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The Tautomerism of 5-Amino-3-oxo-1,2,4-thiadiazole: An Experimental and Theoretical Study

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The 1,2,4-thiadiazole system was the subject of our research as a consequence of the pharmacological activity of some derivatives as GSK3 inhibitors. Therefore, in order to explore the active form responsible for receptor interaction, a systematic study of the tautomerism in the 5-amino-3-oxo-1,2,4-thiadiazole system was performed by using experimental and theoretical methods. Thus, the relative stability of the possible tautomers was studied in the gas phase by density functional theory and local density functional methods. The theoretical study in solution was carried out by using several continuum solvation models. Finally, experimental studies were carried out to unambiguously establish the tautomeric form.

Introduction

Glycogen synthase kinase-3 (GSK-3) is a multifunctional serine/threonine protein kinase found in all eukaryotes that was originally identified by its role in the regulation of glycogen metabolism because of its ability to phosphorylate glycogen synthase, but it is now recognized as a key component in multiple signaling pathways.[1] Thus, GSK-3 participates in a multitude of cellular processes, including cell membrane-to-nucleus signaling, gene transcription, translation, cytoskeletal organization, and cell cycle progression and survival. Two functional aspects make GSK-3 a peculiar kinase: its activity is constitutive and downregulated after cell activation by phosphorylation or interaction with inhibitory proteins, and the enzyme prefers substrates that are specifically prepared, that is, *prephosphorylated*, by other kinases.

GSK-3 is a very promising therapeutic target[2] for the development of selective inhibitors as new drugs for different pathologies as chronic inflammatory processes, cancer, diabetes type II, and neurodegenerative diseases,[3] including Alzheimer's disease[4] or bipolar disorders.[5]

Within the great diversity of chemical structures with GSK-3 inhibition already found, the 1,2,4-thiadiazolidinones (TDZDs) appeared to be the first ATP noncompetitive GSK-3 inhibitors.[6] These compounds are of great interest because they do not show inhibitory activity in other kinases. The potency and the selectivity of TDZDs should allow their use as tool compounds in the resolution of the complex signaling pathway where GSK-3 is implicated. In fact, the identification and development of TDZDs as new drugs hold promise for the treatment of unmet diseases mediated by GSK-3 such as Alzheimer's disease and other neurodegenerative processes in which the Tau protein is involved; chronic inflammatory diseases and cancer have also been recently reported.[7]

As part of our research project, new compounds related to TDZDs were prepared to delimit the structural requirements against GSK-3. In this context, 5-amino-3-oxo-2,3-dihydro-1,2,4-thiadiazole presents an interesting case of amino–imino and keto–enol tautomerism. Although different publications exist about the tautomerism of this system, there remains controversy over the relative stability of the amino and imino forms. In this study, the keto and enol tautomers were not considered, as there is unanimity in the belief that the more stable form is the keto tautomer.[8]

Tautomerism involves a large modification in the reactive characteristics of molecules, including their pharmacological properties. By 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 adjust the activity of molecules that have potential pharmacological properties. The tautomeric forms have different properties and therefore different affinity for the receptor.

To obtain more information regarding the possible structural requirements of the inhibitors of GSK-3 and to explore the bioactive molecule, a tautomeric study of the 1,2,4-thiadiazole system (**1**; Figure 1) was performed from a theoretical and an experimental point of view.

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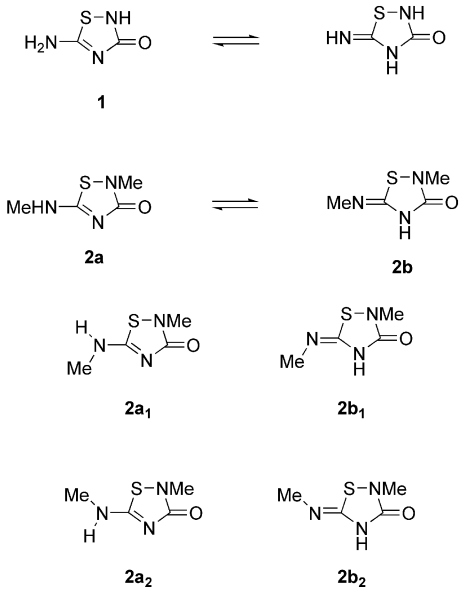


Figure 1. Tautomeric structures of 1,2,4-thiadiazole **1** and 4-methylamino-2-methyl-1,2,4-thiadiazole **2**.

Results and Discussion

Theoretical Study

The theoretical study on the tautomerism of 1,2,4-thiadiazole was carried out on 2-methyl-5-methylamino-3-oxo-2,3-dihydro-1,2,4-thiadiazole where tautomerization to the keto form is blocked. The relative stabilities of the present tautomers of this structure in the gas phase and in solution by means of solvation theoretical models were determined.

For this study, two possible forms for each amino (**2a$_1$**, **2a$_2$**) and imino (**2b$_1$**, **2b$_2$**) tautomer of the heterocyclic system (Figure 1) were considered.

The study of tautomerism in the gas phase of the thiadiazole system was performed by using methods that include electron correlation, a density functional method B3LYP[9] with the bases 6-31G*[10] and 6-31+G** and a local density functional method (LDF)[11] with the DMol program.[12]

The results of the total and relative calculated energies of all tautomer forms in the gas phase are collected in Table 1. The dipole moments of both tautomers are tabulated in Table 2.

Table 2. Calculated dipole moments (μ, Debye) of amino tautomer **2a** and imino tautomer **2b**.

Method	**2a$_1$**	**2a$_2$**	**2b$_1$**	**2b$_2$**
B3LYP/6-31G*	5.64	6.12	0.21	2.56
B3LYP/6-31+G**	6.09	6.67	0.46	3.08
DMol	5.85	6.44	0.31	2.78

The results of the gas-phase calculations of the amino and imino tautomers by using density functional theory based on ab initio methods, explicitly, B3LYP with the 6-31G* and 6-31+G** basis sets, and the local density functional method (LDF) indicate that amino tautomeric forms **2a$_1$** and **2a$_2$** are the most stable. However, the difference in energy of imino tautomeric form **2b$_1$** relative to the most stable form of tautomer **2a** is small (0.6–1.2 kcal mol^{-1}) depending on the method used. Tautomer **2b$_2$** is the least stable. These results indicate that amino tautomers **2a$_1$** and **2a$_2$** and imino tautomer **2b$_1$** should be present in the gas phase as a result of the small energy differences between the tautomeric forms (Table 1). With regard to the amino tautomers, the stability depends on the calculation method used, although the differences are very small. In the case of the imino tautomers, the more stable form is **2b$_1$**. For all tautomers, the DFT method (B3LYP) gives smaller energy differences in the gas phase between the amino and imino tautomers than the DMol method.

Table 1. Calculated energies (E, Hartees and kcal mol^{-1}), solvation energies (E_s, kcal mol^{-1}), and relative energies (E_r, kcal mol^{-1}) of amino tautomer **2a** and imino tautomer **2b** in the gas phase and with different solvation models.

	Method	DMol	B3LYP/ 6-31G*	B3LYP/ 6-31+G**	COSMO/DMol	PCM/B3LYP/ 6-31G*	PCM/B3LYP/ 6-31+G**
2a$_1$	E (Hartees)	–789.961198	–794.301794	–794.331697	–789.984572	–794.321835	–794.355938
	E (kcal)	–495708.55	–498432.32	–498451.08	–495723.22	–498444.89	–498466.29
	E_s				–14.67	–12.57	–15.21
	E_r	0.97	–0.25	–0.28	1.58	0.68	0.10
2a$_2$	E (Hartees)	–789.962750	–794.301402	–794.331243	–789.987089	–79.432292	–794.356105
	E (kcal)	–495709.53	–498432.07	–498450.80	–495724.80	–498445.58	–498466.40
	E_s				–15.27	–13.51	–15.6
	E_r	0.00	0.00	0.00	0.00	0.00	0.00
2b$_1$	E (Hartees)	–789.953915	–794.296281	–794.325839	–789.971008	–79.431011	–794.342782
	E (kcal)	–495703.98	–498428.86	–498447.41	–495714.71	–498437.54	–498458.04
	E_s				–10.73	–8.68	–10.63
	E_r	5.54	3.21	3.39	10.09	8.04	8.36
2b$_2$	E (Hartees)	–789.960787	–794.300765	–794.330237	–789.976833	–79.431397	–794.346098
	E (kcal)	–495708.29	–498431.67	–498450.17	–495718.36	–498439.96	–498460.12
	E_s				–10.07	–8.29	–9.95
	E_r	1.23	0.40	0.63	6.44	5.62	6.28

The values of the dipole moments of the possible tautomers are gathered in Table 2, and they indicate that there are considerable differences in polarity between the tautomeric forms. The relatively low dipole moments of imino tautomer **2b** imply that amino tautomers $\mathbf{2a_1}$ and $\mathbf{2a_2}$ are more favored in aqueous solution (Table 2).

By employing several solvent continuum models, solvation effects were studied to calculate the relative stabilities of the different tautomers in aqueous solution. The self-consistent reaction field (SCRF) method provides a simple solution to the complex process of solvation, and the polarizable continuum model (PCM) can be used to study the solvation energies.[13] The approximation called dielectric PCM defines the cavity as the union of a series of interlocking spheres centered on the atoms, and it uses a numerical representation of the polarization of the solvent. The conductor-like screening model (COSMO)[14] was implemented in DMol,[12] and in this continuum solvation model, the solute molecules form a cavity within a continuum conductor that represents the solvent.

As shown in Table 1, all employed methods give a large energy difference (6 kcal mol^{-1}) between amino tautomer $2\mathbf{a_1}$ and the most stable imino tautomer, $2\mathbf{b_1}$. The least stable imino tautomer is $\mathbf{2b_2}$, and it is 8–10 kcal mol^{-1} higher in energy than $\mathbf{2a_1}$. These results indicate that only amino tautomers $\mathbf{2a_1}$ and $\mathbf{2a_2}$ should be present in aqueous solution because of the large energy difference relative to imino tautomers **2b** (Table 1).

The theoretical results in solution obtained from the continuum solvent models showed a relative stabilization of the amino tautomer, as can be seen from the data obtained with the PCM and COSMO models (Table 1).

The relative stabilization effect of amino tautomer $\mathbf{2a_1}$ is 4.5–5.0 kcal mol^{-1} in relation to $\mathbf{2b_1}$ and 5.2–5.7 kcal mol^{-1} relative to $\mathbf{2b_2}$. With regard to the amino tautomer, the stabilization of $\mathbf{2a_1}$ is slightly smaller: 0.6–0.8 kcal mol^{-1} relative to $\mathbf{2a_2}$.

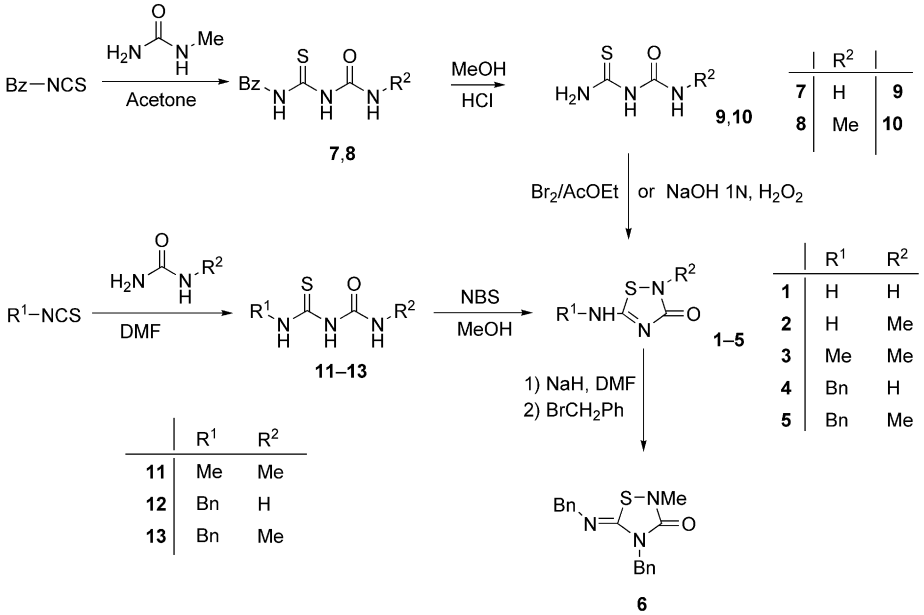
With regard to reproducibility of the experimental results, both solvation models, PCM and COSMO, yield similar stabilization effects of the tautomers in solution. Thus, the PCM method at B3LYP/6-31G* and 6-31+G** gives an energy difference of 4.8–5.0 and 5.2–5.6 kcal mol^{-1} for imino tautomer $\mathbf{2b_2}$ and $\mathbf{2b_1}$, respectively, and 0.4–0.9 kcal mol^{-1} for amino tautomer $\mathbf{2a_2}$. In the case of amino tautomer $\mathbf{2a_1}$, the stabilization is slightly smaller (0.1–0.7 kcal mol^{-1}) relative to $\mathbf{2a_2}$ when the PCM method is used. With the use of the COSMO model, the stabilization of this tautomer is 1.6 kcal mol^{-1}.

Chemistry

To check the theoretical results and to unambiguously establish the tautomeric structure from a experimental point of view by means of ultraviolet spectrophotometry, a series of 5-amino-3-oxo-1,2,4-thiadiazoles was synthesized.

Thus, the parent compound 5-amino-3-oxo-1,2,4-thiadiazole,[15] 2-methyl-1,2,4-thiadiazoles **2**, **3**, **5**, and **6**, and 4-benzylamine-2*H*-1,2,4-thiadiazole **4** were prepared for this study (Scheme 1).

The employed strategy for the synthesis of these derivatives was based on the Párkányi procedure.[15] This method consists of oxidative cyclization of thiobiurets that are prepared in advance by condensation of ureas with isothiocyanates. In the case of the synthesis of thiadiazoles **1** and **2**, it was necessary to prepare first benzoyl thiobiurets **7**[15] and **8**[8d] by condensation of benzoyl isothiocyanate with urea and methylurea, respectively, in acetone under reflux and then perform the debenzoylation reaction to yield thiobiuret precursors **9**[15] and **10**.[8d] The oxidative cycliza-



	R^2
7	H
8	Me

	R^2
9	H
10	Me

	R^1	R^2
1	H	H
2	H	Me
3	Me	Me
4	Bn	H
5	Bn	Me

	R^1	R^2
11	Me	Me
12	Bn	H
13	Bn	Me

Scheme 1.

tion of **9** was carried out under basic peroxide conditions to yield 1,2,4-thiadiazole **1**, whereas the cyclization of thioburet **9** into 2-methylthiadiazole **2** was achieved with molecular bromine, which are milder oxidative conditions than reported.

For the preparation of thiobiurets **11**–**13** it was necessary to employ stronger reaction conditions (refluxing DMF), and these intermediates oxidatively cyclized to 5-amino-3-oxo-2,3-dihydro-1,2,4-thiadiazoles **3**–**5** through N–S bond formation by employing NBS as the oxidizing agent at reflux in methanol (Scheme 1). Finally, the synthesis of 4-benzyliminothiadiazole derivative **6** was achieved by benzylation of **5** in a polar, nonprotic solvent (DMF) in the presence of sodium hydride as the base with benzyl bromide (Scheme 1).

The structures of these compounds were elucidated from their analytical and spectroscopic data (^{1}H and ^{13}C NMR spectra). Unequivocal assignment of all chemical shifts was performed by using HMQC for one-bond correlations. The alkylation site was determined from sequences of HMBC for long distance/carbon correlation. Thus, *N*4-CH_2 correlated with both heterocyclic carbons C-3 and C-5, which showed that imino tautomerization was blocked.

Experimental Tautomeric Study

In aqueous solution, information about the predominant molecular species can be obtained from the UV spectroscopic data. The UV spectra of a series of 1,2,4-thiadiazole derivatives, which were synthesized for this work, were obtained. These compounds include the parent 5-amino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (**1**), derivatives **2** and **3** with the keto tautomer blocked, 4-benzyl-5-benzylimino-2-methyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (**6**) with the imino tautomer blocked, and derivatives **4** and **5** for the study of the influence of the benzyl group in the λ absorption band.

The presence of the amino and imino tautomers can be deduced from the characteristic UV absorption bands. Thus, the amino tautomer shows two characteristics absorption bands at 220 and 250 nm, whereas the imino thiadiazole only shows one band about 245 nm (Table 3). Therefore, it can be concluded that 5-amino-1,2,4-thiadiazoles **1**–**5** exist in aqueous solution as the amino tautomeric form.

Table 3. UV spectroscopic data in water of 5-amino-3-oxo-1,2,4-thiadiazole derivatives **1**–**6**.

Compound	λ_{max} [nm]	
1	217.1	250.2
2	218.7	252.8
3	222.0	253.1
4	222.2	250.4
5	222.7	255.0
6	245.8	

Conclusions

The obtained theoretical results indicate that both amino and both imino tautomers are present in the gas phase, although the amino form is more stable. In water, an important stabilization of the amino tautomer takes place so that the equilibrium is clearly displaced to favor the amino form. These results are in accordance with the experimental UV data, and thus in amino derivatives **1**–**5**, the amino tautomer is present in aqueous solution.

Experimental Section

General: Substrates were purchased from commercial sources and used without further purification. Melting points were determined with a Reichert-Jung Thermovar apparatus and are uncorrected. Flash column chromatography was carried out at medium pressure by using silica gel (E. Merck, Grade 60, particle size 0.040–0.063 mm, 230–240 mesh ASTM). Compounds were detected with UV light (254 nm). ^{1}H NMR spectra were obtained with a Varian XL-300 spectrometer working at 300 MHz. Typical spectral parameters: spectral widths 10 ppm, pulse width 9 μs (57°), data size 32 K. ^{13}C NMR experiments were carried out with the Varian Gemini-300 spectrometer operating at 75 MHz. The acquisition parameters: spectral width 16 kHz, acquisition time 0.99 s, pulse width 9 μs (57°), data size 32 K. Chemical shifts are reported relative to internal Me_4Si. MS (mass spectroscopy): EI (electron ionization), MSD 5973 Hewlett Packard and ESI (electrospray ionization). The UV spectra were obtained with a Perkin–Elmer Lambda 5 spectrophotometer.

Theoretical Calculations: The studied compounds were built with standard bond lengths and angles by using the molecular modeling package Insight II.[17] All the structures were fully optimized without any symmetry restrictions in both the gas phase and the simulated solvent environment until the default convergence criteria of the program were satisfied.

The DFT method was performed by using the Gaussian 98 package.[18] The standard 6-31G* and 6-31+G** basis sets with the density functional calculation (DFT) B3LYP functional[9] were used.

The LDF calculations were carried out by using the DMol program[12] distributed by Accelrys, Inc. A double zeta numerical basis set with polarization functions in all the atoms and the Janak–Moruzzi–Williams (JMW) exchange correlation potential[19] were used. The geometry of the molecules was optimized until the gradient was smaller than 0.001 au.

The solvation effect was studied by using two methods: COSMO[14] and PCM.[13] The study of the solvation with COSMO model was carried out with the DMol program implemented in Cerius2 and the PCM method was performed with the Gaussian 98 package.

General Experimental Procedure for the Synthesis of 5-Amino-3-oxo-2,3-dihydro-1,2,4-thiadiazoles 3–5: A suspension of the appropriate thiobiuret (1 mmol) and NBS (1.25 mmol) was heated at reflux in methanol for 6–15 h depending on the product. The reaction mixture was allowed cool to room temperature (with stirring), the solvent was removed under reduced pressure, and the crude product was purified by column chromatography (silica, dichloromethane/methanol).

2-Methyl-5-methylamino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (3): From **11**. Yield: 20%. M.p. 195–197 °C. ^{1}H NMR (300 MHz, [D_6]-DMSO, 25 °C): δ = 8.90 (br. s, 1 H, NH), 3.20 (br. d, 3 H, CH_3),

3.13 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 169.6 (C-5), 165.2 (C-3), 32.0 (CH_3), 30.0 (CH_3) ppm. MS (EI): *m*/*z* (%) = 145 (6) $[M]^+$. $C_4H_7N_3OS$ (145.18): calcd. C 33.09, H 4.86, N 28.94, S 22.09; found C 33.26, H 5.21, N 29.27, S 22.38

5-Benzylamino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (4): From **12**. Yield: 50%. M.p. 215–217 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 9.43 (br. s, 1 H, NH), 7.2–7.4 (m, 5 H, Ph), 4.53 (s, 2 H, CH_2), 5.70 (br. s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, [D$_6$]-DMSO, 25 °C): δ = 174.4 (C-5), 165.4 (C-3), 137.4 (C-*i*), 128.6 (2 C-*m*), 127.5 (2 C-*o*), 127.1 (C-*p*), 47.55 (*C*H_2Ph) ppm. MS (EI): *m*/*z* (%) = 207 (20) $[M]^+$. $C_9H_9N_3OS$ (207.25): calcd. C 52.16, H 4.38, N 20.27, S 15.47; found C 51.81, H 4.13, N 20.32, S 15.36.

5-Benzylamino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (5): From **13**. Yield: 55%. M.p. 185–187 °C. ^{1}H NMR (300 MHz, [D$_6$]-DMSO, 25 °C): δ = 8.82 (br. s, 1 H, NH), 7.2–7.4 (m, 5 H, Ph), 4.52 (br. s, 2 H, CH_2Ph), 3.02 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 169.7 (C-5), 166.0 (C-3), 137.9 (C-*i*), 128.6 (2 C-*m*), 127.5 (2 C-*o*), 127.4 (C-*p*), 46.8 (*C*H_2Ph), 29.9 (CH_3) ppm. MS (EI): *m*/*z* (%) = 221 (46) $[M]^+$. $C_{10}H_{11}N_3OS$ (221.28): calcd. C 54.28, H 5.01, N 18.99, S 14.49; found C 54.54, H 5.29, N 19.33, S 14.78.

5-Amino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (2): 5-methyl-2-thiobiuret (**10**)[8d] (134 mg, 1 mmol) was dissolved in CH_2Cl_2 (3 mL) and AcOEt (6 mL) at 0 °C and a solution of Br_2/AcOEt (0.5 M, 4 mL, 2 mmol) was added dropwise. The reaction mixture was left stirring at 4 °C for 12 h. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (silica gel; dichloromethane/methanol, 30:1). Yield: 81%. M.p. 267–269 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 9.43 (br. s, 1 H, NH), 3.01 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 170.5 (C-5), 159.50 (C-3), 30.3 (CH_3) ppm. MS (ESI+): *m*/*z* = 132 $[M]^+$. MS: *m*/*z* = 132 $[M]^+$. $C_3H_5N_3OS$ (131.15): calcd. C 27.47, H 3.84, N 32.04, S 24.45; found C 27.25, H 3.46, N 31.90, S 24.21.

4-Benzyl-5-benzylimino-2-methyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (6): A mixture of **5** (221 mg, 1 mmol) and sodium hydride (24 mg, 1 mmol) in anhydrous DMF (2.5 mL) was stirred at room temperature for 1 h. Benzyl bromide (0.12 mL, 1 mmol) was then added, and the mixture was heated at reflux with stirring for 24 h. The reaction mixture was cooled to room temperature, and the solvent was then removed under reduced pressure. The resulting crude product was purified by flash chromatography (hexane/AcOEt, 3:1). Yield: 40%. M.p. 220–222 °C. ^{1}H NMR (300 MHz, [D$_6$]-DMSO, 25 °C): δ = 7.4–7.5 (m, 5 H, Ph), 4.87 (s, 2 H, CH_2Ph), 3.03 (s, 3 H, CH_3), 7.3–7.1 (m, 5 H, Ph), 4.19 (s, 2 H, CH_2Ph) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 166.1 (C-5), 158.2 (C-3), 137.2 (C-*i*), 129.4 (C-*o*), 128.2 (C-*m*), 126.1 (C-*p*), 50.2 (*C*H_2Ph), 31.8 (CH_3), 140.1 (C-*i*), 128.0 (2 C-*m*), 126.8 (2 C-*o*), 124.6 (C-*p*), 47.8 (*C*H_2Ph) ppm. MS (EI): *m*/*z* (%) = 311 (75) $[M]^+$. $C_{17}H_{17}N_3OS$ (311.40): calcd. C 65.57, H 5.50, N 13.49, S 10.30; found C 65.80, H 5.57, N 13.77, S 10.19.

General Experimental Procedure for the Synthesis of Thiobiurets 11–13: To a solution methylurea or benzylurea (1 mmol) in anhydrous DMF (2.5 mL) was added the corresponding isothiocyanate (1 mmol). The reaction mixture was heated at reflux for 10–24 h and then left to cool to room temperature. Afterwards, distilled water (2.5 mL) was added, and the precipitated product was filtered off with suction and purified by flash chromatography.

1,5-Dimethyl-2-thiobiuret (11): Yield: 35%. M.p. 145–147 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.22 (br. m, 1 H, CH_3N*H*CS), 9.78 (br. s, 1 H, CSN*H*CO), 6.61 (br. m, 1 H, CH_3N*H*CS), 2.97 (d, $^3J_{H\text{-}H}$ = 4.6 Hz, 3 H, NHCH_3), 2.60 (d, $^3J_{H\text{-}H}$ = 4.7 Hz, 3 H, CH_3NH) ppm. ^{13}C NMR (75 MHz, [D$_6$]-DMSO, 25 °C): δ = 180.6 (C2), 155.0 (C4), 31.4 (CH_3), 25.7 (CH_3) ppm. MS (EI): *m*/*z* (%) = 147 (100) $[M]^+$.

1-Benzyl-2-thiobiuret (12): Yield: 40%. M.p. 156–158 °C (ref.[16] 158–160 °C). ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.83 (br. m, 1 H, CH_2N*H*CS), 9.88 (br. s, 1 H, $CONH_2$), 7.5–7.2 (m, 5 H, Ph), 4.79 (d, $^3J_{H\text{-}H}$ = 5.5 Hz, 2 H, CH_2-Ph), 6.96 (br. s, 1 H, NH_2), 6.38 (br. s, 1 H, NH_2) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 180.7 (C-2), 155.6 (C-2), 137.8 (C-*i*), 128.5 (2 C-*m*), 127.5 (2 C-*o*), 127.2 (C-*p*), 47.6 (*C*H_2Ph) ppm. MS (EI): *m*/*z* (%) = 209 (40) $[M]^+$.

1-Benzyl-5-methyl-2-thiobiuret (13): Yield: 70%. M.p. 181–183 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.74 (br. m, 1 H, CH_2N*H*CS), 9.93 (br. s, 1 H, CSN*H*CO), 6.69 (br. m, 1 H, CON*H*CH_3), 7.3–7.2 (m, 5 H, Ph), 4.76 (d, $^3J_{H\text{-}H}$ = 5.5 Hz, 2 H, CH_2Ph), 2.62 (d, $^3J_{H\text{-}H}$ = 4.6 Hz, 3 H, CH_3NH) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 179.5 (C-2), 160.2 (C-4), 140.2 (C-*i*), 130.1 (2 C-*m*), 129.0 (2 C-*o*), 128.2 (C-*p*), 55.2 (*C*H_2Ph), 25.1 (CH_3) ppm. MS (EI): *m*/*z* (%) = 223 (100) $[M]^+$.

5-Methyl-2-thiobiuret (10): 1-benzoyl-5-methyl-2-thiobiuret[8d] (**8**; 237 mg, 1 mmol) was dissolved in an aqueous solution of NaOH (1 N, 1 mL, 1 mmol) and absolute ethanol (2 mL), and the reaction mixture was heated at 50 °C for 10 min. Distilled water (10 mL) was then added, and the reaction mixture was neutralized with 5% HCl. The mixture was extracted with AcOEt (3 × 10 mL), the resulting organic phase was dried with anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Yield: 97 mg (73%). M.p.180–182 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 9.74 (br. s, 1 H, CSN*H*CO), 9.41 (br. s, 1 H, $CSNH_2$), 8.89 (br. s, 1 H, $CSNH_2$), 6.65 (br. m, 1 H, N*H*CH_3), 2.60 (d, $^3J_{H\text{-}H}$ = 4.6 Hz, 3 H, CH_3NH) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 181.7 (C-2), 155.0 (C-4), 26.1 (CH_3) ppm. MS (EI): *m*/*z* (%) = 134 (100) $[M]^+$.

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