2**:**3**:**4**, whilst in the disubstituted derivatives obtained, *N,N*-dialkylation predominated over the *N,O*-disubstitution in a ratio 16:1 for compounds **5**:**6**.

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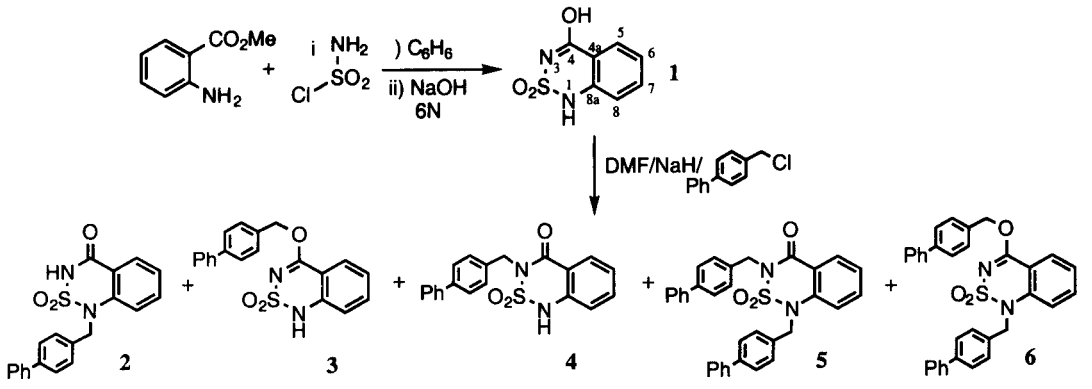


Scheme 1

The structures of these compounds were elucidated from their analytical and spectroscopic data (^1H and ^{13}C NMR) which are collected in Table 1 and in the Experimental. Unequivocal assignment of all chemical shifts was done using 2-dimensional experiments such as COSY or HMQC for one-bond correlation. We have checked that the CH_2 of substituent at $N(1)$ in **2** gave a ^1H resonance at higher field, but a ^{13}C resonance at lower field, than the corresponding signals observed for the same substituent at $N(3)$ in the isomer **4**. This was previously observed in some related diacyclonucleosides.[12] The alkylation site was determined by means of NOE experiments and from sequences of HMBC for long distance/carbon correlation. Thus, $N1$-CH_2 correlated exclusively with the quaternary carbon C-8a, while $N3$-CH_2 or O-CH_2 correlated with the heterocyclic carbon C-4. When alkylation takes place at $N1$, additional NOE effects could be observed between signals corresponding to $N1$-CH_2 and H-8.

Table 1. Some representative ^{13}C NMR (δ_C) and ^1H NMR (δ_H) data of benzothiadiazinone dioxides **1-6**

No. Solvent	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	N_1-CH_2	N_3-CH_2	O-CH_2	N_1-CH_2	N_3-CH_2	O-CH_2
1 DMSO-d6	164.33	113.73	128.23	122.76	135.48	117.72	140.62	-	-	-	-	-	-
THF-d8	165.07	118.68	131.13	125.26	136.93	120.37	142.26	-	-	-	-	-	-
MeOD-d4	166.14	115.93	129.75	124.27	136.58	119.02	141.90	-	-	-	-	-	-
CP/MAS	166.96	113.58	126.92	124.01	131.05	116.97	138.81	-	-	-	-	-	-
2 DMSO-d6	165.66	119.61	131.82	119.10	137.11	113.65	142.18	46.11	-	-	4.99	-	-
3 DMSO-d6	162.23	109.46	129.07	122.08	135.99	116.73	142.14	-	-	69.36	-	-	5.52
4 DMSO-d6	162.33	117.56	129.32	123.44	135.80	120.29	139.80	-	44.29	-	-	5.06	-
5 $CDCl_3$	162.18	122.80	130.58	126.40	134.73	122.03	140.65	55.79	46.27	-	4.91	5.00	-
6 $CDCl_3$	166.83	113.58	130.40	123.05	136.83	117.04	141.52	50.24	-	71.62	5.25	-	5.55

TAUTOMERISM IN SOLUTION

The ^{13}C NMR data of benzothiadiazinone **1** recorded in different solvents are gathered in Table 1. The ^{13}C chemical shifts differences found, specially in those signals belonging to the thiadiazine ring (C-4, C-4a and C-8a), evidence a prototropic process in which the equilibrium tautomer population is modified by the nature of the solvent. By comparison of the C-4a chemical shift of **1** with those of the model compounds **5** and **6**, in which the prototropy is blocked, it can be concluded that benzothiadiazinone **1** exists mainly as the $N1$(H)-hydroxy form in the polar aprotic solvent, dimethyl sulphoxide, while in non-polar solvent, such as tetrahydrofuran, the population of the keto form increases considerably. Protic solvents, such as methanol, increase the rate of the prototropic exchange, and the C-4a chemical shift reflects the existence of an equal population of the two main tautomers ($N1$(H)-hydroxy and keto) in solution.

Tautomerism in monosubstituted benzothiadiazinones **2-4** is also present. Protrotopy in the $N3$-

substituted parent heterocycle (1,2,6-thiadiazin-4-one dioxide) has been previously studied,[13] and the keto form is more abundant than the hydroxy and the CH- possible tautomers. In our case, and taking into account mainly the C-4a chemical shift, by comparison with those of the model compounds, we observe that *N*1- and *N*3-monosubstituted derivatives (compounds **2** and **4**) are principally in the keto form. The upfield shift of the corresponding signal for compound **3**, which is probably due to anisotropy of the benzene ring, does not allow us to assign the tautomeric form in this case.

TAUTOMERISM IN SOLID STATE

The ^{13}C CP/MAS spectrum of derivative **1** (Table 1) indicates that in the solid state, which can be considered as a highly polar medium, the benzothiadiazinone **1** exits as a single tautomer, since each carbon appears as a unique signal. By comparison of the C-4a chemical shift in the solid state with those found in solution, in particular with compounds **5** and **6** in which the protrotopy is completely blocked, it can be concluded that compound **1** exits as the tautomer *N*1(H)-hydroxy in the solid state.

TAUTOMERISM IN GAS PHASE (THEORETICAL CALCULATIONS)

The tautomeric equilibrium of benzothiadiazine **1** has been also studied in the gas-phase using molecular orbital calculations following the *ab initio* method at HF/6-31G* level, which has been shown to provide excellent geometrical descriptors in systems containing the sulfamido moiety,[14] and one density functional theory based, the *ab initio* method BLYP/6-31G*.[15]

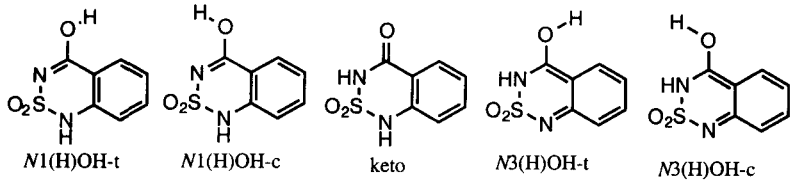


Figure 1

The geometries of the three possible tautomers of compound **1** together with two different conformations for the hydroxy forms depicted in Figure 1 were fully optimized at the *ab initio* level indicated. The relative energies together with their calculated dipole moment are collected in Table 2.

Based on relative energy of tautomers, some conclusion can be drawn. The *N*3(H)OH tautomer is quite unstable, probably due by the unfavorable repulsions arising from 1,4 interactions between hydrogens. In all cases, the keto form was found to be the most stable tautomer in the gas phase. However, since the *N*1(H)OH form is more polar than the keto one, electrostatic stabilization in polar solvents or in the solid state could favour this OH-tautomer, which is in agreement with the experimental results previously described.

Table 2. Relative energies (ΔE kcal mol^{-1}) and dipole moment (μ D) of different tautomers of benzothiadiazinone **1**

Method	keto		*N*1(H)OH-t		*N*1(H)OH-c		*N*3(H)OH-t		*N*3(H)OH-c	
	ΔE	μ	ΔE	μ	ΔE	μ	ΔE	μ	ΔE	μ
HF/3-21G*	0.00	5.33	10.85	6.87	23.46	10.02	35.39	9.71	36.77	8.18
HF/6-31G*	0.00	5.04	6.14	6.81	15.62	9.61	29.95	9.48	31.46	8.21
BLYP/6-31G*	0.00	4.03	4.51	5.25			20.83	7.44		

Monosubstituted benzothiadiazinone tautomerism was also studied theoretically . The *ab initio* calculations at HF/6-31G* level gave energy differences (Figure 2), which showed a clear preference for the keto tautomer in *N*1- and *N*3-monosubstituted compounds. In the *O*-alkyl derivative, the *N*1(H) tautomer is preferred because of better π bond delocalisation in the fused benzene ring.

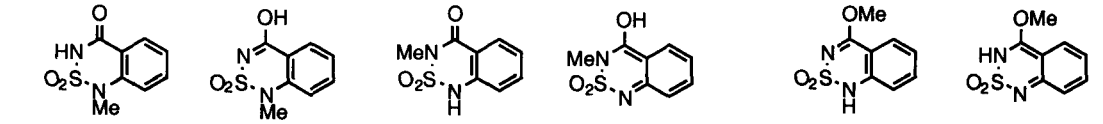


Figure 2

On the other hand, relative energies calculated at HF/6-31G* level for dimethyl derivatives (Figure 3) could account for the experimental ratio found for compounds **5** and **6**, in which the *N,N*-disubstituted benzothiadiazinone **5** was the major product and the *N3,O*-derivative was not be detected.

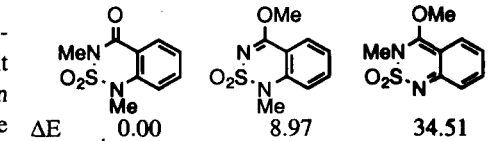


Figure 3

ACID-BASE PROPERTIES OF BENZOTHIADIAZINONE

Finally, the pK_a values of benzothiadiazinone **1** have been experimentally determined in water using UV spectroscopy.[17] Considering the two ionizable groups present in benzothiadiazinone system, the ionization sequence was established by comparison of the pK_a values of the partially blocked compound *N3*-isopropyl benzothiadiazinone and the ionization sequence of related thienothiadiazinone. Therefore, the ionization sequence should begin by the formation of *N*1-monoanion (pK_a 0.96) followed by the deprotonation of *N*3 (pK_a 7.95) yielding the corresponding dianion (Figure 4).

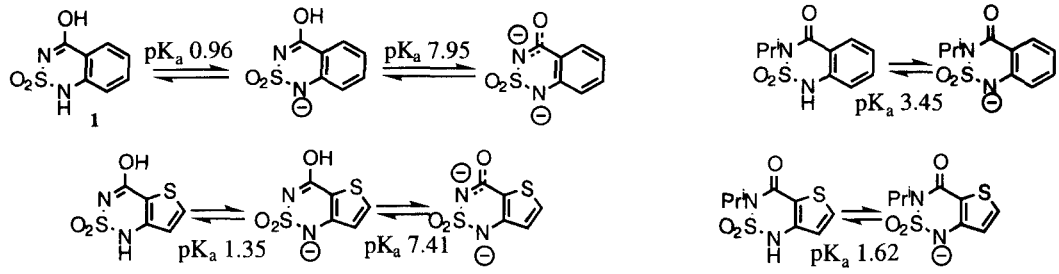


Figure 4

In order to complete these studies, we have also performed *ab initio* calculation at 6-31G* level of the *N*1- and *N*3-benzothiadiazinone monoanions. The relative energy of these two charged forms shows that the *N*1-monoanion is energetically more favoured by 5.02 kcal mol^{-1}, which is in agreement with the proposed ionization sequence.

Likewise, considering some representative distances, which are collected in Figure 5, we could explain the regioselectivity observed in alkylation reaction. Hence, in the *N*3-monoanion, the carbonyl bond character reflected in the C-4,O distance, together with the single bond character between C-4 and N3, suggest that the charge delocalization is more centred on the nitrogen atom than the oxygen. This is consistent with the experimentally determined benzylation ratio of 16:1 for compounds **5**:**6**.

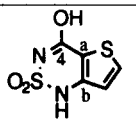
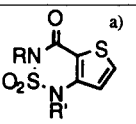
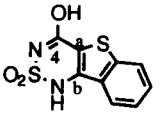
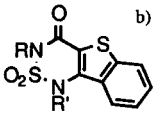
d(C-4,O) = 1.19 Å	d(C-4,O) = 1.31 Å	d(C-4,O) = 1.21 Å	d(C-4,O) = 1.21 Å
d(C-4,N3) = 1.39 Å	d(C-4,N3) = 1.27 Å	d(C-4,N3) = 1.36 Å	d(C-4,N3) = 1.34 Å

Figure 5

CONCLUSIONS

Concerning the tautomerism of benzothiadiazinone, since the *N*1(H)-hydroxy form is more polar than the keto one, polar solvents or the solid state favours this hydroxy tautomer, while in absence of such effects, as in the gas phase, the most stable (keto) tautomer, is the most abundant. This conclusion can be extrapolated to other fused thiadiazinones (Table 5), in which, considering the quaternary carbon adjacent to the C=O chemical shift, we observe the same previously described behaviour, *i.e.* a more abundant population of the *N*1(H)-hydroxy tautomer in polar environments.

Table 5. Some representative ^{13}C NMR (δ_C) data of thieno- and benzothieno-thiadiazinone dioxides

	Solvent	C-4	C-a	C-b		Solvent	C-4	C-a	C-b
	DMSO-d_6	160.82	110.20	146.22		$CDCl_3$	157.34	114.36	144.75
	THF-d_8	161.26	114.41	146.74					
	MeOD-d_4	162.48	112.62	147.14					
	DMSO-d_6	161.96	106.89	142.40		$CDCl_3$	158.47	111.72	137.27
	THF-d_8	162.34	109.39	142.36					
	MeOD-d_4	163.41	111.03	142.09					

a) R = Bn, R' = acetoxyethoxymethyl (ref. 18); b) R = R' = Bn (ref. 19)

EXPERIMENTAL

Melting points were determined with a Reichert-Jung Thermovar apparatus and are uncorrected. Flash column chomatography was carried out at medium pressure using silica gel (E. Merck, Grade 60, particle size 0.040-0.063 mm, 230-240 mesh ASTM) with the indicated solvent as eluent. Preparative thin-layer chromatography (TLC) was performed on 20 x 20 cm glass plates coated with a 2 mm layer of silica gel PF_{254} (Merck). IR spectra were obtained with a Perkin-Elmer 681 infrared spectrophotometer. ^1H NMR spectra were obtained on Varian XL-300 spectrometer working at 300 MHz. Typical spectral parameters were spectral width 10 ppm, pulse width 9 μs (57°), data size 32 K. NOE difference spectra were measured under the same conditions, using a presaturation time of 3 s. ^{13}C NMR experiments were carried out on the Varian XL-300 spectrometer operating at 75 MHz. The acquisiton parameters were spectral width 16 kHz, acquisition time 0.99 s, pulse width 9 μs (57°), data size 32 K. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si and *J* values are reported in Hertz. Mass spectra (MS) were obtained by electronic impact at 70 eV in a VG 12-250 spectrometer (VG Masslab). Elemental analyses were carried out in a Perkin-Elmer 240C equipment in the Centro de Química Orgánica "Manuel Lora Tamayo" (CSIC).

1-(4-Biphenylylmethyl)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide (**2**), *4-(4-biphenylylmethoxy)-1H-2,1,3-benzothiadiazine 2,2-dioxide* (**3**), *3-(4-biphenylylmethyl)-2,1,3-benzothiadiazin-4(1H)-one 2,2-dioxide* (**4**), *1,3-di(4-biphenylylmethyl)-2,1,3-benzothiadiazinone 2,2-dioxide* (**5**), *1-(4-biphenylylmethyl)-4-(4-biphenylylmethoxy)-2,1,3-benzothiadiazine 2,2-dioxide* (**6**). To a suspension of sodium hydride (0.04 g, 1.6 mmol) in DMF, was added benzothiadiazine dioxide **1** (0.26 g, 1.3 mmol) and 4-biphenylylmethyl chloride (0.39 g, 1.9 mmol). The reaction mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure. The residue was dissolved in water and the aqueous phase was extracted with dichlorometane (2 x 10 ml). The organic phase was dried over sodium sulfate and the solvent evaporated under reduced pressure. The residue was chromatographed on silica gel column eluting with CH_2Cl_2. Two fractions were separated. The first one which consisted of dibenzylderivatives **5** and **6** was separated by TLC eluting with CH_2Cl_2/hexane 1:1. The faster running band afforded derivative (**5**) (yield 0.04 g, 7 %), as a white solid, m.p. 180-182 °C; UV (MeOH) λ_{max}: 251.8, 203.5 nm. IR (KBr) ν_{max}: 1140, 1350 (SO_2); 1620 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.91 (s, 2H, N_1-CH_2), 5.00 (s, 2H, N_3-CH_2), 8.07 (dd, 1H, J_{H5H6} = 7.9, J_{H5H7} = 1.4, H-5), 7.06-7.50 (m, 21H, Ar-H, H-6, H-7, H-8). MS m/z (rel intensity): 167 (100), 530 (M^+, 5). Anal. Calcd. for $C_{33}H_{26}N_2O_3S$: C, 74.72; H, 4.90; N, 5.27; S, 6.04. Found: C, 74.69; H, 4.96; N, 5.20; S, 5.90.

From the lower running band, derivative (**6**) (yield 0.003 g, 0.4 %) was isolated as a white solid, m.p. 182-183 °C; UV (MeOH) λ_{max}: 254.8, 216.2 nm. IR (KBr) ν_{max}: 1170, 1360 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.25 (s, 2H, N_1-CH_2), 5.55 (s, 2H, *O*-CH_2), 7.06 (dd, 1H, J_{H6H8} = 0.7, J_{H7H8} = 8.5, H-8), 7.08 (t, 1H, J_{H5H6} = 7.9, J_{H6H7} = 7.3, H-6), 7.24-7.65 (m, 19H, Ar-H, H-7), 7.97 (dd, 1H, J_{H5H6} = 7.9, J_{H5H7} = 1.5, H-5). MS m/z (rel intensity): 167 (100), 530 (M^+, 8). Anal. Calcd. for $C_{33}H_{26}N_2O_3S$: C, 74.72; H, 4.90; N, 5.27; S, 6.04. Found: C, 74.44; H, 5.10; N, 5.15; S, 5.99.

The second column fraction was rechromatographed on a silica gel column using CH_2Cl_2/MeOH (50:1) as eluent. From the first fraction was isolated derivative (**3**) (yield 0.04 g, 10 %), as a white solid, m.p. 190-192 °C; UV (MeOH) λ_{max}: 254.3, 217.3 nm. IR (KBr) ν_{max}: 1140, 1320 (SO_2); 3220 (NH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.52 (s, 2H, *O*-CH_2), 7.13 (d, 2H, *J* = 8.3, H-8), 7.17 (t, 2H, *J* = 7.4, H-6), 7.38-7.74 (m,

10H, Ar-H, H-7), 7.89 (d, 1H, J = 8.0, H-5). MS m/z (rel intensity): 167 (100), 364 (M^+, 11). Anal. Calcd. for $C_{20}H_{16}N_2O_3S$: C, 65.94; H, 4.39; N, 7.68; S, 8.80. Found: C, 65.71; H, 4.21; N, 7.38; S, 8.58.

From the second fraction derivative (**4**) (yield 0.02 g, 4 %), was isolated as a syrup; UV (MeOH) λ_{max}: 252.1, 203.3 nm. IR (KBr) ν_{max}: 1160, 1340 (SO_2); 1610 (C=O); 3210 (NH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 5.06 (s, 2H, N-CH_2), 7.14 (d, 2H, J = 8.1, H-8), 7.25 (t, 2H, J = 7.3, H-6), 7.32-7.67 (m, 10H, Ar-H, H-7), 7.99 (d, 1H, J = 7.9, H-5). MS m/z (rel intensity): 167 (100), 364 (M^+, 14). Anal. Calcd. for $C_{20}H_{16}N_2O_3S$: C, 65.94; H, 4.39; N, 7.68; S, 8.80. Found: C, 65.71; H, 4.22; N, 7.75; S, 8.73.

From the third fraction was isolated derivative (**2**) (yield 0.008 g, 2 %), as a syrup; UV (MeOH) λ_{max}: 251.4, 204.1 nm. IR (KBr) ν_{max}: 1130, 1360 (SO_2); 1650 (C=O); 3230 (NH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 4.99 (s, 2H, N-CH_2), 6.79 (dd, 1H, J_{H6H8} = 0.6, J_{H7H8} = 8.3, H-8), 6.87 (t, 1H, J_{H5H6} = 7.7, J_{H6H7} = 7.3, H-6), 7.24-7.63 (m, 10H, Ar-H, H-7), 7.89 (dd, 1H, J_{H5H7} = 1.7, H-5). MS m/z (rel intensity): 167 (100), 364 (M^+, 16). Anal. Calcd. for $C_{20}H_{16}N_2O_3S$: C, 65.94; H, 4.39; N, 7.68; S, 8.80. Found: C, 66.06; H, 4.16; N, 7.79; S, 9.03.

Theoretical Calculations

The *ab initio* calculations were performed using the GAUSSIAN94 package.[16] The standard 6-31G* basis set at HF level and with the BLYP[15] functional were used.

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REFERENCES

1. Alhambra, C.; Luque, F. J.; Estelrich, J.; Orozco, M. *J. Org. Chem.*, **1995**, *60*, 969-976.
2. Luyten, I.; Pankiewicz, K. A.; Watanabe, K. A.; Chattopadhyaya, J. *J. Org. Chem.*, **1998**, *63*, 1033-1040.
3. Ono, M.; Kawakami, M. *J. Biochem.*, **1977**, *81*, 1247-1251.
4. Martinez, A.; Esteban, A. I.; De Clercq, E. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1031-1032.
5. Martinez, A.; Esteban, A. I.; Castro, A.; Gil, C.; Conde, S.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1999**, *42*, 1145-1150.
6. Goldman, R. C.; Klein, L. L. *Ann. Rep. Med. Chem.*, **1994**, *29*, 155-164.
7. Elguero, J.; Marzin, C.; Katrizky, A. R.; Linda, P. *The tautomerism of Heterocycles*; Academic Press: New York, 1986.
8. Castro, A.; Martinez, A. *J. Chem. Soc., Perkin Trans. II*, **1994**, 1561-1564.
9. De la Cruz, A.; Elguero, J.; Goya, P. Martinez, A. *Tetrahedron*, **1992**, *48*, 6135-6150.
10. Elguero, J.; Goya, P.; Rozas, I.; Martinez, A. *Chem. Ber.*, **1989**, *122*, 919-924.
11. Cohen, E.; Klarberg, B. *J. Am. Chem. Soc.*, **1974**, *84*, 1994-2002.
12. Esteban, A. I.; Juanes, O.; Conde, S.; Martinez, A. *Tetrahedron*, **1994**, *48*, 13865-13870.
13. Elguero, J.; Jimeno, M. L.; Nieves, R.; Ochoa, C. *J. Chem. Soc., Perkin Trans. II*, **1988**, 859-863.
14. Alkorta, I.; Garcia-Gomez, C.; Paéz, J.A.; Goya, P. *J. Phys. Org. Chem.*, **1996**, *9*, 203-211.
15. Delley, B. *J. Chem. Phys.*, **1991**, *94*, 7245-7250.
16. Gaussian 94, Revision B.3, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. Gaussian, Inc., Pittsburgh PA, **1995**.
17. Albert, A.; Serjeant, E.P. *UV Determination of Ionization Constants*; Chapman and Hall: London, 1971
18. Martinez, A.; Esteban, A. I.; Castro, A. Gil, C.; Conde, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *Bioorg. Med. Chem.*, **1999**, in press.
19. Martinez, A.; Castro, A.; Gil, C.; Segarra, V.; Miralpeix, M.; Domenech, T.; Beleta, J.; Ryder, H.; Palacios, J. M.; Miró, X.; Bonet, C.; Casacuberta, J. M.; Azorín, F.; Piña, B.; Puigdomenech, P. *J. Med. Chem.*, **1999**, submitted.