Scientific paper

Evaluation of Michael-type Acceptor Reactivity of 5-Benzylidenebarbiturates, 5-benzylidenerhodanines, and Related Heterocycles Using NMR

Emilija Arsovska,[1] Jurij Trontelj,[1] Nace Zidar,[1] Tihomir Tomašić,[1] Lucija Peterlin Mašič,[1] Danijel Kikelj,[1] Janez Plavec[2] and Anamarija Zega[1,*]

[1] *University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia*

[2] *National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia*

* *Corresponding author: E-mail: anamarija.zega@ffa.uni-lj.si*

Received: 01-02-2014

Abstract

Despite existing experimental and computational tools to assess the risk, the non-specific chemical modification of protein thiol groups remains a significant source of false-positive hits, particularly in academic drug discovery. Herein, we describe the application of a simple NMR method in a systematic study on the reactivity of 5-benzylidenebarbiturates, 5-benzylidenerhodanines, and their related oxo-heterocycles, which have been associated with numerous biological activities and have recently gained a reputation as unselective promiscuous binders. Using this method, we confirmed the reactivity of 5-benzylidenebarbiturates, which are known to easily form Michael adducts with nucleophiles. In contrast, 5-benzylidene five-membered oxo-heterocycles revealed almost insignificant reactivity. We can conclude that the distinct binding profile of the most controversial compounds, 5-benzylidenerhodanines, is not necessarily related to their unspecific Michael acceptor reactivity.

Keywords: Drug discovery, False positives, Michael acceptors, 5-benzylidene heterocycles, NMR spectroscopy

1. Introduction

One of the problems in the early stages of drug discovery is the number of nonspecific, false-positive hits. Such compounds, which turn out to be dead ends when attempts are made to optimise their activity, are very costly in terms of time and resources. Thus, it is imperative that medicinal chemists recognise and eliminate these problematic molecules from further consideration at the earliest possible stage.[1] It appears that nonspecific biological activity may arise through cross-reactivity between the compound and the target protein. However, for some compounds it is not always obvious that they will be prone to react with a protein; hence, such compounds can escape traditional functional group filters designed to capture problematic molecules.[2–4] For example, heterocyclic cores tend to mask the appearance of Michael acceptor functionality in 5-benzylidenebarbiturates and 5-benzylidenerhodanines, scaffolds often appearing in the literature in the compounds with a wide range of biological effects which recently gained a reputation of being unselective and promiscuous binders.[2–6]

It is believed that 5-benzylidenerhodanines undergo facile reaction with nucleophiles via Michael addition to the exocyclic double bond (Figure 1). Indeed, in the crystal structure of 5-benzylidenerhodanine-based inhibitors in complex with the HCV RNA polymerase NS5B, a covalent bond between the inhibitor and a cysteine residue in the allosteric binding site has been observed. However, this inhibition was found to be reversible.[7]

Furthermore, there are crystal structures of rhodanine-based inhibitors with different enzymes available, where 5-benzylidenerhodanine fragment does not form covalent bonds with the target proteins. This was observed in the co-crystal structures of compounds that were designed in our lab as inhibitors of the bacterial enzyme MurD ligase (PDB codes: 2Y1O, 2WJP, and 2Y68).[9,10]

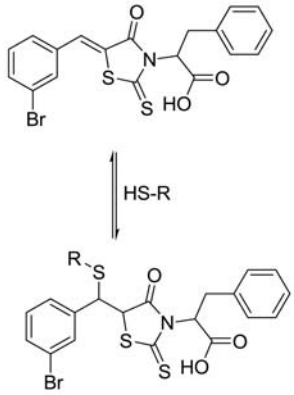


Figure 1. Reaction of 5-benzylidenerhodanine with nucleophile (DTT) via Michael addition to the exocyclic double bond.[8]

Still, rhodanines have been identified as thiol-reactive compounds through the use of ALARM NMR, an assay to detect reactive molecules by NMR.[11] A systematic study on the promiscuity of rhodanines, hydantoins, thiohydantoins, and thiazolidine-2,4-diones was recently performed by Mendgen et al..[12] These researchers evaluated the Michael acceptor reactivity of the exocyclic double bond of 14 compounds that showed activity against one or more enzymes using glutathione as an exemplary biological nucleophile. In no case was the addition of glutathione detected, which indicated that the electrophilicity of this exocyclic double bond conjugated to the carbonyl group is not significant. However, the reactivity of the investigated compounds on their target proteins could not be completely excluded because the reactivity of cysteine sulphur varies depending on the protein environment.[12]

The reactivity of 5-arylmethylidenebarbituric acids as Michael acceptors is less questionable. These compounds contain a strongly polarised exocyclic double bond with a partial positive charge on the methyne carbon atom, and it is known that they can form Michael adducts with nucleophiles, such as amines,[13–15] thiols,[16] and water.[17]

Recently, Avonto et al. developed an NMR spectroscopic method[18] to identify Michael acceptor sites using cysteamine.[19] With a pKa of 8.3, cysteamine has similar reactivity as many surface thiols of proteins and is therefore a model thiol for a reactivity screen. The method sorts compounds in a reversible and an irreversible thiol sinks and predicts their potential ability to modify proteins. Authors suggested that this rapid NMR spectroscopic test could be used as a pre-screen for more complex assays for the prediction of the reactivity of compounds, such as ALARM NMR spectroscopy, which requires a labelled protein substrate and 2D NMR spectroscopic measurements,[11] the ^{13}C NMR approach,[20] UV-based glutathione trapping experiments[21] and kinetic assessment of UV-active compounds towards thiols.[22]

Having identified various 5-benzylidenebarbiturate-[23] and 5-benzylidenerhodanine-based compounds[9,10,23–26] as hits in our screening campaigns against enzyme targets in bacterial peptidoglycan biosynthesis, we decided to perform a systematic study on the reactivity of some 5-benzylidenebarbiturates, 5-benzylidenerhodanines, and their parent analogue heterocycles (Figure 2) using this simple and quick NMR spectroscopic method.[18]

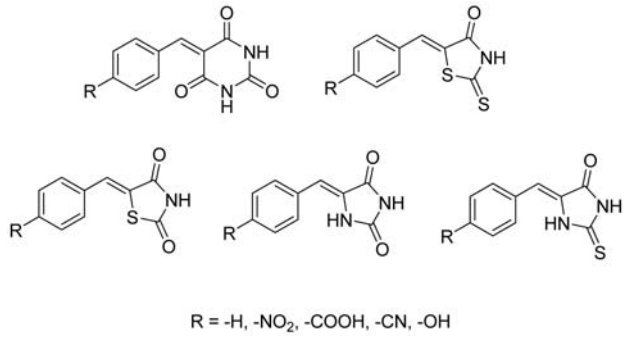


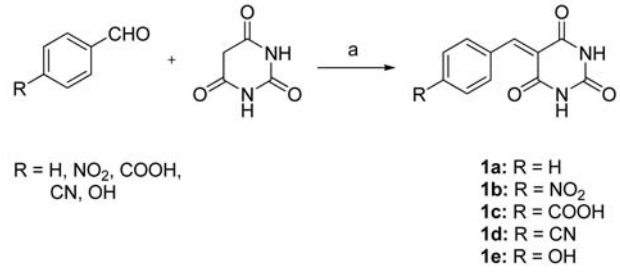
Figure 2. Heterocyclic Michael acceptor compounds studied in this work.

2. Results and Discussion

2. 1. Chemistry

For our study, we have synthesised a series of unsubstituted as well as *p*-nitro-, *p*-cyano-, *p*-hydroxy-, and *p*-carboxy- substituted 5-benzylidenebarbiturates, -rhodanines, -hydantoins, -thiohydantoins, and -thiazolidine-2,4-diones (Figure 2).

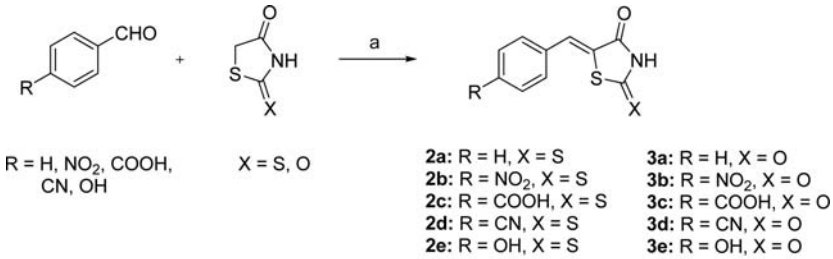
The 5-benzylidenebarbiturates **1a-1e** were synthesised via Knoevenagel condensation between the barbituric acid and different benzaldehydes with heating the reagents in water at 100 °C for 12 h (Scheme 1).[17]



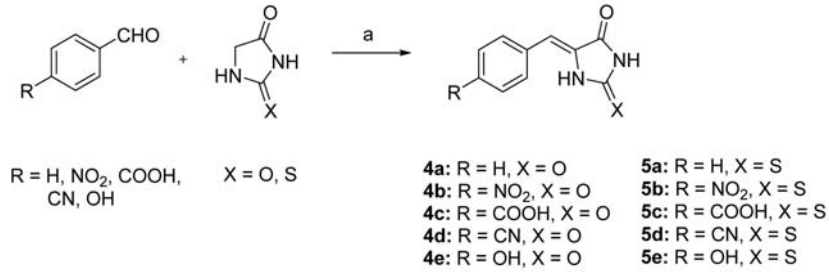
Scheme 1. Reagents and conditions: a) H_2O, reflux, 12 h

The 5-benzylidene thiazolidin-4-one-based compounds **2a-2e** and **3a-3e** were prepared via condensation between 2-thioxothiazolidine-4-one (rhodanine) or thiazolidine-2,4-dione and different benzaldehydes. The products were synthesised under microwave heating at 150 °C for 20 min, and utilizing glacial acetic acid and piperidine as catalysts (Scheme 2). [26]

When the same reaction conditions were used for the synthesis of hydantoin and thiohydantoin derivatives, the desired products **4a–4e** and **5a–5e** were not obtained.



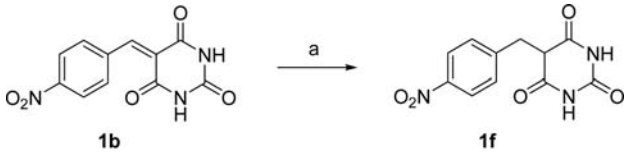
Scheme 2. Reagents and conditions: a) EtOH, AcOH, piperidine, 30 W, 18 bar, 150 °C, 20 min.



Scheme 3. Reagents and conditions: a) AcOH, CH_3COONH_4, reflux, 20 h.

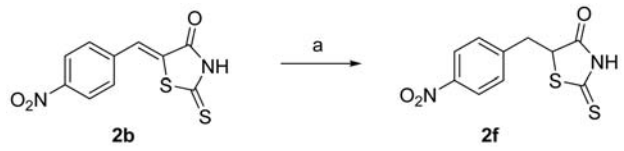
After testing different reaction condition and solvents, the target compounds were finally obtained when imidazolidine-2,4-dione or 2-thioxoimidazolidine-4-one and suitable benzaldehydes were heated under reflux in glacial acetic acid for 20 h using ammonium acetate as the catalyst (Scheme 3).[10]

The reduction of 5-(4-nitrobenzylidene) barbituric acid (**1b**) to 5-(4-nitrobenzyl) barbituric acid (**1f**) was performed with sodium borohydride in ethanol as the solvent at room temperature for 2 h (Scheme 4).[27]



Scheme 4. Reagents and conditions: a) $NaBH_4$, EtOH, rt, 2 h.

For the synthesis of the 5-(4-nitrobenzyl)-2-oxothiazolidin-4-one derivative with the reduced exocyclic double bond (**2f**), 5-(4-nitrobenzylidene)-2-oxo-thiazolidin-4-one (**2b**) was reduced using diethyl 2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (Hantzsch ester) and activated silica gel (Scheme 5).[25]



Scheme 5. Reagents and conditions: a) diethyl 2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, silica gel 60, toluene, 100 °C, 24 h.

2. 2. NMR Spectroscopy

To unambiguously assign the spectra and establish the presence of the vinyl group, heteronuclear correlation NMR experiments (HSQC and HMBC) were first performed for all compounds (data not shown). Vinyl protons exhibit proton chemical shifts between δ6.51 and 8.33 ppm, whereas the respective carbon resonances occur between δ105.1 and 129.0 ppm (Supporting Information, Section 1).

The Avontos NMR assay conditions were employed as described in the literature.[18] Our compounds were treated with one equivalent of cysteamine in DMSO-d_6. The ^{1}H NMR signal intensity of the vinyl proton was then measured to estimate the reactivity of the compound. The treatment of 5-benzylidenebarbiturates with cysteamine led to the instantaneous formation of Michael adducts (Figure 3a), as was observed in the ^{1}H NMR spectra through the disappearance of the vinyl proton signal (Figure 4).

In contrast, 5-benzylidenerhodanines, -thiohydantoins, -hydantoins, and -thiazolidine-2,4-diones (Figure 3b) gave only sluggish and incomplete reactions with cysteamine, which were observable only as a modulation of the intensity of the vinyl signal in the ^{1}H NMR spectra (Supporting Information, Section 2 and 4, Table 1). Besides alteration of the vinyl signal, changes in the chemical shifts and additional signals were observed. To obtain the exact structural information of the products that were expected to be Michael adducts, we performed HMBC and HSQC experiments of the reaction mixture after addition of cysteamine (Supporting Information, Section 3, Figure 7b and 8b). The spectra revealed the formation of either the expected cysteamine adduct (Figure 3) only or addi-

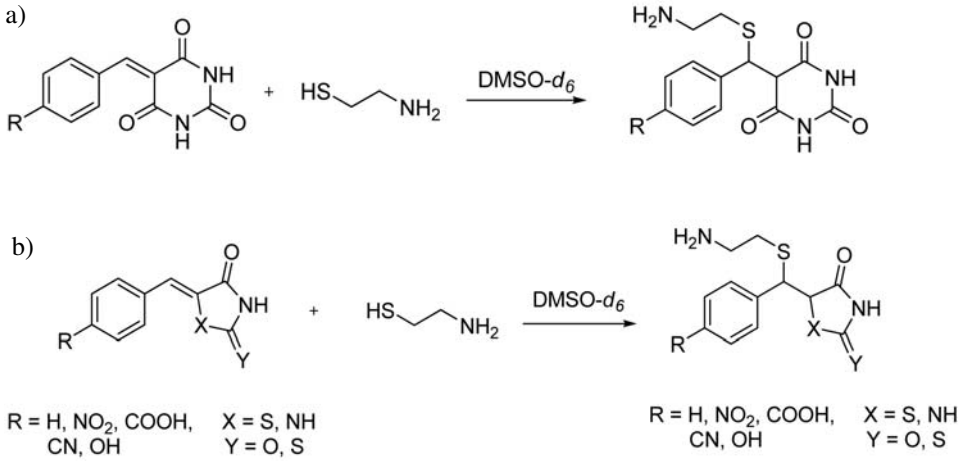


Figure 3. Reactions of heterocyclic derivatives containing an exocyclic double bond, with cysteamine.

tionally, an adduct with the amino group of cysteamine (Supporting Information, Section 3, Figure 9b). The changes in the chemical shifts of the aromatic and vinyl protons in 5-benzylidenerhodanines, -thiohydantoins, -hydantoins, and -thiazolidine-2, 4-diones can be referred to the changes in the tautomeric form of five-membered heterocycles.[28]

The formation of a Michael adduct and the consequent modulation of the intensity of the vinyl signal was also dependent on the substitution on the aromatic ring of the tested compounds. This can be explained by the differences in the reactivity of the exocyclic double bond as a result of the electronic properties of the substituents on the aromatic ring. It has been demonstrated that the Lewis acidity and hence the reactivity of 5-arylmethylidenebarbituric acids toward Michal addition increase in the presence of electron-withdrawing substituents. Thus, nitro, carboxyl, and cyano groups enhance the Lewis acidity of benzylidene carbon, whereas the electron-donating hydroxyl group renders exocyclic doube bond less susceptible to nucleophilic attack.[17] Consequently, changes in spectra were most clear in heterocyclic compounds with the *p*-nitro substituent on the aromatic ring, thus we decided to focus and fully present here results on *p*-nitro-substituted 5-benzylidenebarbiturates, -rhodanines, -hydantoins, -thiohydantoins, and -thiazolidine-2,4-diones (Table 1).

To obtain further insight into the role of the exocyclic double bond in the reaction between 5-benzylidene-substituted heterocycles with cysteamine, 5-benzylidene-

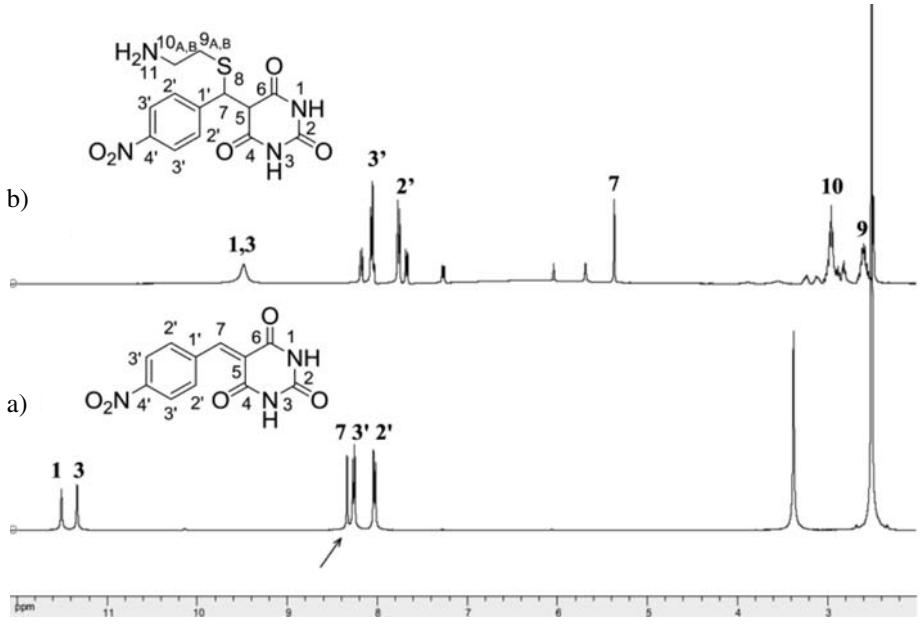


Figure 4. ^{1}H NMR spectra of **1b** in DMSO-d_6 before (A) and 5 minutes after the addition of 1 equivalent of cysteamine (B). The arrow denotes the NMR signal of the vinyl proton of **1b** that disappears during the course of the reaction.

barbiturate **1b** and 5-benzylidenerhodanine **2b** were reduced to obtain the corresponding benzyl derivatives **1f** and **2f**, respectively. As expected, the treatment of **1f** and **2f** with one equivalent of cysteamine in DMSO-d_6 did not lead to any changes in the NMR spectra (Supporting Information, Section 2, Figure 6b).

Table 1. Quantification of the analytes after the addition of cysteamine.

Analyte	Relative amount of reactant (%) NMR	Relative amount of conjugate[a] (%) NMR
1b-cysteamine	0	71.9
2b-cysteamine	76.3	15.2
3b-cysteamine	72.4	15.3
4b-cysteamine	88.1	7.0
5b-cysteamine	94.3	3.2

The relative amounts were quantified 5 minutes after the mixing of the reactants in the NMR tube. The quantification of the cysteamine conjugates was performed by integrating the signals of adducts and comparing the values to those from the integrals of the corresponding parent compounds. For statistical reasons, each measurement was repeated three times.

[a] relative amount of the main adduct with the thiol group of cysteamine

We can conclude that 5-benzylidenerhodanines and similar heterocyclic analogues are not entirely nonreactive, although the electrophilicity of this Michael-acceptor system is much less significant compared with that of 5-benzylidenebarbiturates. Still, it should be emphasised that a concentration of an enzyme in an enzyme assays is always much lower than the concentration of the tested compounds; therefore, even if the Michael addition reaction occurs in a very small extent, it can affect the results of the test and consequently give a false-positive result.

The experimental protocol developed by Avonto et al. suggests the dilution of the adduct in DMSO-d_6 with deuterochloroform as a test for the reversibility of the Michael adduct formation, since in the equilibrium, a change of the polarity would reverse the reaction, with reappearance of the olefin resonance lost during the addition reaction in DMSO-d_6. The adduct stability is a critical determinant for the biological profile of thiol-trapping agents and a criterion for the potential application of such agents in medicinal chemistry.[29] However, due to the low solubility of our compounds in the DMSO/chloroform mixture, we were unable to perform these experiments, which prevented us from classifying our test compounds at either reversible or irreversible thiol sinks.

According to our standard experimental protocol, the spectra of the compounds were measured 5 minutes after the addition of cysteamine. However, it usually took longer to complete the enzymatic assay for the assessment of the activity of our compounds; hence, the mixtures were incubated for 15 min.[23] To place the method into perspective, the reaction of cysteamine was also studied on a longer time scale (after 10 minutes, 30 minutes, 1 hour, and 24 hours), but no important changes were observed in the spectra of any of the tested compounds.

Additionally, by using the NMR method developed by Avonto et al.,[18] we analysed compound **6**, which contains the 5-benzylidene thiazolidine-2,4-dione moiety (Figure 5) and was designed as an inhibitor of the bacterial MurD ligase. The crystal structure of the enzyme-inhibitor complex revealed that the thiazolidine-2,4-dione moiety of **6** (Figure 5) is located in the uracil-binding pocket in the active site of the enzyme, where it forms hydrogen bonds, stacking, and hydrophobic interactions with the active site residues, but makes no covalent bonds with the nucleophilic amino acid residues of the protein.[24]

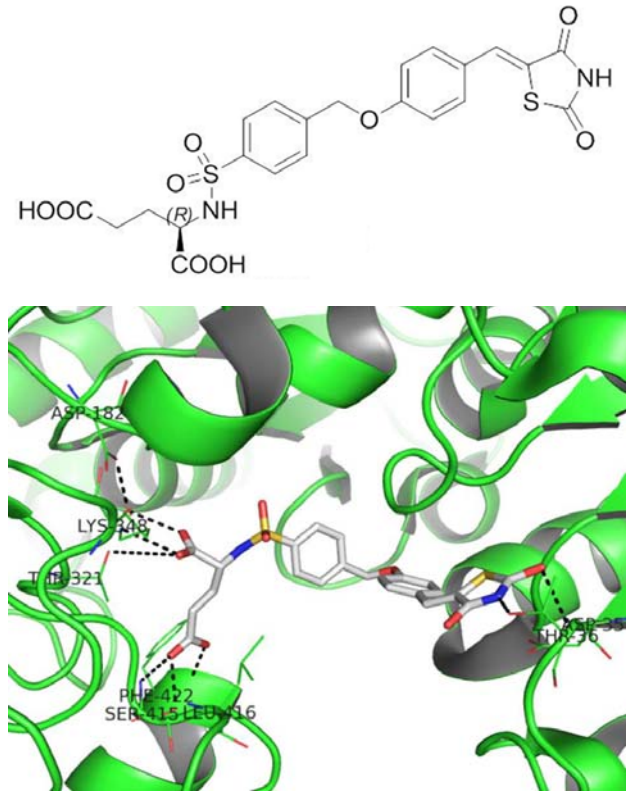


Figure 5: Crystal structure of the thiazolidin-2,4-dione-based inhibitor **6** in the active site of *E. coli* MurD (PDB ID code: 2Y67) .[24] The interactions with the amino acid residues in the active site are presented as dashed lines.

We confirmed this findings through our NMR experiment where compound **6** gave only a slow and incomplete reaction with cysteamine even after the addition of an excess (5 eq) of cysteamine (Supporting Information, Figure 5b).

The applied NMR spectroscopic method can be used as a simple and rapid experimental tool for the assessment of hit compounds for their potential to covalently

modify protein targets via cysteine thiol group addition to Michael acceptors-the reactivity can be easily estimated by the measurement of the ^{1}H NMR signal intensity of the vinyl proton. Additionally, the method provides important structural information, particularly if more than one reactive site is present in a molecule. However, the reactivity of compounds with their target proteins could not be completely excluded using this method because the reactivity of cysteine thiol group importantly varies depending on the protein environment.

3. Conclusion

In summary, we have evaluated the Michael-acceptor reactivity of benzylidene-substituted oxo-heterocyclic compounds, potential Michael acceptors, in the presence of cysteamine as an exemplary biological nucleophile using Avonto et al.[18] NMR method.

Our findings indicate that although 5-benzylidenerhodanines and similar heterocyclic compounds are not entirely nonreactive for addition of cysteamine, their electrophilicities are significantly lower compared to those of 5-benzylidenebarbiturates. We have confirmed that compound **6**, a potent thiazolidine-2,4-dione-based MurD inhibitor, does not act as a Michael acceptor for cysteamine. We can conclude that the distinct binding profile of the most controversial compounds, 5-benzylidenerhodanines, is not necessarily related to their unspecific Michael acceptor reactivity.

4. Experimental Section

4. 1. Chemistry

The chemicals were obtained from Acros, Aldrich Chemical Co., Apollo Scientific, and Fluka and used without further purification. The analytical thin-layer chromatography was performed on silica gel Merck 60 F_{254} precoated plates (0.25 mm). The flash column chromatography was carried out on silica gel 60 (particle size of 0.040–0.063 mm; Merck, Germany). The melting points were determined on a Reichert hot stage microscope and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer at 295 K and 400 MHz and are reported in ppm using the solvent as the internal standard (DMSO-d_6 at 2.50 ppm). The ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer at 295 K and 100 MHz and are reported in ppm using the solvent as the internal standard (DMSO-d_6 at 39.5 ppm). ^{1}H/^{13}C HSQC and HMBC spectra were recorded on a Bruker Avance III 400 MHz spectrometer at 295 K and 400 MHz and are reported in ppm using the solvent as the internal standard (DMSO-d_6 at 2.50 ppm). The ^{1}H/^{15}N HMBC spectrum was recorded on a Varian DDR2 600 MHz spectrometer at 295 K. The mass spectra data were recorded using a Q-Tof Premier (Waters-Micromass, Manchester, UK).

General procedure for the preparation of 5-benzylidene barbituric acids 1a–1e.

A suspension of the corresponding benzaldehyde (1.00 mmol) and barbituric acid (128 mg, 1.00 mmol) in water (20 mL) was refluxed for 10 h. The mixture was then cooled to room temperature, and the product was filtered off and washed with EtOH.

General procedure for the preparation of 5-benzylidenerhodanines 2a–2e and 5-benzylidenethiazolidin-4-one 3a–3e.

To a suspension of rhodanine (**2a–2e**; 700 mg, 5,26 mmol) or thiazolidine-2,4-dione (**3a–3e**; 616 mg, 5.26 mmol) and the corresponding benzaldehyde (5.78 mmol) in absolute ethanol (5 mL) in a 10-mL process vial, glacial acetic acid (25 μL, 0.26 mmol) and piperidine (26 μL, 0.26 mmol) were added. The vial was sealed, placed in a microwave reactor, and heated at 150 °C for 20 minutes (maximum power of 30 W). The reaction mixture was cooled, and the precipitate filtered off and dried.

General procedure for the preparation of 5-benzylidene hydantoins 4a–4e and 5-benzylidene thiohydantoins 5a–5e.

To a stirred suspension of the corresponding benzaldehydes (3.33 mmol) and imidazolidine-2,4-dione (500 mg, 5 mmol) or 2-thioxoimidazolidin-4-one (580 mg, 5 mmol) in glacial acetic acid (5 mL) ammonium acetate (507 mg, 6.6 mmol) was added and the reaction mixture was heated under reflux for 20 h. The solution was cooled, and the precipitate was filtered off, washed with water and ethanol, and dried.

(Z)-4-((2,5-dioxoimidazolidin-4-ylidene)methyl)benzoic acid (4c). Yield: 52%; yellow solid; m. p. > 300 °C; IR (KBr) ν = 3155, 1778, 1723, 1656, 1606, 1387, 1266, 1183, 1118, 1104, 1012, 885 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz): δ 13.05 (s, 1H, COOH), 11.37 (s, 1H, NH), 10.73 (s, 1H, NH), 7.93 (d, 2H, J = 8.4 Hz, Ar-H-2,6), 7.73 (d, 2H, J = 8.4 Hz, Ar-H-3,5), 6.46 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.9, 165.4, , 155.7, 137.3, 129.8, 129.5, 129.4, 129.2, 106.7. MS (ESI) (%) = 231.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_7N_2O_4$: 231.0406, found: 231.0409.

Z)-4-((5-oxo-2-thioxoimidazolidin-4-ylidene)methyl) benzoic acid (5c). Yield: 52%; yellow crystals; m. p. >300 °C ; IR (KBr) ν = 3312, 2985, 2478, 1731, 1694, 1651, 1495, 1377, 1266, 1171, 1114, 1087, 882 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz): δ 13.03 (s, 1H, COOH), 12.49 (s, 1H, NH), 12.34 (s, 1H, NH), 7.94 (d, 2H, J = 8.4 Hz, Ar-H-2,6), 7.84 (d, 2H, J = 8.4 Hz, Ar-H-3,5), 6.51 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.6,

166.8, 165.7, 136.6, 130.5, 130.0, 129.5, 129.1, 109.7. MS (ESI) (%) = 247.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_7N_2O_3S$: 247.0177, found: 247.0175.

4. 2. NMR Experiments

The compound (0.10 mmol) was dissolved in DMSO-d_6 (500 μL) in a standard 5-mm NMR tube, and the spectrum was recorded at 400 MHz. Cysteamine (7.7 mg, 0.10 mmol, 1 mol equiv) was then added, and the ^{1}H NMR spectrum was recorded 5 min after the addition. A positive assay was evidenced by the disappearance of a particular ^{1}H NMR absorption for olefin system of the substrate. All of the original spectra are presented in the Supporting Information.

The ^{1}H/^{15}N HMBC spectrum was recorded at 600 MHz 15 min after the addition of cysteamine (7.7 mg, 0.10 mmol) to the tube with compound **1b** (26.2 mg, 0.10 mmol) dissolved in DMSO-d_6 (500 μL).

6. Acknowledgements

This work was financially supported by the Ministry of Higher Education, Science and Technology of the Republic of Slovenia and by Ad Futura Foundation.

5. References

1. R. Šink, S. Gobec, S. Pečar, A. Zega, *Curr. Med. Chem.* **2010**, *17*, 4231–4255.
2. J. B. Baell, G. A. Holloway, *J. Med. Chem*. **2010**, *53*, 2719–2740.
3. J. B. Baell, *Future Med. Chem*. **2010**, *2*, 1529–1546.
4. R. F. Bruns, I. A. Watson, *J. Med.Chem.* **2012**, *55*, 9763–9772.
5. T. Tomašić, L. Peterlin Mašič, *Curr. Med. Chem*. **2009**, *16*, 1596–1629.
6. T. Tomašić, L. Peterlin Mašič, *Expert Opin. Drug. Discov*. **2012***, 7,* 549–560.
7. J. P. Powers, D. E. Piper, Y. Li, V. Mayorga, J. Anzola, J. M. Chen, J. C. Jaen, G. Lee, J. Liu, M. G. Peterson, G. R. Tonn, Q. Ye, N. P. C. Walker, Z. Wang, *J. Med. Chem.* **2006**, *49*, 1034–1046.
8. E. E. Carlson, J. M. My, L. L. Kiessling, *Chem. Biolog.* **2006,** *13*, 825–837.
9. T. Tomašić, R. Šink, N. Zidar, A. Fic, C. Contreras-Martel, A. Dessen, D. Patin, D. Blanot, M. Müller-Premru, S. Gobec, A. Zega, D. Kikelj, L. Peterlin Mašič, *ACS Med. Chem. Lett.*, **2012**, *8*, 626–630.
10. T. Tomašić, N. Zidar, R. Šink, A. Kovač, D. Blanot, C. Contreras-Martel, A. Dessen, M. Müller-Premru, A. Zega, S. Gobec, D. Kikelj, L. Peterlin Mašič, *J. Med. Chem*. **2011**, *54,* 4600–4610.
11. J. R. Huth, R. Mendoza, E. T. Olejniczak, R.W. Johnson, D. A. Cothron, Y. Liu, C. G. Lerner, J. Chen, P. J. Hajduk, *J. Am. Chem. Soc*. **2005**, *127*, 217–224.
12. T. Mendgen, C. Steuer, C. D. Klein, *J. Med. Chem*. **2012**, *55*, 743–753.
13. R. Cremlyn, J. P. Bassin, F. Ahmed, M. Hastings, I. Hunt, T. Mattu, *Phosphorus Sulfur,* **1992**, *73*, 161–172.
14. B. Schreiber, H. Martinek, P. Wolschann, P. Schuster, *J. Am. Chem. Soc*. **1979**, *101*, 4708–4713.
15. R. Bednar, E. Haslinger, U. Herzig, O. E. Polansky, P. Wolschann, *Monatsh. Chem.* **1976**, *107*, 1115–1122.
16. A. R. Katritzky, I. Ghiviriga, D. C. Oniciu, F. Soti, *J. Heterocyc. Chem.* **1996**, *33*, 1927–1934.
17. N. Zidar, D. Kikelj, *Acta Chim. Slov*. **2011**, *58*, 151–157.
18. C. Avonto, O. Taglialatela-Scafati, F. Pollastro, A. Minassi, V. Di Marzo, L. De Petrocellis, G. Appendino, *Angew. Chem., Int. Ed.* **2011**, *50*, 467–471.
19. J. A. H. Schwöbel, Y. K. Koleva, S. J. Enoch, F. Bajot, M. Hewitt, J. C. Madden, D. W. Roberts, T. W. Schultz, M. T. D. Cronin, *Chem. Rev*. **2011**, *111*, 2562–2596.
20. K. P. Cusack, L. D. Arnold, C. E. Barberis, H. Chen, A. M. Ericsson, G. S. Gaza-Bulseco, T. D. Gordon, C. M. Grinnell, A. Harsch, M. Pellegrini, E. Tarcsa, *Bioorg. Med. Chem. Lett*. **2004,** *14,* 5503–5507.
21. T. W. Schultz, J. W. Yarbrough, R. S. Hunter, A. O. Aptula, *Chem. Res.Toxicol*. **2007**, *20*, 1359–1363.
22. S. Amslinger, N. Al-Rifai, K. Winter, K. Wörmann, R. Scholz, P. Baumeister, M. Wild, *Org. Biomol. Chem*., **2013**, *11*, 549–554.
23. T. Tomašić, N. Zidar, A. Kovač, S. Turk, M. Simčič, D. Blanot, M. Müller-Premru, M. Filipič, S. Golič Grdadolnik, A. Zega, M. Anderluh, S. Gobec, D. Kikelj, L. Peterlin Mašič, *ChemMedChem*, **2010***, 5*, 286–295.
24. N. Zidar, T. Tomašić, R. Šink, A. Kovač, D. Patin, D. Blanot, C. Contreras-Martel, A. Dessen, M. Müller-Premru, A. Zega, S. Gobec, L. Peterlin Mašič, D. Kikelj, *Eur. J. Med. Chem*. **2011**, *46*, 5512–5523.
25. T. Tomašić, A. Kovač, M. Simčič, D. Blanot, S. Golič Grdadolnik, S. Gobec, D. Kikelj, L. Peterlin Mašič, *Eur. J. Med. Chem*. **2011**, *46*, 3964–3975.
26. T. Tomašić, N. Zidar, M. Müller-Premru, D. Kikelj, L. Peterlin Mašič*, Eur. J. Med. Chem*. **2010**, *45*, 1667–1672.
27. H. Q. Cui, G. F. Ruda, J. Carrero-Lerida, L. M. Ruiz-Perez, I. H. Gilbert, D. Gonzalez-Pacanowska, *Eur. J. Med. Chem.* **2010**, *45*, 5140–5149.
28. Tautomeric forms were verified using ACD/NMR Predictor, version 12.01, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www. acdlabs.com, 2012.
29. S. Amslinger, *Chem Med Chem,* **2010**, *5*, 351–356.

Povzetek

Kljub obstoječim eksperimentalnim in računalniškim orodjem za detekcijo lažno pozitivnih zadetkov, ostaja nespecifična kemijska modifikacija tiolnih skupin proteinov pomemben vir lažno pozitivnih spojin. V članku smo opisali uporabo preproste NMR metode v sistematični študiji reaktivnosti 5-benzilidenbarbituratov, 5-benzilidenrodaninov, in sorodnih okso-heterociklov, struktur ki so povezane s številnimi biološkimi aktivnostmi in so pred kratkim pridobile sloves neselektivnih, promiskuitetnih ligandov. Z uporabo te metode smo potrdili reaktivnost 5-benzilidenbarbituratov za katere je znano, da lahko tvorijo Michaelove adukte z nukleofili. Nasprotno so 5-benziliden petčlenski oksoheterocikli izkazali skoraj nepomembno reaktivnost. Zaključimo lahko, da profil vezave najbolj spornih spojin, 5-benzilidenrodaninov, ni nujno povezan z njihovo nespecifično Michael-akceptorsko reaktivnostjo.

Supporting Information

Evaluation of Michael-type Acceptor Reactivity of 5-Benzylidenebarbiturates, 5-benzylidenerhodanines, and Related Heterocycles Using NMR

Emilija Arsovska,[1] Jurij Trontelj,[1] Nace Zidar,[1] Tihomir Tomašić,[1] Lucija Peterlin Mašič,[1] Danijel Kikelj,[1] Janez Plavec[2] and Anamarija Zega[1,*]

[1] *University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia*

[2] *National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia*

* *Corresponding author: E-mail: anamarija.zega@ffa.uni-lj.si*

Received: 01-02-2014

Section 1.

^{1}H and ^{13}C chemical shifts of the parent compounds and Michael adducts.

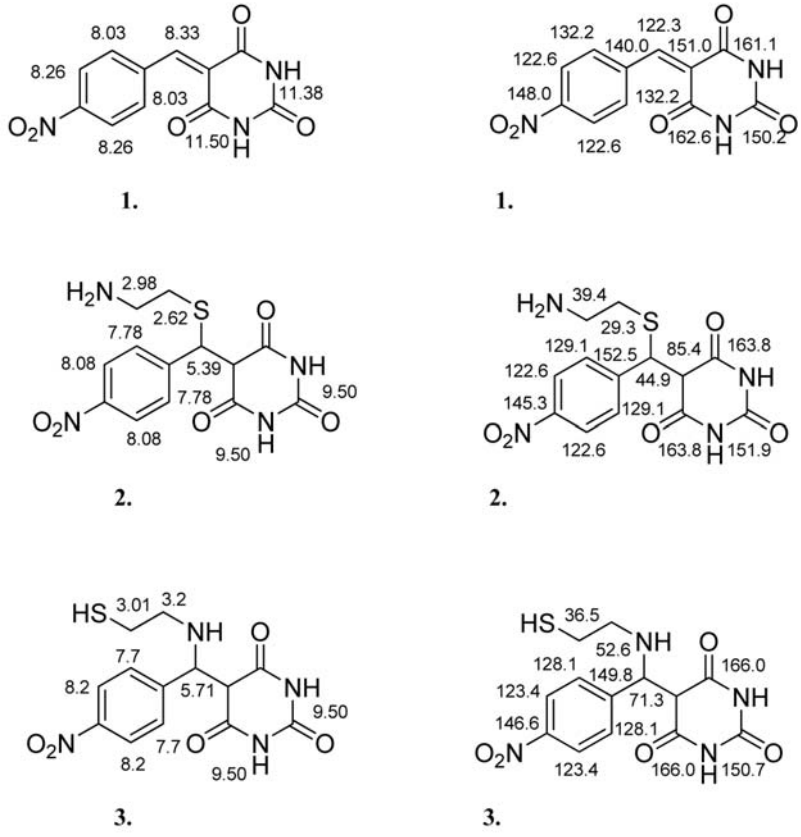


Figure 1a. Assigned ^{1}H (left) and ^{13}C (right) NMR Spectra of **1b** (**1**), its Michael adduct with the thiol group of cysteamine (**2**) and its Michael adduct with the amino group of cysteamine (**3**).

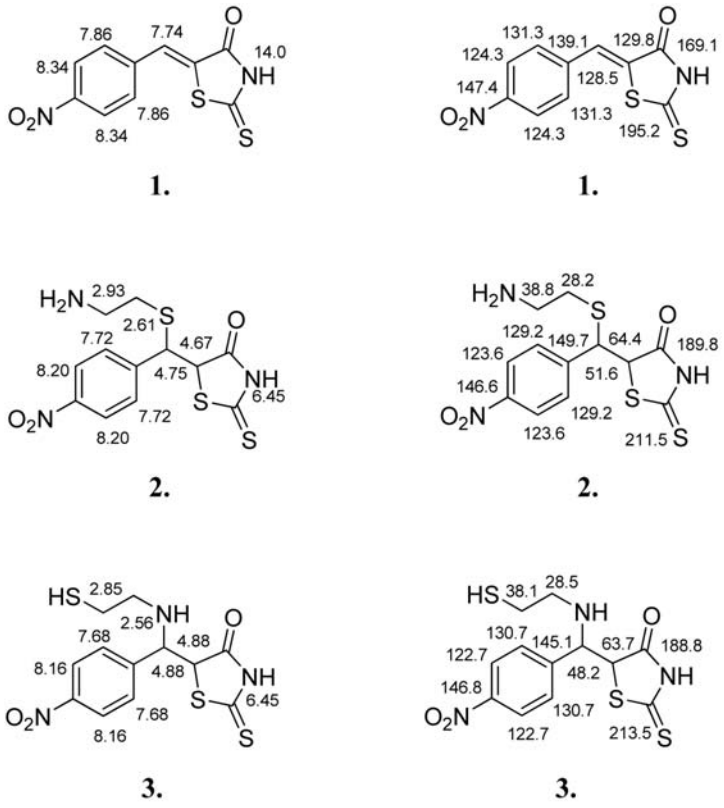


Figure 2a. Assigned ^{1}H (left) and ^{13}C (right) NMR Spectra of **2b** (**1**), its Michael adduct with the thiol group of cysteamine (**2**) and its Michael adduct with the amino group of cysteamine (**3**).

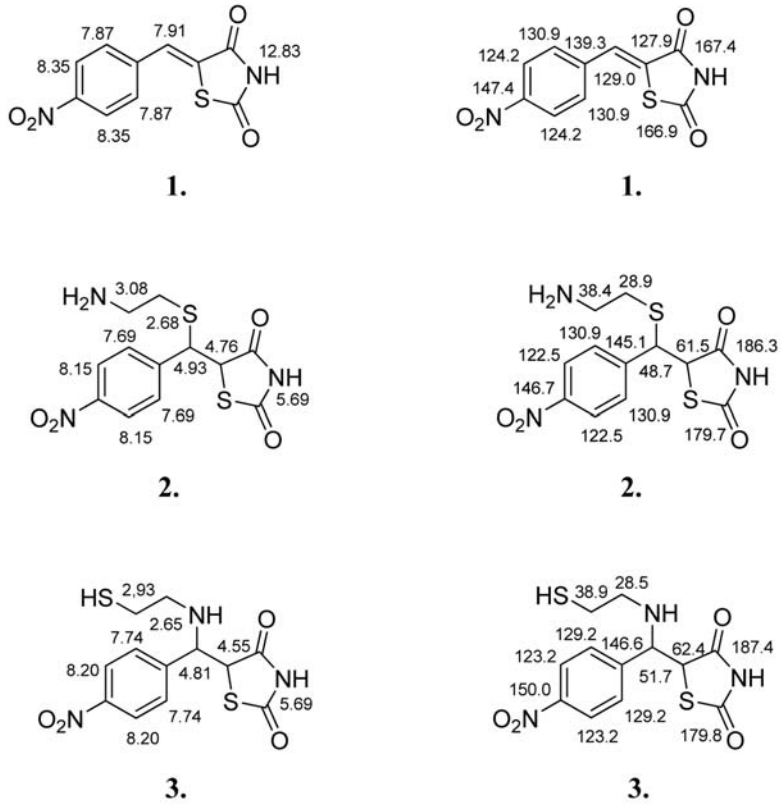


Figure 3a. Assigned ^{1}H (left) and ^{13}C (right) NMR Spectra of **3b** (**1**), its Michael adduct with the thiol group of cysteamine (**2**) and its Michael adduct with the amino group of cysteamine (**3**).

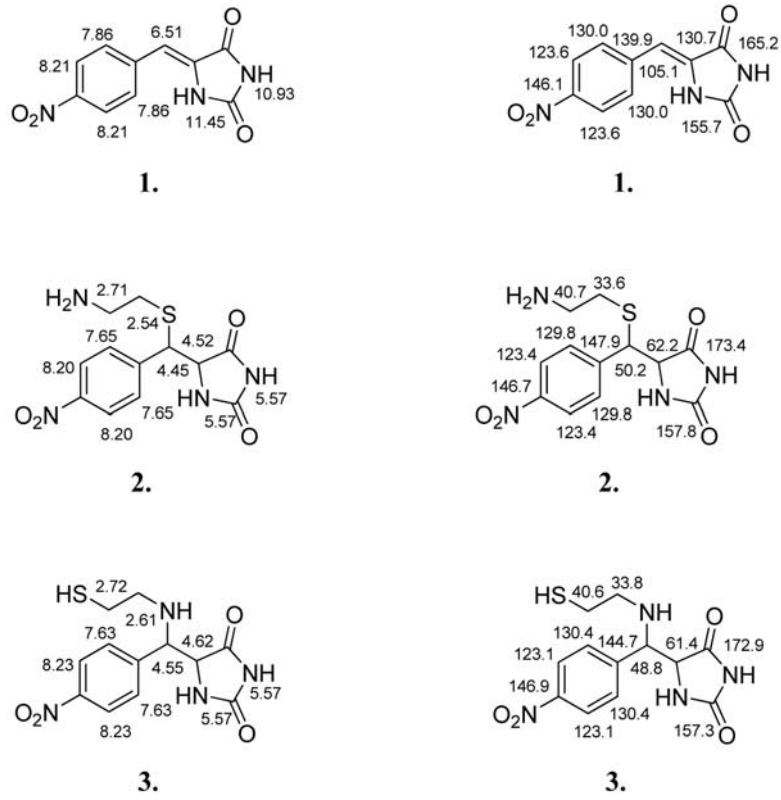


Figure 4a. Assigned ^{1}H (left) and ^{13}C (right) NMR Spectra of **4b** (**1**), its Michael adduct with the thiol group of cysteamine (**2**) and its Michael adduct with the amino group of cysteamine (**3**).

Figure 5a. Assigned ^{1}H (left) and ^{13}C (right) NMR Spectra of **5b** (**1**), because this compound was almost nonreactive, the Michael adducts could not be assigned properly.

Section 2:

Original NMR spectra.

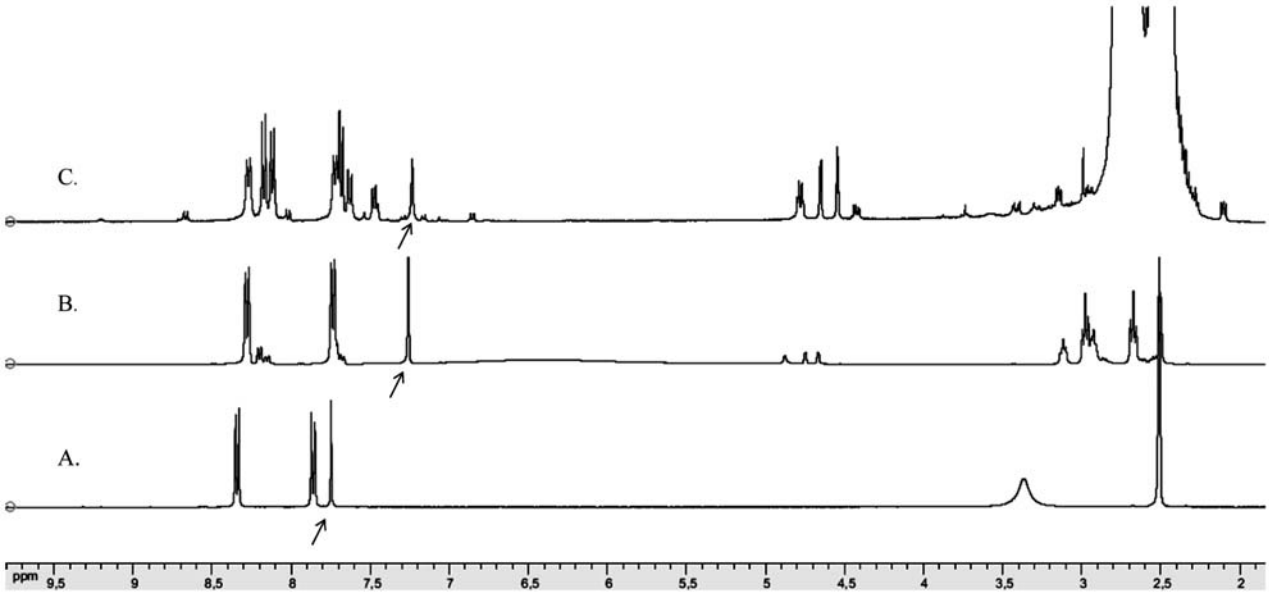


Figure 1b. ^{1}H NMR spectra of **2b** in DMSO-d_6 before (A), 5 minutes after the addition of 1 equivalent of cysteamine (B), and 5 minutes after the addition of 20 equivalents of cysteamine (C). The arrow notes the NMR signal of the vinyl proton.

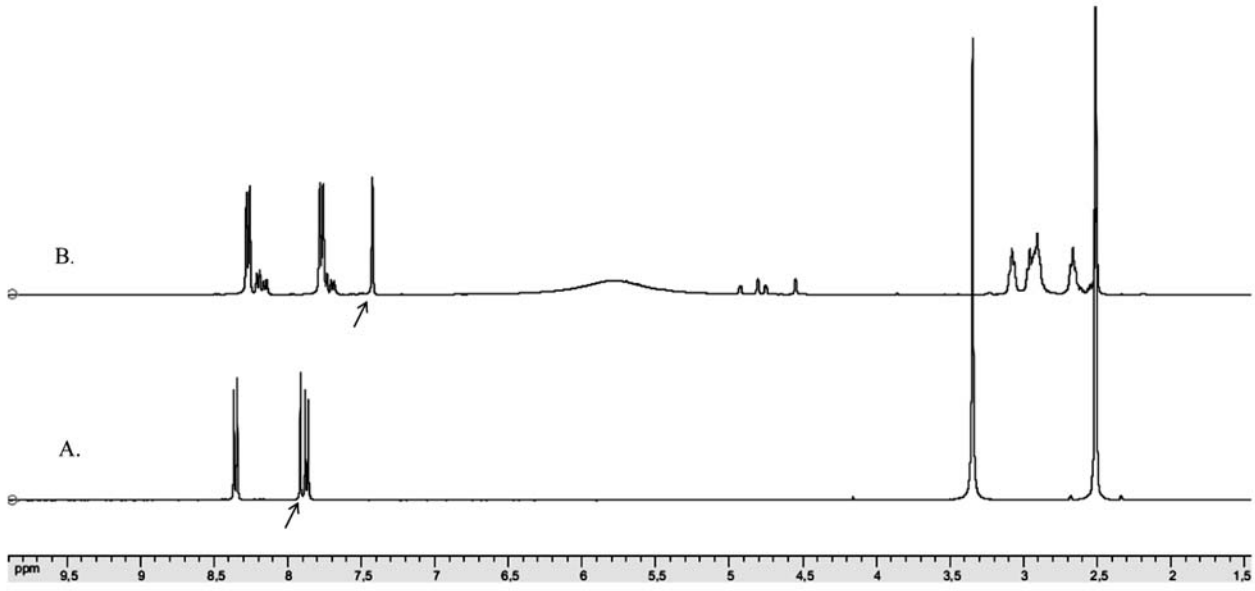


Figure 2b. ^{1}H NMR spectra of **3b** in DMSO-d_6 before (A) and 5 minutes after the addition of 1 equivalent of cysteamine (B). The arrow notes the NMR signal of the vinyl proton.

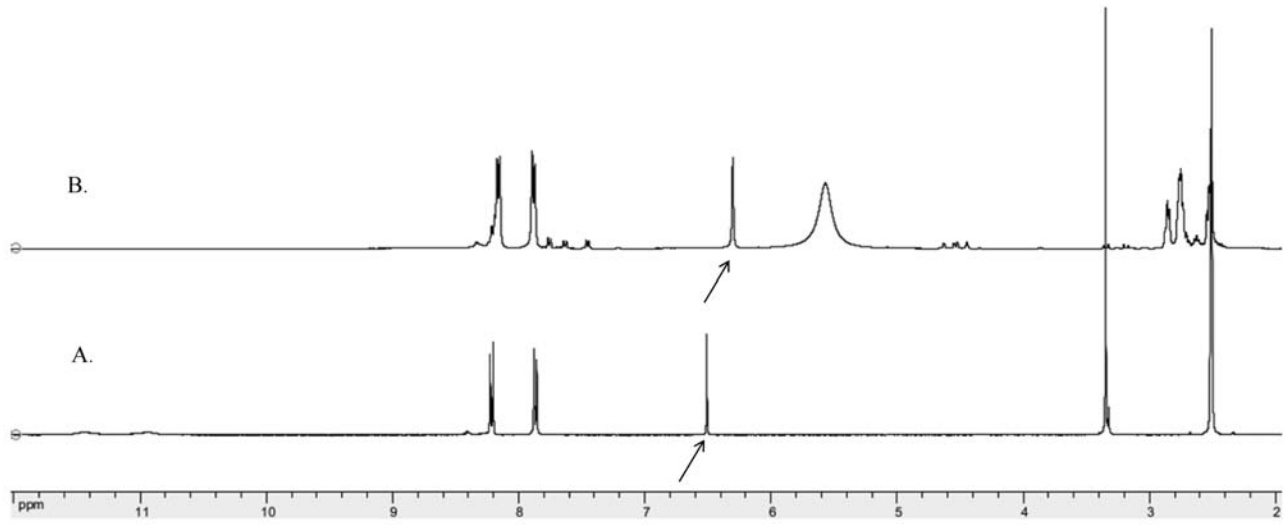


Figure 3b. ^{1}H NMR spectra of **4b** in DMSO-d_6 before (A) and 5 minutes after the addition of 1 equivalent of cysteamine (B). The arrow notes the NMR signal of the vinyl proton.

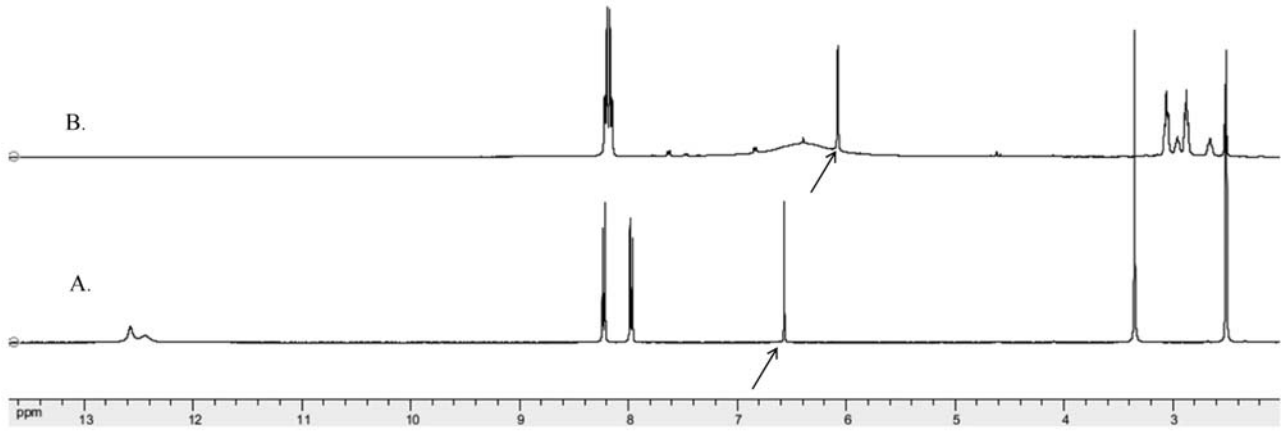


Figure 4b. ^{1}H NMR spectra of **5b** in DMSO-d_6 before (A) and 5 minutes after the addition of 1 equivalent of cysteamine (B). The arrow notes the NMR signal of the vinyl proton.

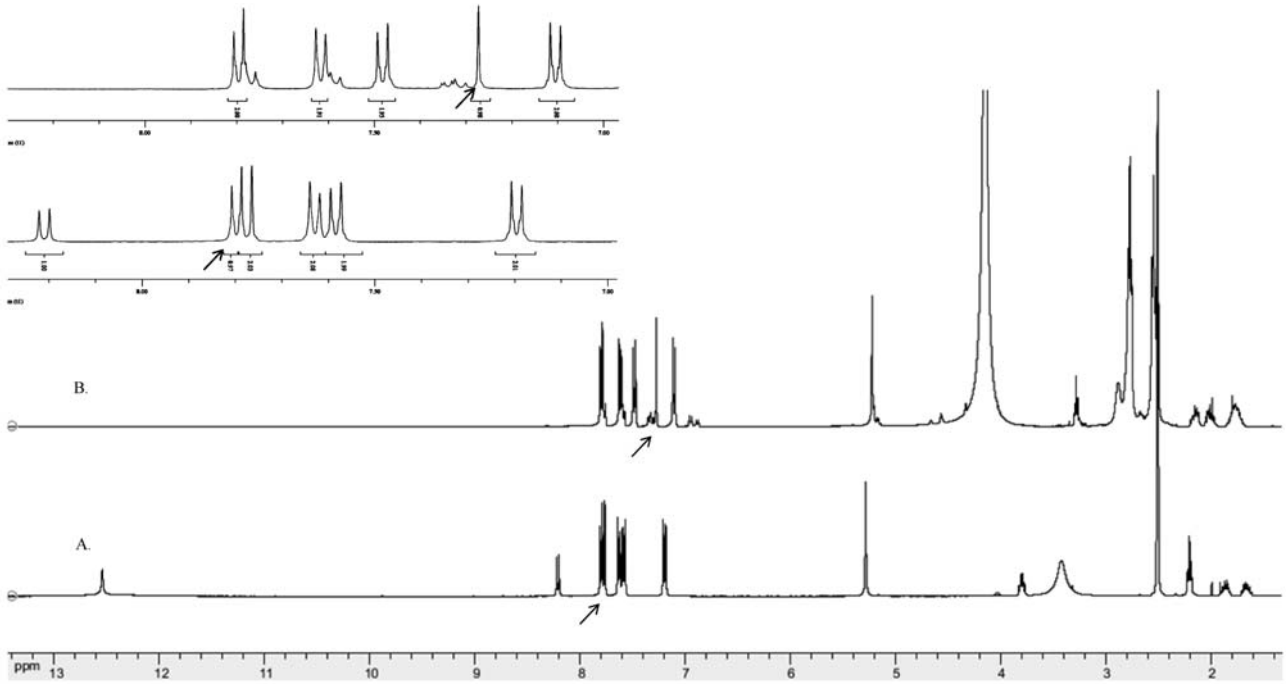


Figure 5b. ^{1}H NMR spectra of compound **I** in DMSO-d_6 before (A) and 5 minutes after the addition of 5 equivalents of cysteamine (B). The arrow notes the NMR signal of the vinyl proton.

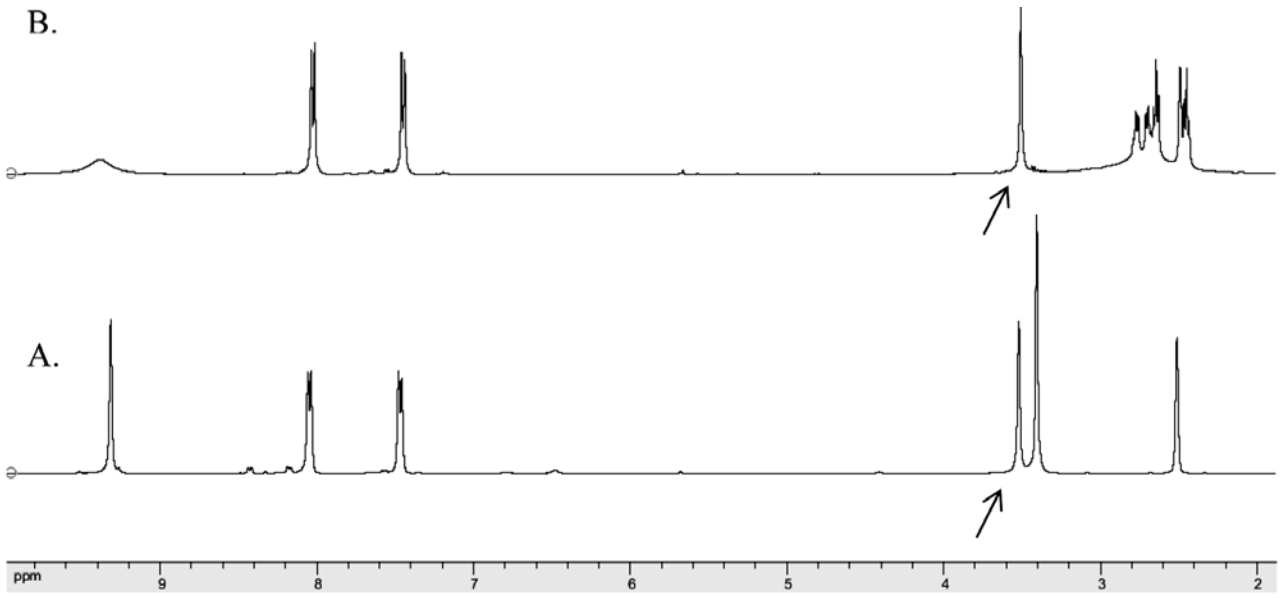


Figure 6b. ^{1}H NMR spectra of **1f** in DMSO-d_6 before (A) and 5 minutes after the addition of 1 equivalent of cysteamine (B). The arrow notes the NMR signal of the methylene proton.

Section 3:

We confirmed the formation of adducts between **1b** and cysteamine with the ^{1}H-^{13}C HMBC NMR, . ^{1}H-^{13}C HSQC NMR spectra and the ^{1}H-^{15}N HMBC NMR spectra.

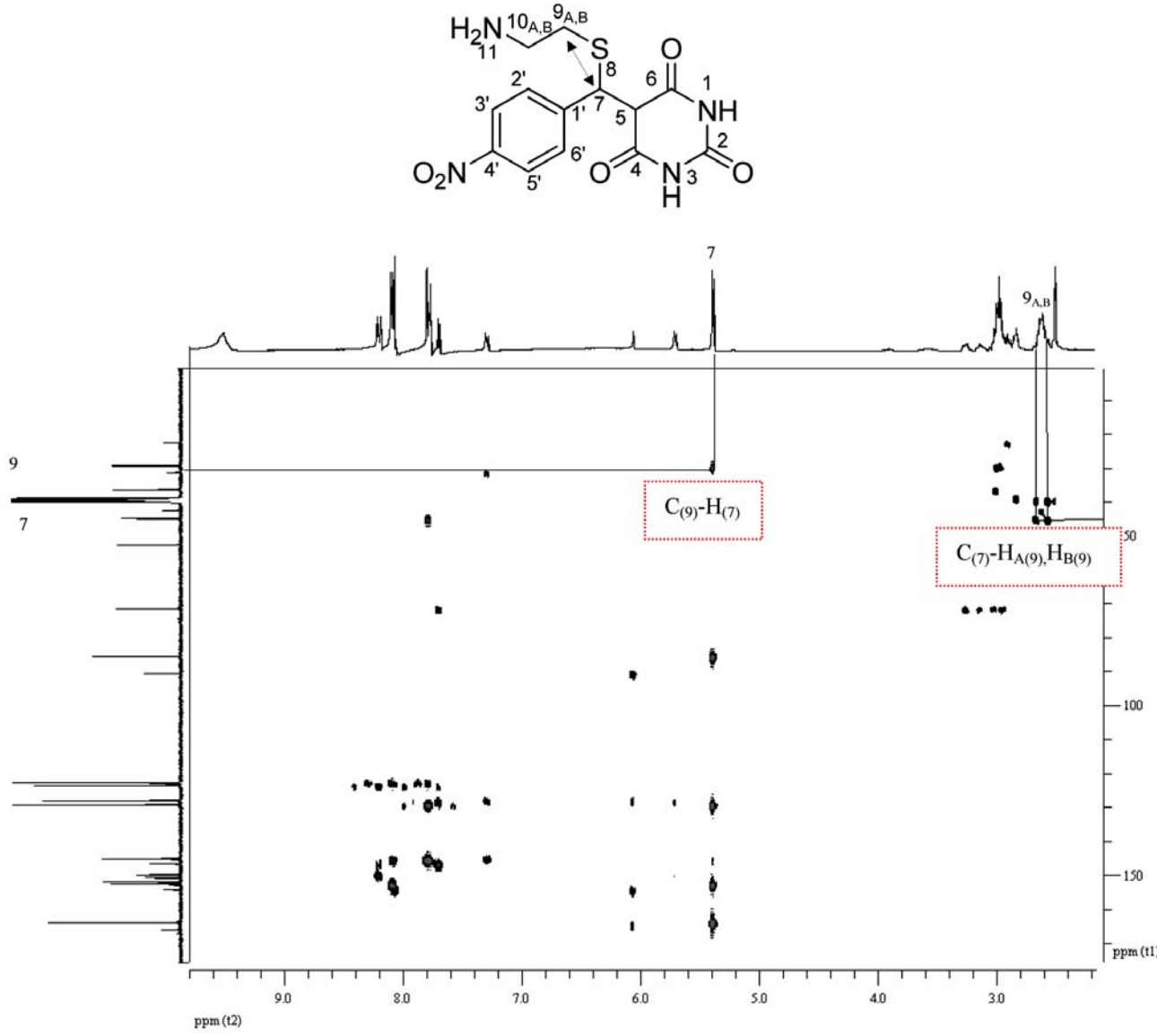
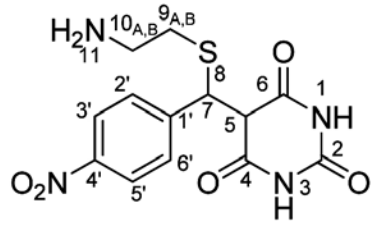


Figure 7b. ^{1}H-^{13}C HMBC NMR spectra reveal the formation of Michael adduct between **1b** and the thiol group of cysteamine.



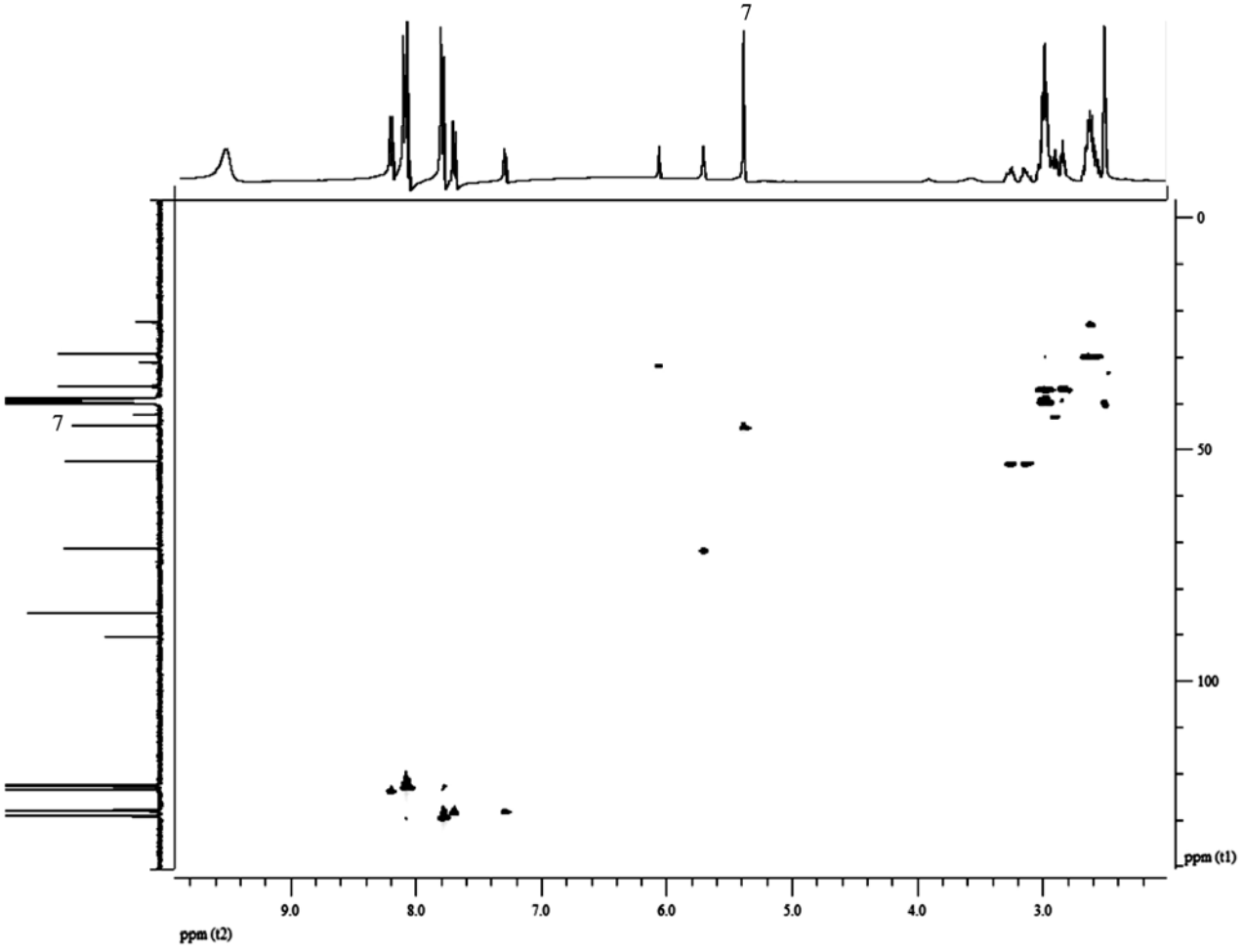


Figure 8b. ^{1}H-^{13}C HSQC NMR spectra reveal the formation of Michael adduct between **1b** and the thiol group of cysteamine,

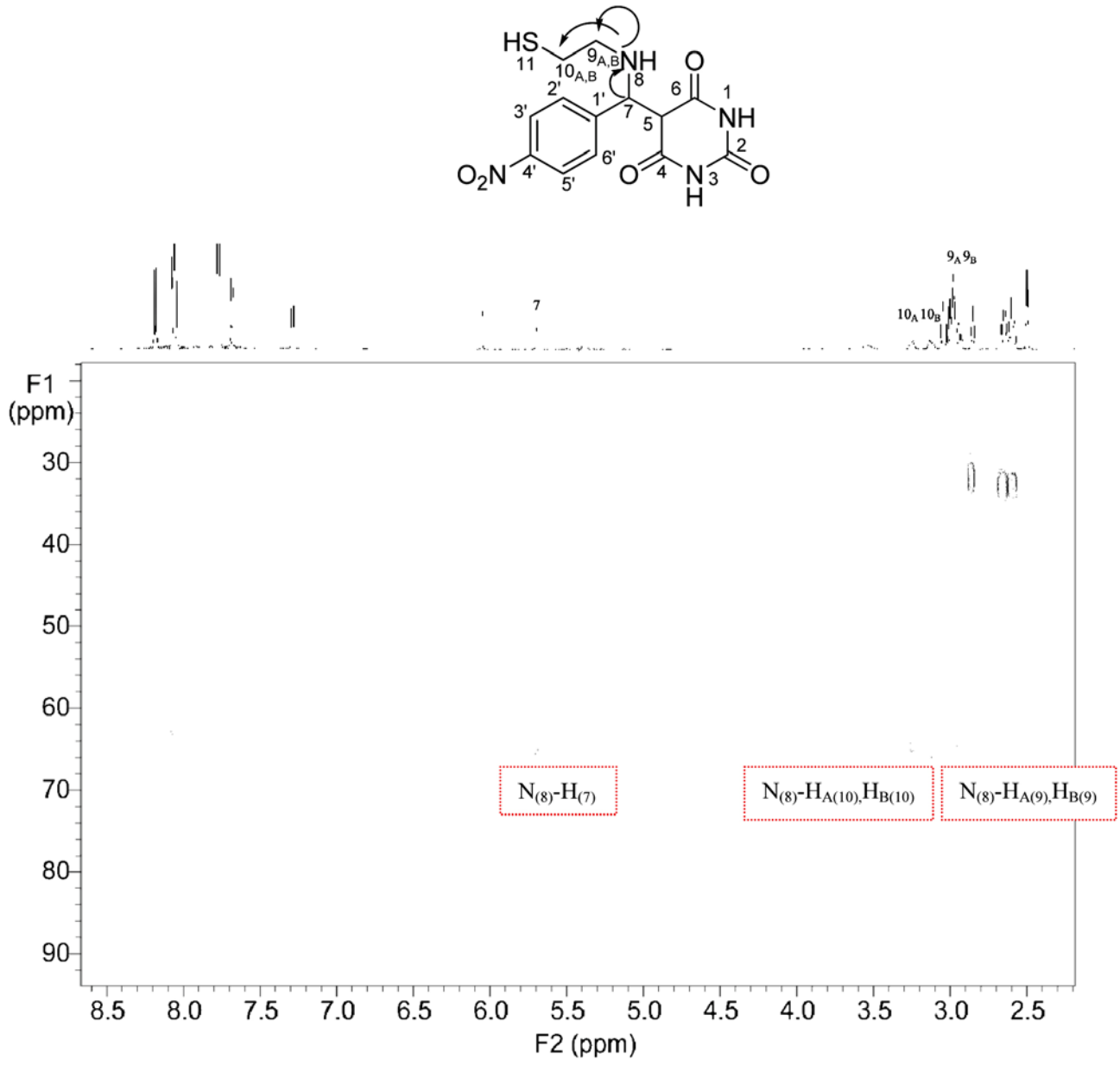


Figure 9b. ^{1}H-^{15}N HMBC NMR spectra reveal the formation of Michael adduct between **1b** and the amino group of cysteamine.

Section 4:

Table 1. Quantification of the analytes after the addition of cysteamine- the table with all compounds and their reactivities.

Analyte	Relative amount of reactant (%) NMR	Relative amount of conjugate[a] (%) NMR
1a-cysteamine	0	46
1b-cysteamine	0	71.9
1c-cysteamine	0	51.2
1d-cysteamine	0	53.4
1e-cysteamine	0	29.7
2a-cysteamine	92.6	5.5
2b-cysteamine	76.3	15.2
2c-cysteamine	91.7	4.5
2d-cysteamine	79.4	13.8
2e-cysteamine	97.1	1.9
3a-cysteamine	80.6	12.9
3b-cysteamine	72.4	15.3
3c-cysteamine	85.5	8.5
3d-cysteamine	76.3	15.2
3e-cysteamine	93.5	3.7
4a-cysteamine	95.2	3.1
4b-cysteamine	88.1	7.0
4c-cysteamine	95.2	2.8
4d-cysteamine	94.3	4.7
4e-cysteamine	95.2	1.8
5a-cysteamine	94.3	2.7
5b-cysteamine	94.3	3.2
5c-cysteamine	94.3	2.8
5d-cysteamine	94.2	2.9
5e-cysteamine	99	1

The relative amounts were quantified 5 minutes after the mixing of the reactants in the NMR tube. The quantification of the cysteamine conjugates was performed by integrating the signals of adducts and comparing the values to those from the integrals of the corresponding parent compounds. For statistical reasons, each measurement was repeated three times. [a]relative amount of the main adduct with the thiol group of cysteamine

Section 5:

Analysis of compounds

5-Benzylidenepyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (1a). White crystals; mp 265–268 °C (lit.[1] 256–258 °C); ^{1}H NMR (400 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 11.40 (s, 1H, NH), 11.25 (s, 1H, NH), 8.29 (s, 1H, CH=C), 8.07–8.09 (m, 2H, Ar-H-2,6), 7.57–7.61 (m, 3H, Ar-H-3,4,5). ^{13}C NMR (100 MHz, [D$_6$]DMSO, DMSO-d_6) δ 163.4, 161.5, 154.6, 150.2, 133.1, 132.6, 132.2, 128.0, 119.1. HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_7N_2O_3$: 215.0457, found: 215.0454.

5-(4-Nitrobenzylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (1b). White crystals; mp 289–292 °C (lit.[2] 290–293 °C); ^{1}H NMR (400 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 11.50 (s, 1H, NH), 11.33 (s, 1H, NH), 8.33 (s, 1H, CH=C), 8.26 (d, 2H, *J* = 8.8 Hz, Ar-H-3,5), 8.03 (d, 2H, *J* = 8.8 Hz, Ar-H-2,6). ^{13}C NMR (100 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 162.6, 161.1, 151.0, 150.2, 148.0, 140.0, 132.2, 122.6, 122.3. HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_6N_3O_5$: 260.0307, found: 260.0304.

4-((2,4,6-Trioxotetrahydropyrimidin-5(6*H*)-ylidene)methyl)benzoic Acid (1c). White crystals; mp > 300°C (lit.[3] >300 °C); ^{1}H NMR (400 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 13.22 (s, 1H, COOH), 11.46 (s, 1H, NH), 11.29 (s, 1H, NH), 8.31 (s, 1H, CH=C), 8.00 (d, 2H, *J* = 8.4 Hz, Ar-H-2,6), 7.96 (d, 2H, *J* = 8.4 Hz, Ar-H-3,5). ^{13}C NMR (100 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 166.7, 163.0, 161.3, 152.7, 150.2, 137.1, 132.6, 131.9, 128.5, 121.0. HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{12}H_7N_2O_5$: 259.0355, found: 259.0349.

4-((2,4,6-Trioxotetrahydropyrimidin-5(6*H*)-ylidene)methyl)benzonitrile (1d). White crystals; mp > 300 °C (lit.[4] 320 °C); ^{1}H NMR (400 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 11.49 (s, 1H, NH), 11.32 (s, 1H, NH), 8.29 (s, 1H, CH=C), 7,99 (d, 2H, *J* = 8.4 Hz, Ar-H-2,6), 7.90 (d, 2H, *J* = 8.4 Hz, Ar-H-3,5). ^{13}C NMR (100 MHz, [D6]DMSO, DMSO-d_6) δ = 162.7, 161.2, 151.6, 150.2, 137.9, 131.9, 131.5, 121.9, 118.5, 112.7. HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{12}H_6N_3O_3$: 240.0409, found: 240.0412.

5-(4-Hydroxybenzylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (1e). White crystals; mp >300 °C (lit.[5] >300 °C); ^{1}H NMR (400 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 11.26 (s, 1H, NH), 11.14 (s, 1H, NH), 10.81 (s, 1H, OH), 8.33 (d, 2H, *J* = 8.9 Hz, Ar-H-2,6), 8.21 (s, 1H, CH=C), 6.88 (d, 2H, *J* = 8.9 Hz, Ar-H-3,5). ^{13}C NMR (100 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 164.1, 163.0, 162.3, 155.4, 150.2, 138.3, 123.7, 115.5, 114.2. HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_7N_2O_4$: 231.0406, found: 231.0404.

5-(4-nitrobenzyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (1f). To a suspension of compound **1b** (261 mg, 1.00 mmol) in ethanol (20 mL), $NaBH_4$ (113 mg, 3.00 mmol) was added. The reaction mixture was stirred at RT for 2 h. The ?nal suspension was filtered and washed with ethanol (20 mL) and dichloromethane to obtain compound **1f** (220 mg, 85%) as a yellow solid; mp >300 °C ; IR (KBr) ν_{max} = 2923, 2849, 1601, 1492, 1451, 1371, 1069, 1028, 906, 757, 701, 540 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 8.91 (s, 2H, NH), 8.04 (d, 2H, *J* = 8.8 Hz, Ar-H-3,5), 7.75 (d, 2H, *J* = 8.8 Hz, Ar-H-2,6), 3.48 (s, 2H, CH2CH), 3.33 (s, 1H, COCHCO). ^{13}C NMR (100 MHz, [D$_6$]DMSO, DMSO-d_6) δ = 164.6, 154.1, 152.2, 144.9, 129.2, 122.7, 83.5, 29.0. MS (ESI) (%) = 262.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_8N_3O_5$: 262.0464, found: 262.0469.

(Z)-5-benzylidene-2-thioxothiazolidin-4-one(2a). Yield: 62%; yellow crystals; m. p. 205–209°C (lit.[6]

205–206 °C) ; IR (KBr) ν = 3447, 3272, 1708, 1606, 1508, 1406, 1342, 1287, 1223, 1181, 1000, 844 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO, DMSO-d_6): δ = 13.87 (s, 1H, NH), 7.65 (s, 1H, CH=C), 7.59–7.62 (m, 2H, Ar-H-2,6), 7.48–7.57 (m, 3H, Ar-H-3,4,5). ^{13}C NMR (100 MHz, [D_6]DMSO, DMSO-d_6) δ = 195.6, 169.3, 132.9, 131.6, 130.7, 130.4, 129.4, 125.5. MS (ESI) (%) = 222.0 (MH^+). HRMS (ESI-): *m/z* [*M*+H]$^+$ calcd for $C_{10}H_8NOS_2$: 222.0047, found: 222.0052.

(Z)-5-(4-Nitrobenzylidene)-2-thioxothiazolidin-4-one (2b). Yield: 75%; brown crystals; m. p. 256–260°C (lit.[7] 250–252 °C). IR (KBr) ν = 1698, 1588, 1435, 1195, 673,550, 517 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO, DMSO-d_6): δ = 14,02 (s, 1H, NH), 8.34 (d, 2H, J = 8.9 Hz, Ar-H-3,5), 7.86 (d, 2H, *J* = 8.9 Hz, Ar-H-2,6), 7.74 (s, 1H, CH=C). ^{13}C NMR (100 MHz, [D_6]DMSO, DMSO-d_6) δ = 195.2, 169.1, 147.4, 139.1, 131.3, 129.8, 128.5, 124.3. MS (ESI) (%) = 265.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_5N_2O_3S_2$: 264.9742, found: 264.9739.

(Z)-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl) benzoic acid (2c). Yield: 60%; yellow crystals; m. p. >300°C (lit.[8] 307–308 °C); IR (KBr) ν = 3024, 1728, 1690, 1607, 1593, 1475, 1411, 1374, 1290, 1244, 1179, 836 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO, DMSO-d_6): δ = 13.95 (s, 1H, NH), 13.26 (s, 1H, COOH), 8.06 (d, 2H, *J* = 8.5 Hz, Ar-H-2,6), 7.71 (d, 2H, *J* = 8.5 Hz, Ar-H-3,5). ^{13}C NMR (100 MHz, [D_6]DMSO, DMSO-d_6) δ = 195.3, 169.2, 166.5, 136.8, 131.8, 130.4, 130.0, 129.9, 127.8. MS (ESI) (%) = 264.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_6NO_3S_2$: 263.9789, found: 263.9783.

(Z)-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl) benzonitrile (2d). Yield: 68%; yellow crystals; m. p. 290–295 °C (lit.[9] 286 °C); IR (KBr) ν = 3114, 2234, 1714, 1605, 1444, 1282, 1191, 1066, 831 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO, DMSO-d_6): δ = 14.00 (s, 1H, NH), 7.99 (d, 2H, *J* = 8.4 Hz, Ar-H-2,6), 7.77 (d, 2H, *J* = 8.2 Hz, Ar-H-3,5), 7.70 (s, 1H, CH=C). ^{13}C NMR (100 MHz, [D_6]DMSO, DMSO-d_6) δ = 195.2, 169.2, 137.3, 133.0, 130.7, 129.1, 129.0, 118.3, 112.1. MS (ESI) (%) = 245.0 ([M-H]). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_5N_2OS_2$: 244.9843, found: 244.9837.

(Z)-5-(4-hydroxybenzylidene)-2-thioxothiazolidin-4-one (2e). Yield: 39%; yellow crystals; m. p. 280–285 °C (lit.[10] 274 °C); IR (KBr) ν = 3368, 3083, 2848, 1687, 1567, 1514, 1435, 1369, 1278, 1221, 1165, 1065, 1014, 829 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO, DMSO-d_6): δ = 13.72 (s, 1H, NH), 10.44 (s, 1H, OH), 7.57 (s, 1H, CH=C), 7.47 (d, 2H, *J* = 8.8 Hz, Ar-H-2,6), 6.93 (d, 2H, *J* = 8.8 Hz, Ar-H-3,5). ^{13}C NMR (100 MHz, [D_6]DMSO, DMSO-d_6) δ = 195.5, 169.4, 160.3, 133.0, 132.4, 123.9, 120.9, 116.5. MS (ESI) (%) = 236.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_6NO_2S_2$: 235.9840, found: 235.9843.

(Z)-5-(4-nitrobenzyl)-2-thioxothiazolidin-4-one (2f). Diethyl 2,6-dimetyl-1,4-dihydro-3,5-pyridinedicarboxylate (0. 427 g, 1.69 mmol) and silica gel (1.3 g, 1 g/mmol), which was previously activated by heating at 120°C for 5 h, were added to a stirred suspension of **2b** (0.345 g; 1.3 mmol) in toluene (50 mL). The mixture was heated to 100°C for 24 h under an argon atmosphere in the dark. The reaction mixture was then cooled and filtered. The filter cake was rinsed with ethyl acetate. The combined filtrate and rinse were evaporated to dryness. The residue was redissolved in ethylacetate (30 mL) and washed with 1 M HCl (3 × 30 mL) and brine (30 mL). The organic phase was dried over Na_2SO_4 and filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography using dichloromethane/methanol (20:1) as the eluent. Compound **2f** was obtained as a yellow solid (0.162 g, 47%); m. p. 126–130 °C (lit.[11] 125–128 °C); IR (KBr): ν = 2978, 2923, 1724, 1601, 1592, 1559, 1491, 1444, 1371, 1352, 1298, 1223, 1108, 1028, 865, 757, 699, 540 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO, DMSO-d_6): δ = 13.24 (s, 1H, CSNHCO), 8.20 (d, 1H, *J* = 8.7 Hz, Ar-H-3,5), 7.54 (d, 1H, *J* = 8.7 Hz, Ar-H-2,6), 5.12 (dd, 1H, J_1 = 8.3 Hz, J_2 = 5.3 Hz, SCHCO), 3.52 (dd, 1H, AB system, ^2J = 14.1 Hz, ^3J =5.3 Hz, HB from Ar-CH2CH), 3.38 (dd, 1H, AB system, ^2J = 14.1 Hz, ^3J =8.3 Hz, HA from Ar-CH2CH). MS (ESI) (%) = 267.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_7N_2O_3S_2$: 266.9898, found: 266.9891.

(Z)-5-benzylidenethiazolidine-2,4-dione (3a). Yield: 50%; yellow crystals; m. p. 245–250°C (lit.[12] 245–247 °C); IR (KBr) ν = 3028, 1697, 1594, 1492, 1332, 1297, 1212, 1165, 1020, 919, 812 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO, DMSO-d_6): δ = 12.64 (s, 1H, NH), 7.81 (s, 1H, CH=C), 7.59–7.62 (m, 2H, Ar-H-2,6), 7.52–7.56 (m, 2H, Ar-H-3,5), 7.47-7.51 (m, 1H, Ar-H-4). ^{13}C NMR (100 MHz, [D_6]DMSO, DMSO-d_6) δ = 167.9, 167.3, 133.0, 131.7,130.4, 130.0, 129.3, 123.5. MS (ESI) (%) = 204.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_6NO_2S$: 204.0119, found: 204.0114.

(Z)-5-(4-nitrobenzylidene)thiazolidine-2,4-dione (3b). Yield: 34%; yellow crystals; m. p. 272–276 °C (lit.[12] 267–270 °C); IR (KBr) ν = 3257, 1716, 1608, 1951, 1508, 1412, 1310, 1212, 1137, 1106, 1008, 919, 843 cm^{-1}. ^1H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 12.83 (s, 1H, NH), 8.35 (d, 2H, *J* = 8.8 Hz, Ar-H-3,5), 7.91 (s, 1H, CH=C), 7.87 (d, 2H, *J* = 8.8 Hz, Ar-H-2,6). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 167.4, 166.9, 147.4, 139,3, 130.9, 129,0, 127.9, 124.2. MS (ESI) (%) = 249.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_5N_2O_4S$: 248.9970, found: 248.9964.

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)benzoic acid (3c). Yield: 23%; yellow solid; m. p. > 300°C; IR (KBr) ν = 3423, 3053, 1687, 1611, 1568, 1422, 1323, 1289, 1162, 1126, 1010, 908, 852, 773, 698, 660, 611, 518, 480. ^{1}H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 13.22 (s, 1H, COOH), 12.74 (s, 1H, NH), 8.06 (d, 2H, *J* = 8.3 Hz, Ar-H-2,6), 7.84 (s, 1H, CH=C), 7.72 (d, 2H, *J* = 8.3 Hz, Ar-H-3,5). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 167.7, 167.2, 166.6, 137.0, 131.6, 130.3, 130.0, 126.0. MS (ESI) (%) = 248.0 ([M-H]$^-$,). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_6NO_4S$: 248.0018, found: 248.0014.

(Z)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)benzonitrile (3d). Yield: 37%; yellow crystals; m. p. 256–260°C; IR (KBr) ν = 3154, 3069, 2775, 2234, 1755, 1714, 1694, 1605, 1557, 1501, 1412, 1346, 1316, 1211, 1147, 970, 924, 834 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 12.79 (s, 1H, NH), 7.99 (d, 2H, *J* = 8.3 Hz, Ar-H-2,6, 7.86 (s, 1H, CH=C), 7.78 (d, 2H, *J* = 8.3 Hz, Ar-H-3,5). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 167.4, 167.0, 137.5, 133.0, 130.4, 129.6, 127.2, 118.4, 112.0. MS (ESI) (%) = 229.0 ([M-H]$^-$,). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_5N_2O_2S$: 229.0072, found: 229.0076.

(Z)-5-(4-hydroxybenzylidene)thiazolidine-2,4-dione (3e). Yield: 44%; yellow solid; m. p. >300°C (lit.[12] 310–312 °C); IR (KBr) ν = 3405, 2997, 1677, 1572, 1509, 1339, 1279, 1211, 1154, 1022, 900, 826 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 12.47(s, 1H, NH), 10.32 (s, 1H, OH), 7.70 (s, 1H, CH=C), 7.46 (d, 2H, *J* = 8.7 Hz, Ar-H-2,6), 6.92 (d, 2H, *J* = 8.7 Hz, Ar-H-3,5). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 168.1, 167.5, 159.8, 132.4, 132.3, 123.9, 118.9, 116.3. MS (ESI) (%) = 220.0 ([M-H]$^-$,). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_6NO_3S$: 220.0068, found: 220.0066.

(Z)-5-benzylideneimidazolidine-2,4-dione (4a). Yield: 57%; brown solid; m. p. 220–223°C (lit.[13] 220–224 °C); IR (KBr) ν = 3526, 3452, 3208, 2757, 1714, 1656, 1454, 1381, 1256, 1197, 1098, 1009, 880 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 11.27 (s, 1H, NH), 10.56 (s, 1H, NH), 7.61–7.63 (m, 2H, Ar-H–2,6), 7.39–7.42 (m, 2H, Ar-H-3,5), 7.31–7.35 (m, 1H, Ar-H-4), 6.42 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 165.5, 155.7, 132.9, 129.3, 128.7, 128.3, 127.9, 108.2. MS (ESI) (%) = 189.0 (MH$^+$,). HRMS (ESI+): *m/z* [*M*+H]$^+$ calcd for $C_{10}H_9N_2O_2$: 189.0664, found: 189.0665.

(Z)-5-(4-nitrobenzylidene)imidazolidine-2,4-dione (4b). Yield: 45%; yellow solid; m. p. >300°C (lit.[13] mp >335 °C); IR (KBr) ν = 3330, 3195, 3031, 2436, 1744. 1664, 1592, 1504, 1392, 1341, 1248, 1199, 1089, 1008, 876 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 11.45 (s, 1H, NH), 10.94 (s, 1H, NH), 8.21 (d, 2H, *J* = 8.9 Hz, Ar-H-3,5), 7.87 (d, 2H, *J* = 8.9 Hz, Ar-H-2,6), 6.51 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 165.2, 155.7, 146.1, 139.9, 130.7, 130.0, 123.6, 105.1. MS (ESI) (%) = 232.0 [M-H]$^-$,). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_6N_3O_4$: 232.0358, found: 232.0354.

(Z)-4-((2,5-dioxoimidazolidin-4-ylidene)methyl)benzonitrile (4d). Yield: 54%; yellow solid; m. p. > 300 °C (lit.[13] > 250 °C); IR (KBr) ν = 3194, 3150, 3043, 2762, 2222, 1717, 1665, 1376, 1287, 1260, 1096, 1018, 877 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 11.33 (s, 1H, NH), 10.89 (s, 1H, NH), 7.85 (d, 2H, *J* = 8.4 Hz, Ar-H-2,6), 7.79 (d, 2H, *J* = 8.4 Hz, Ar-H-3,5), 6.45 (s, 1H, CH=C), ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 165.2, 155.7, 137.7, 132.3, 130.2, 129.7, 118.7, 109.9, 105.8. MS (ESI) (%) = 214.0 (MH$^+$). HRMS (ESI): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_6N_3O_2$: 212.0460, found: 212.0456.

(Z)-5-(4-hydroxybenzylidene)imidazolidine-2,4-dione (4e). Yield: 48%; yellow solid; m. p. >300°C (lit.[13] 311–314 °C); IR (KBr) ν = 3347, 3170, 3057, 2740, 1751, 1709, 1657, 1586, 1515, 1367, 1270, 1223, 1019, 881 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 11.13 (s, 1H, NH), 10.34(s, 1H, OH), 9.88 (s, 1H, NH), 7.48 (d, 2H, J = 8.7 Hz, Ar-H-2,6), 6.79 (d, 2H, J= 8.7 Hz, Ar-H-3,5), 6.35 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 165.6, 158.0, 155.6, 131.2, 125.3, 123.8, 115.7, 109.3. MS (ESI) (%) = 203.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_7N_2O_3$: 203.0457, found: 203.0454.

(Z)-5-benzylidene-2-thioxoimidazolidin-4-one (5a). Yield: 57%; white crystals; m. p. 267–270°C (lit.[14] 272–274°C); IR (KBr) ν = 3232, 1723, 1642, 1477, 1342, 1249, 1186, 1172, 1091, 924 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 12.40 (s, 1H, NH), 12.17 (s, 1H, NH), 7.73–7.76 (m, 2H, Ar-H-2,6), 7.37–7.45 (m, 3H, Ar-H-3,4,5), 6.49 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 166.9, 165.4, 155.7, 137.3, 129.8, 129.5, 129.2, 106.7, MS (ESI) (%) = 203.0 ([M-H]$^-$). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_7N_2OS$: 203.0279, found: 203.0276.

(Z)-5-(4-nitrobenzylidene)-2-thioxoimidazolidin-4-one (5b). Yield: 45%; yellow crystals; m. p. >300°C (lit.[15] 294°C); IR (KBr) ν = 3204, 3004, 1743, 1654, 1517, 1480, 1334, 1175, 1084, 967 cm^{-1}. ^{1}H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 12.58 (s, 1H, NH), 12.43 (s, 1H, NH), 8.23 (d, 2H, *J* = 8.6 Hz, Ar-H-3,5), 7.97 (d, 2H, *J* = 8.6 Hz, Ar-H-2,6), 6.57 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 180.0, 165.6, 146.6, 139.1, 130.8, 130.2, 123.6, 107.9. MS (ESI) (%) = 250.0 (MH$^+$,). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_6N_3O_3S$: 248.0130 found: 248.0123.

(Z)-4-((5-oxo-2-thioxoimidazolidin-4-ylidene)methyl) benzonitrile (5d). Yield: 54%; yellow crystals; m. p. >300°C; IR (KBr) ν = 3224, 3009, 2235, 1731, 1651, 1509, 1494, 1364, 1342, 1251, 1183, 1093, 973, 873 cm^{-1}. ^1H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 12.52 (s, 1H, NH), 12.36 (s, 1H, NH), 7.91 (d, 2H, *J* = 8.6 Hz, Ar-H-2,6), 7.86 (d, 2H, *J* = 8.6 Hz, Ar-H-3,5), 6.52 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 179.9, 165.6, 137.1, 132.4, 130.5, 129.8, 118.7, 110.7, 108.7. MS (ESI) (%) = 230.0 (MH^+). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{11}H_6N_3OS$: 228.0232, found: 228.0229.

(Z)-5-(4-hydroxybenzylidene)-2-thioxoimidazolidin-4-one (5e). Yield: 50%; yellow crystals; m. p. >300°C; IR (KBr) ν = 3322, 3169, 1719, 1649, 1603, 1585, 1500, 1367, 1253, 1174, 967 cm^{-1}. ^1H NMR (DMSO- d_6, 400 MHz, DMSO-d_6): δ 12.27(s, 1H, NH), 11.99(s, 1H, NH), 10.07(s, 1H, OH), 7.64 (d, 2H, *J* = 8.8 Hz, Ar-H-2,6), 6.81 (d, 2H, *J* = 8.8 Hz, Ar-H-3,5), 6.43 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6, 100 MHz, DMSO-d_6) δ 178.2, 165.8, 159.0, 132.4, 125.1, 123.3, 115.8, 112.9. MS (ESI) (%) = 221.0 (MH^+). HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{10}H_7N_2O_2S$: 219.0228, found: 219.0230.

References

1. M. L. Deb, P. J. Bhuyan, *Tetrahedron Lett*. **2005**, *46*, 6453–6456.
2. T. S. Jin, R. Q. Zhao, T. S. Li, *Asian J. Chem*. **2007**, *19*, 3815–3820.
3. E. Gursu, N. Ulusoy, *Acta Pharm. Turc*. **1996**, *38*, 107–109.
4. K. Rehse, W. D. Kapp, *Arch. Pharm*. **1982**, *315*, 346–353.
5. J. T. Li, H. G. Dai, D. Liu, T. S. Li, *Synthetic Commun*. **2006,** *36*, 789–794.
6. J. F. Zhou, F. X. Zhu, Y. Z. Song, Y. L. Zhu, *ARKIVOC*, **2006**, *14,* 175–180.
7. T. Tomašić, N. Zidar, R. Šink, A. Kovač, D. Blanot, C. Contreras-Martel, A. Dessen, M. Müller-Premru, A. Zega, S. Gobec, D. Kikelj, L. Peterlin Masič, *J. Med. Chem*. **2011**, *54,* 4600–4610.
8. F. J. Allan, G. G. Allan, J. B. Thomson, *Can. J. Chem*. **1958**, *36*, 1579–1583.
9. J. A. Pinson, O. Schmidt-Kittler, J. Zhu, I. G. Jennings, K. W. Kinzler, B. Vogelstein, D. K. Chalmers, P. E. Thompson, *ChemMedChem*. **2011**, *6*, 514–522.
10. R. Andreasch, *J. Chem. Soc., Abstr.,* **1919,** *116*, 96–98.
11. N. Zidar, T. Tomašič, R. Šink, V. Rupnik, A. Kovač, S. Turk, D. Patin, D. Blanot, C. Contreras Martel, A. Dessen, M. Müller Premru, A. Zega, S. Gobec, L. Peterlin Mašič, D. Kikelj, *J. Med. Chem*. **2010**, *53*, 6584–6594.
12. D. H. Yang, B.Y. Yang, Z.C. Chen, S.Y. Chen, *Org. Prep. Proc. Int.,* **2006**, *38*, 81–85.
13. J. C. Thenmozhiyal, P. T. Wong, W. Chui, *J. Med. Chem.,* **2004**, *47*, 1527–1535.
14. Y. Sun, L. Gao, M. Ding, *Syn. Commun*. **2006**, *36,* 1185– 1191.
15. L. C. Santos, F. T. Uchoa, A. R. P. A. Canas, I. A. Sousa, R. O. Moura, M. C. A. Lima, S. L. Galdino, I. R. Pitta, J. Barbe, *Heterocycl. Comm*. **2005**, *11*, 121–128.