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Regioselectivity evaluation of the (Z)-5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione alkylation in alkaline environment

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ABSTRACT

Thiazolidine-2,4–dione represents a key heterocyclic core in medicinal chemistry because it has the ability to bind to a wide variety of protein targets and has been intensively studied for developing bioactive multitargeting agents. The N-alkylation of imides and O-alkylation of phenols are important reactions frequently used in the synthesis of biologically active compounds, like the thiazolidine-2,4–dione derivatives. Based on literature data, by treating (Z)-5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione with alkyl halides in a 1:1 molar ratio, either O-alkylation or N-alkylation reactions can take place. Six (Z)-5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione derivatives were synthesized by alkylating (Z)-5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione with aliphatic halogenated derivatives in alkaline environment, using a 1:1 molar ratio and then were physically and spectrally analyzed. The spectral data and the results obtained from the theoretical evaluation of the parent compound's acidity support the formation of N-alkylated derivatives in the reaction conditions.

1. Introduction

Of the five membered heterocyclic ring systems, thiazolidine-2,4–dione is an extensively studied moiety for developing new bioactive agents in the treatment of a broad spectrum of pathologies like diabetes and its complications, microbial infections, inflammation, arthritis, cancer, melanoma, etc. [1]. Thiazolidine-2,4–dione represents a privileged scaffold in medicinal chemistry due to its vast biological profile with multifarious pharmacological properties. Several thiazolidine-2,4–dione derivatives were reported as potential antitumor agents because of their inhibitory activity towards the phosphoinositide 3-kinase gamma, mitogen-activated protein kinase, Pim kinase and histone deacetylase and also as potential agonists of the nuclear peroxisome proliferator-activated receptors (PPARs), which induce antiproliferative, antiangiogenic and prodifferentiation pathways in many types of tissue, thus downregulating carcinogenesis [2]. Thiazolidine-2,4–dione was the pharmacophore moiety in compounds acting as PPARγ agonists/modulators, protein-tyrosine phosphatase 1B and aldose reductase-2 inhibitors, α-glucosidase inhibitors, free fatty acid receptor agonists, $\beta-3$ agonists, effective in the treatment of diabetes and its complications [3]. For the compounds bearing the thiazolidine-2,4–dione nucleus were found applications in the infectious diseases therapy like antifungal agents [4,5], antibacterial agents acting as autoinducer-2 quorum sensing, MurD ligase or peptide deformylase inhibitors, antimalarial agents, antiviral agents acting as HIV-1 reverse transcriptase inhibitors. Supplementary, this scaffold was reported responsible for the pharmacological activity of compounds acting as tyrosinase inhibitors useful for treating melanoma, antioxidant agents and xanthine oxidase inhibitors, anti-inflammatory, COX-2 inhibitors and neuroprotective agents [1,6].

Most thiazolidine-2,4–dione derivatives reported in the literature as biologically active compounds were developed through structural modifications in positions 3 (the free –NH moiety) and 5 (the free $-CH_2$ moiety) of the thiazolidine-2,4–dione nucleus, usually by N-alkylation and Knoevenagel condensation, respectively. [1,6]

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The O-alkylation of phenols and N-alkylation of imides in alkaline environment are intensively studied by the researchers in organic and medicinal chemistry [7-9] because they can be widely used for obtaining bioactive compounds, like the ones mentioned above. (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione has two potential acid groups, one being the phenol and the other the imide. In alkaline environment, they will both manifest their Brønsted-Lowry acidity by releasing the proton, thus creating an anionic structure, the nucleophile. This one is capable of interaction with the methylene (-CH_2-) group from an alkyl halide used as alkylating agent, the electrophile, according to a SN2 mechanism and following a two-step reaction. First, the anionic rich electronic species attacks the methylene moiety of the electrophile in an addition reaction that is followed afterwards by an elimination reaction of the halide anion [10]. The rate of the SN2 reaction depends on both the concentration of electrophile and nucleophile species in the reaction environment. The formation of the anionic nucleophile species in alkaline environment is directly linked to the solvent used and to the intrinsic properties of the substrate. It is known that aprotic polar solvents like dimethylformamide (DMF) or dimethylsulfoxide (DMSO) are able to expose the anionic species, increasing its reactivity towards the electrophilic partner, in order to perform the first step of the SN2 reaction [9].

According to the literature, two possible types of compounds can be obtained after alkylation of (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione (compound **2**) in alkaline environment with aliphatic chlorinated derivatives, using a 1:1 molar ratio. Some papers reported the selective formation of ethers, by alkylation of the phenolic oxygen, [11–16] while others suggested that N-alkylation took place, when using equimolecular amounts of reagents [17-21] (Fig. 1). The use of a reaction ratio of two equivalents of alkylating agent to one equivalent of compound **2**, led to the loss of regioselectivity, giving double O and N-alkylated derivatives [22].

In order to evaluate the veracity of one hypothesis or the other, 6 compounds were synthesized, all being (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione derivatives and were analyzed spectrally (^{1}H NMR, ^{13}C NMR, MS, IR). The present study is mostly focused on the analysis of the ^{1}H NMR spectra of the synthesized compounds in the 8.5–14 ppm range. We have chosen this spectral area because here are found the protons signals of the starting compound **2**, given by the groups in its structure that could be alkylated: the signal characteristic to the phenolic OH (broad, at 10.310 ppm) and the imide NH (broad, at 12.431 ppm). We focused on these broad signals present in the ^{1}H NMR spectrum of the parent molecule (**2**), the disappearance of these signals, respectively the apparition of new signals, depending on the substituent that was introduced on the parent molecule. Furthermore, it has been studied in the aromatic zone of the ^{1}H NMR spectrum how the benzylidene proton and benzyl protons are shifted in compounds **5a-f**, as a result of the substitution at the level of the nitrogen or oxygen atom of the compound **2**.

2. Results and discussion

All the compounds were synthesized and characterized.

2–chloro-N-((2-phenylthiazol-4-yl)methyl)acetamide (**4f**): white solid; m.p. 105 °C; yield=77%; FT IR (KBr) ν_{max} cm^{-1}: 1661 (C=O); MS: m/z = 267.6 (M + 1).

(Z)−5-(4-hydroxybenzylidene)−3-((2-(phenylamino)thiazol-4-yl)methyl)thiazolidine-2,4–dione (**5b**): yellow solid; m.p. 229–230 °C; yield = 48%; ^{1}H NMR (DMSO–d_6, 500 MHz) δ: 10.370 (br, 1H, OH), 10.198 (s, 1H, NH), 7.899 (s, 1H, -CH=), 7.563–7.523 (m, 4H, Ar), 7.232 (t, J = 8.5 Hz, 2H, Ar), 6.946 (d, J = 8.5 Hz, 2H, Ar), 6.902 (t, J = 7.5 Hz, 1H, Ar), 6.730 (s, 1H, Ar), 4.760 (s, 2H, -CH_2-); ^{13}C NMR (DMSO–d_6, 125 MHz) δ: 167.65, 165.985, 164.033, 160.694, 145.876, 141.544, 134.167, 133.124, 129.316, 124.375, 121.715, 117.298, 117.095, 116.934, 104.511, 41.783; FT IR (KBr) ν_{max} cm^{-1}: 3414 (Ar-OH), 1725, 1675 (C=O); MS: m/z = 410.3 (M + 1).

(Z)−2-(5-(4-hydroxybenzylidene)−2,4-dioxothiazolidin-3-yl)-N-(4-methylbenzo[d]thiazol-2-yl) acetamide (**5c**): yellow solid; m.p. 273–274 °C; yield = 66%; ^{1}H NMR (DMSO–d_6, 500 MHz) δ: 12.986 (br, 1H, NH), 10.499 (br, 1H, OH), 8.138 (ds, 1H, Ar), 7.907 (s, 1H, -CH=), 7.769 (d, J = 9 Hz, 1H, Ar), 7.534 (d, J = 8.5 Hz, 2H, Ar), 7.472 (dd, 1H, Ar), 6.932 (d, J = 8.5 Hz, 2H, Ar), 4.701 (s, 2H, -CH_2-); ^{13}C NMR (DMSO–d_6, 125 MHz) δ: 167.749, 166.412, 165.824, 160.946, 158.846, 147.829, 134.811, 133.633, 133.215,

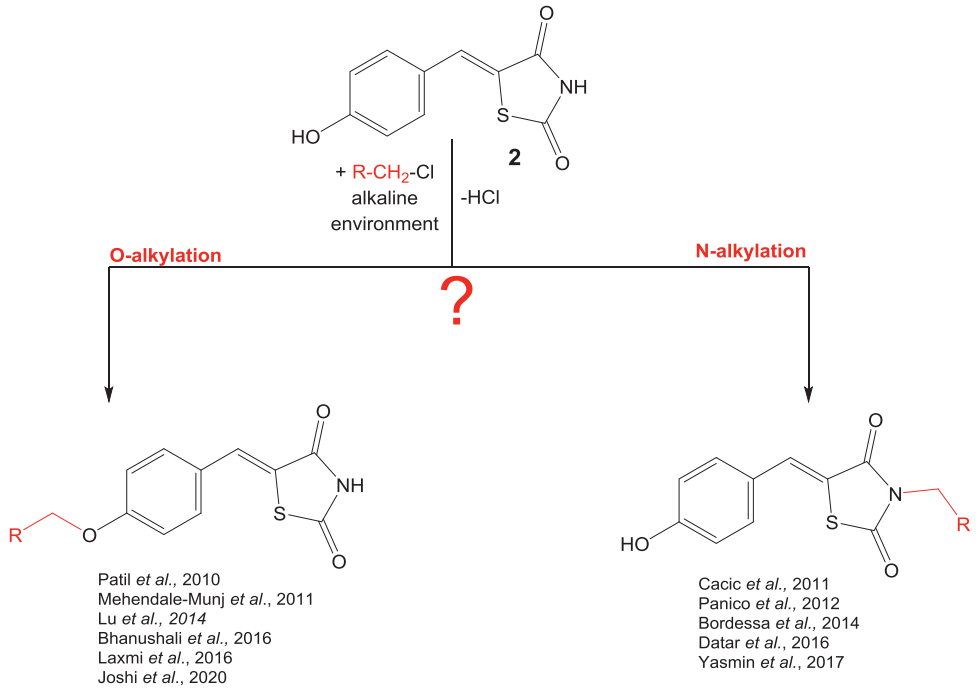


Fig. 1. The two possible types of alkylation products of (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4-dione, reported in the literature.

132.984, 128.350, 127.076, 124.172, 122.401, 122.023, 116.990, 116.647, 115.759, 44.044, 31.172; FT IR (KBr) ν_{max} cm^{-1}: 3408 (Ar-OH), 1734, 1708, 1672 (C=O); MS: m/z = 426.1 (M + 1).

(Z)−2-(5-(4-hydroxybenzylidene)−2,4-dioxothiazolidin-3-yl)-N-(thiazol-2-yl)acetamide (**5d**): yellow solid; m.p. carbonization > 280 °C; yield = 58%; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 12.705 (br, 1H, NH), 10.689 (br, 1H, OH), 7.892 (s, 1H, -CH_2-), 7.524 (d, J = 9 Hz, 2H, Ar), 7.501 (d, J = 3.5 Hz, 1H, Th), 7.270 (d, J = 3.5 Hz, 1H, Th), 7.009 (d, J = 9 Hz, 2H, Ar), 4.639 (s, 2H, -CH_2-); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 167.770, 165.859, 164.488, 163.221, 161.093, 138.219, 134.748, 133.152, 124.081, 117.032, 116.598, 114.499, 43.806; FT IR (KBr) ν_{max} cm^{-1}: 3426 (Ar-OH), 1735, 1699, 1467 (C=O); MS: m/z = 362.1 (M + 1).

(Z)-ethyl 2-(2-(5-(4-hydroxybenzylidene)−2,4-dioxothiazolidin-3-yl)acetamido)−4-methylthiazole-5-carboxylate (**5e**): yellow solid; m.p. carbonization > 265 °C; yield=63%; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 13.073 (br, 1H, NH), 10.618 (br, 1H, OH), 7.884 (s, 1H, -CH_2-), 7.515 (d, J = 9 Hz, 2H, Ar), 6.997 (d, J = 8.5 Hz, 2H, Ar), 4.669 (s, 2H, -CH_2-), 4.229 (q, J = 7.5 Hz, 2H, -CH_2-), 2.551 (s, 3H, -CH_3), 1.264 (t, J = 7 Hz, 3H, Th); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 167.728, 166.092, 165.789, 162.423, 161.058, 159.483, 156.571, 134.797, 133.159, 124.088, 117.011, 116.563, 115.052, 61.073, 43.946, 17.440, 14.626; FT IR (KBr) ν_{max} cm^{-1}: 3408 (Ar-OH), 1714, 1711, 1675, 1658 (C=O); MS: m/z = 448.3 (M + 1).

(Z)−2-(5-(4-hydroxybenzylidene)−2,4-dioxothiazolidin-3-yl)-N-((2-phenylthiazol-4-yl) methyl)acetamide (**5f**): yellow solid; m.p. 232–233 °C; yield = 61%; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 10.373 (br, 1H, OH), 8.899 (t, J = 5.5 Hz, 1H, NH), 7.947 (m, 2H, Ar), 7.882 (s, 1H, -CH=), 7.535–7.506 (m, 5H, Ar), 7.457 (s, 1H, Th), 6.945 (d, J = 8.5 Hz, 2H, Ar), 4.465 (d, J = 6 Hz, 2H, -CH_2-), 4.377 (s, 2H, -CH_2-); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 167.847, 167.665, 166.048, 165.845, 160.771, 155.192, 134.286, 133.481, 133.145, 130.758, 129.736, 126.572, 124.284, 117.039, 116.941, 116.130, 43.946, 39.774; FT IR (KBr) ν_{max} cm^{-1}: 3401 (Ar-OH), 1728, 1681, 1663 (C=O); MS: m/z = 452.3 (M + 1).

The molecular peaks found for all final compounds **5a-f** suggested that alkylation took place at just one out of the two possible atoms (N or O) of the (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione.

Analyzing the IR spectrum, the two peaks of the potential aryl-alkyl ether, which could appear in case of the O-alkylation, given by the symmetric stretch (near 1040 cm^{-1}) and the asymmetric stretch (near 1250 cm^{-1}) of the etheric bonds C–O–C are not found, suggesting that alkylation took place on the nitrogen atom for all final compounds **5a-f** and not on the phenolic oxygen.

Comparing the ^{1}H NMR spectrum of the (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione (compound **2**) with its parent compound thiazolidine-2,4–dione (compound **1**), the most deshielded proton gave a broad signal at 12.023 ppm in the compound **1,** respectively at 12.431 ppm in the compound **2**, by the NH proton. The other supplementary broad signal found for compound **2** in the ^{1}H NMR spectrum, corresponding to a less deshielded proton at 10.310 ppm is given by the phenolic hydrogen. After assignment of the protons from the structure of compound **2** that gave these broad signals, further analysis of the ^{1}H NMR spectrum of the resulted **5a-f** compounds can be performed, to trace the appearance or disappearance of signals of interest.

Structurally, compounds **4a** and **4b** are chloromethyl azoles. These compounds have no amide groups in their structures. The reason for choosing these two compounds was that they would not give in the ^{1}H NMR spectrum signals of the amide NH group, which could interfere with the observation in the same spectral zone of signal characteristic to the NH proton of thiazolidinedione moiety or the phenolic OH proton. Compound **4b** has, in addition to **4a**, a secondary aromatic amine. This compound's synthesis and its spectral analysis (Tables 1–3) allowed us to analyze this type of signal and to observe how it can influence the interpretation of the ^{1}H NMR spectrum in the spectral area of interest, 8.5–14 ppm. In the case of compound **5a**, it is obvious that the single broad signal given by a deshielded proton is given by the phenolic moiety at 10.446 ppm. Because the broad signal characteristic to its much deshielded proton at 12.431 ppm totally disappeared, it is obvious that the alkylation took place at the nitrogen atom. A similar situation is found for the compound **5b**, where the broad signal of the deshielded proton of the phenol can be found at 10.370 ppm. The supplementary peak given by the proton from the amino group is found as a strong sharp signal at 10.198 ppm (Table 1). Guided by the appearance and the chemical shift of this proton, this type of proton cannot be confused with the proton of an amide or with that of a thiazolidinedione.

This second type of alkylating agents used, the chloroacetamide derivatives **4c-f** aimed at obtaining the other final compounds (**5c-f**), bearing an amide group. Based on these facts, the signals characteristic to the proton from the amide group in the 8.5–14 ppm spectral area were analyzed. In the compounds **5c-e**, the aromatic amide gave the supplementary expected signals as broad, between 12.705–13.078 ppm and the phenolic proton between 10.508–10.689 ppm. For compound **5f**, the aliphatic amide gave the supplementary expected signal as sharp at 8.899 ppm and the phenolic proton at 10.372 ppm (Table 1).

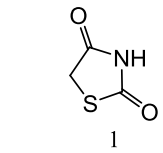
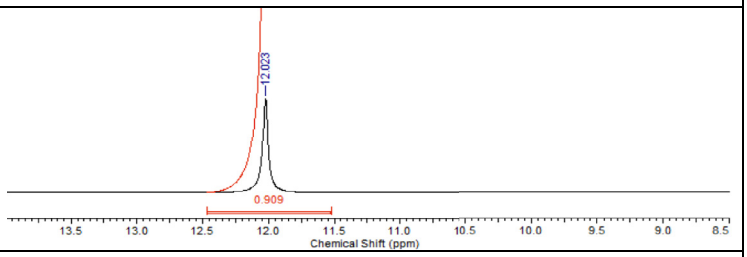
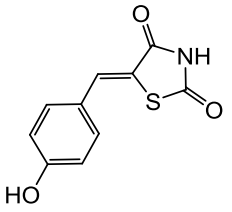
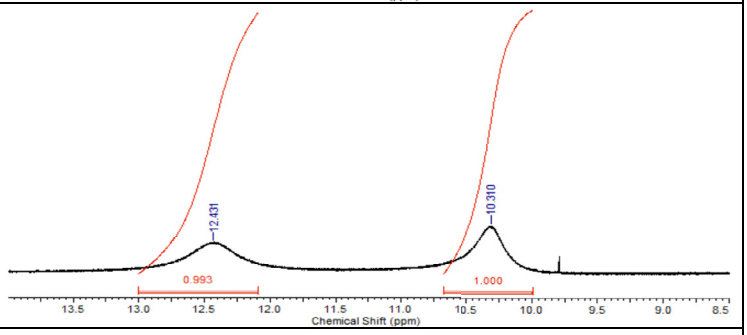
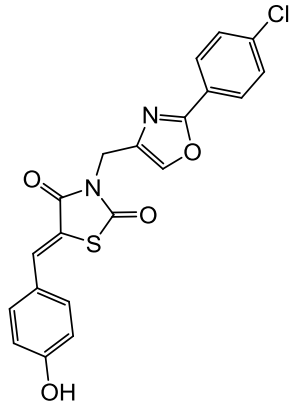
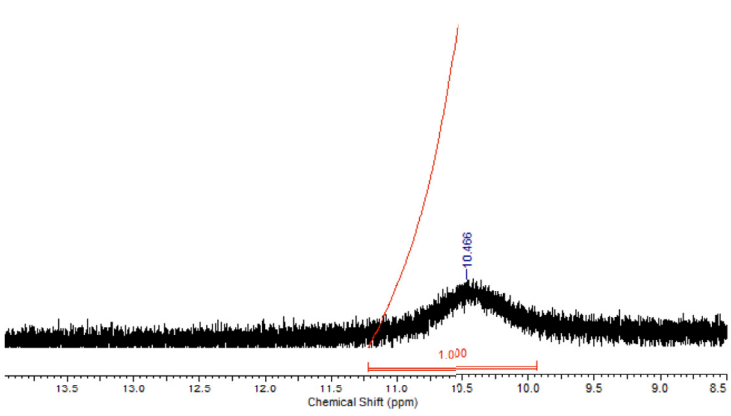
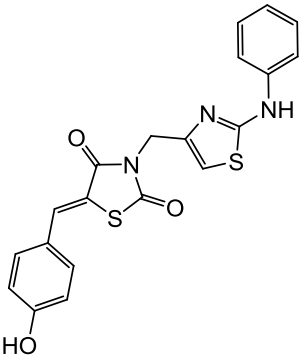
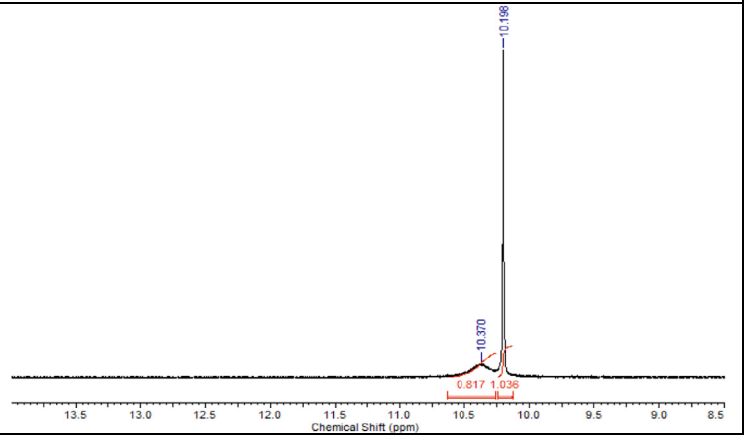
The electrons conjugation effect found in the parent compound **2** between the benzylidene nucleus and thiazolidine-2,4–dione is present in the final compounds **5a-f** too. The alkylation of compound **2** (whether N-alkylation or O-alkylation would take place) would not make the conjugation of electrons disappear, but the substitution on the nitrogen or oxygen atoms would influence the conjugation of electrons and consequent the changes in the chemical shift of protons can be found.

For a better analysis of the signals, we have used the spectral data of a supplementary compound (**6**), previously reported by our group [4,23]. This compound has a similar structure with the parent compound **2**, but has the oxygen atom substituted with a carbon atom. Compound **6** can be considered an O-alkylated derivative of compound **2**, but it was obtained by another synthetic route, independent of compound **2** and independent of the hypothesis studied in the present paper, being obtained by Knoevenagel condensation between *p*-methoxybenzaldehyde and thiazolidin-2,4–dione in alkaline environment. Spectral data of compound **6** was used in the present paper for spectral comparison, to strengthen the previously formulated conclusion, that under the reaction conditions presented in the hypothesis, takes place the N-alkylation of compound **2**, not the O-alkylation.

The benzylidene proton from the parent compound **2** was found at 7.699 ppm, while in compounds **5a-f** it was found between 7.882–7.907 ppm. Comparing to the chemical shift of this proton from the compound **2**, in compounds **5a-f**, the benzylidene proton appears with 0.208–0.183 ppm (average 0.192 ppm) downfield. On the other hand, compared to the signal given by the benzylidene proton in compound **6** (substituted at the oxygen atom substituted with methyl), it appears at 7.687 ppm, with a deviation of only 0.012 ppm. Therefore, the benzylidene proton being negligibly influenced by the presence of the carbon atom on the phenolic oxygen, the shift to higher values is due to the substitution at the level of the nitrogen atom in the case of compounds **5a-f**.

The four benzylidene protons on the aromatic nucleus that appear as two coupled doublets (J = 8.5 Hz or J = 9 Hz), are found in the spectrum of the parent compound **2** at 7.454 ppm and 6.919 ppm, respectively. Two benzylidene protons are found in compounds **5a-f** at 0.059–0.099 ppm (average 0.074 ppm) downfield compared to the parent compound **2**, while in compound

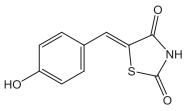
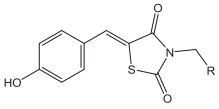
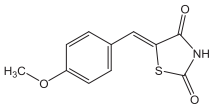
Table 1
The region 8.5–14 ppm from the ^{1}H NMR spectrum of the studied compounds.

Compound	^{1}H NMR spectrum between 8.5-14 ppm
 1	
 2	
 5a	
 5b	

6 they are found with 0.187 ppm more deshielded compared to the parent compound **2**. The other two protons (*meta*-benzylidene) are found in the compounds **5a-f** at 0.017–0.090 ppm (average 0.048 ppm) downfield compared to the parent compound **2**, while in compound **6** they are found 0.164 ppm downfield.

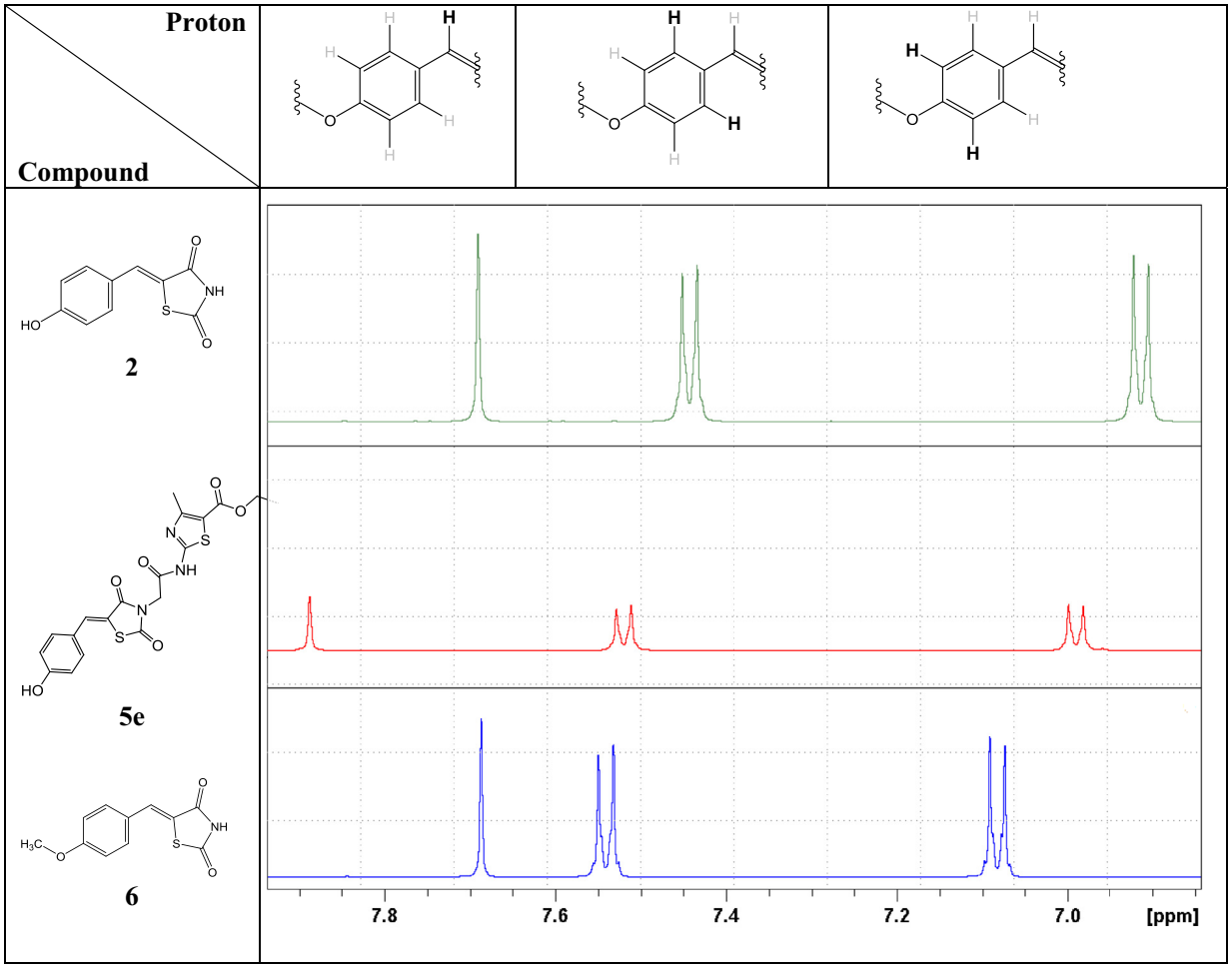
For a better observation of the numerical data presented in the Table 2 and to understand better the shifting due to substitution on the two different sites, a graphical depiction of the ^{1}H NMR spectrum of the compounds **2, 5e** and **6** was presented in Table 3. Moreover, in Table 4 ^{1}H-^{1}H COSY NMR spectrum for the compounds **2, 5e** and **6** are presented in comparison. The choice to depict the ^{1}H NMR spectrum of the compound **5e** was made because it has a simple structure, with no supplementary structural moieties such benzene rings which would give signals in the analyzed spectral zone and the shift of the benzylidene and the benzylic protons can be traced without difficulty. All spectral data

Table 2
Comparison of some distinctive signals from the ^{1}H NMR spectrum of the compounds **2, 5a-f** and **6**.

Compound		NH CS / DRC2 (ppm)	OH CS / DRC2 (ppm)	Benzylidene CS / DRC2 (ppm)	Benzylic CS / DRC2 (ppm)	CS / DRC2 (ppm)
	**2**	12.431 / 0	10.310 / 0	7.699 / 0	7.454 / 0	6.919 / 0
	**5a**	missing	10.466 / 0.156	7.882 / 0.183	7.513 / 0.059	6.936 / 0.017
	5b	missing	10.370 / 0.060	7.899 / 0.200	7.532 / 0.078	6.946 / 0.027
	5c	missing	10.499 / 0.189	7.907 / 0.208	7.553 / 0.099	6.977 / 0.058
	5d	missing	10.689 / 0.379	7.892 / 0.193	7.524 / 0.071	7.009 / 0.090
	5e	missing	10.618 / 0.308	7.884 / 0.185	7.515 / 0.061	6.997 / 0.078
	5f	missing	10.373 / 0.063	7.882 / 0.183	Overlapping	6.941 / 0.021
	**6**	12.265 / 0.166	missing	7.687 / 0.012	7.641 / 0.187	7.083 / 0.164

CS: Chemical Shift.
DRC2: Deviation Relative to Compound 2.

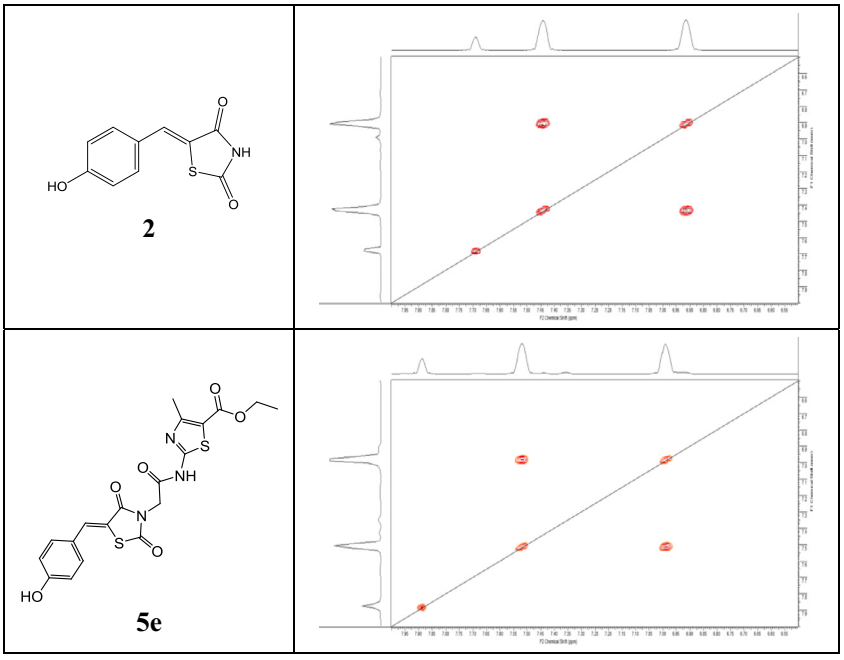
Table 3
Comparison of some distinctive signals from the ^{1}H NMR spectrum of the compounds **2, 5e** and **6**.



of the compounds from this paper can be found in the Supplementary material.

Significant shift to a higher value of the benzylidene proton in compound **5e** can be observed as a result of N-alkylation. The same proton, in the case of compound **6** used as the reference of O-alkylated compound, appears at the same value as in the case of parent compound **2**. The same shifting effect can be observed in the case of benzyl protons. Due to N-alkylation, there is a shift effect towards higher values for those protons, compared to the parent compound **2**, depicted for compound **5e** in Table 3. In the case

Table 4
Comparison of $^{1}H–^{1}H$ COSY NMR spectrum of the compounds **2, 5e** and **6** in the 6.5–8 ppm region.



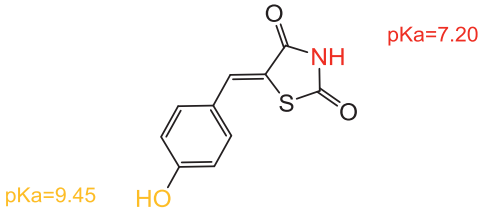


Fig. 2. The computed pKa of the two acidic groups of the (Z)–5-(4-hydroxybenzylidene)-thiazolidine-2,4-dione.

of compound **6** used as a reference for an O-alkylated compound, this effect is much more intense on benzyl protons, compared with benzyl protons from compound **5e**.

Therefore, if in the case of compounds **5a-f** they had been obtained by O-alkylation and not by N-alkylation, as established previously, a different pattern in terms of the chemical shift in the signals given by those four protons on the aromatic nucleus and by the benzylidene proton could have been observed, similar to compound **6**.

Theoretical *in silico* evaluation of the acidity of compound **2** provided the pKa of the two discussed moieties above. The strongest acidic proton is the one from the thiazolidine-2,4–dione ring, with a pKa = 7.20. The phenolic OH is computed to be less acidic, with a pKa = 9.45 (Fig. 2). A low pKa of a group suggests an increased acidity and a relatively higher amount of its conjugated base in the medium. A higher quantity of an acid's conjugated base in the reaction medium (a potential nucleophile) will lead to a higher amount of its derivative to be obtained in a reaction with a nucleophile.

Analyzing the ionization steps of compound **2** from the initial acidic structure to its correspondent conjugated bases, it can be assumed that the first acidic proton to mobilize in the presence of a base in the reaction medium will be that from the thiazolidine-dione (the strongest acidic function in the molecule) and not from the phenol (Fig. 3– Species 2 vs. Species 3). The highest percent of monoionized compound **2** is found at pH=8.3, where 86.2% of the four microspecies possible for the discussed compound is represented by the ionized thiazolidine-2,4–dione (Fig. 3- Species 2), while at the same pH the phenol ionized species (Fig. 3- Species 3) is only 0.7%. Comparing the two percentages, the species 2 is found to be at that pH at about 122 fold more abundant then species 3. Analyzing the data representing the four microspecies distribution used for plotting Fig. 3, the phenoxide microspecies (species 3) can be found in a negligible amount in the reaction medium. Throughout the entire range of pH 7–10, species 2 is always found in quantities over 100 times larger than species 3.

The pKa obtained for the two acid protons, quite different from each other and the consequent distribution between the resulted microspecies, guides us from this point of view that in an alkylation reaction of the (Z)–5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione (compound **2**), performed in alkaline medium, the preferential product obtained will be due to N-alkylation, because the rate of the SN2 reaction is dependent on the concentration of the species. All data resulted from the microspecies calculation used in depiction of Fig. 3 is provided in the Supplementary material as percent of species through 0–14 pH range, as resulted from www.chemicalize.org calculation.

3. Conclusions

In order to evaluate how the alkylation reaction takes place on (Z)–5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione, 6 compounds were synthesized, 5 of which have not been reported previously in the literature, using different aliphatic chlorinated alkylating agents in alkaline environment.

All compounds were obtained by N-alkylation and none of them were resulted by O-alkylation, based on the analysis of the spectral data. A supplementary derived hypothesis evaluated during the present study was if there could be some potential steric hindrance which could lead to O-alkylation instead on N-alkylation, because some of the alkylating agents used were chloroacetamide derivatives (compounds **4c-f**), different to the rest of alkylation agents that were non-chloroacetamide derivatives (compounds **4a-b**). Analyzing the resulted spectral data, there is no difference between those two types of alkylating agents in terms

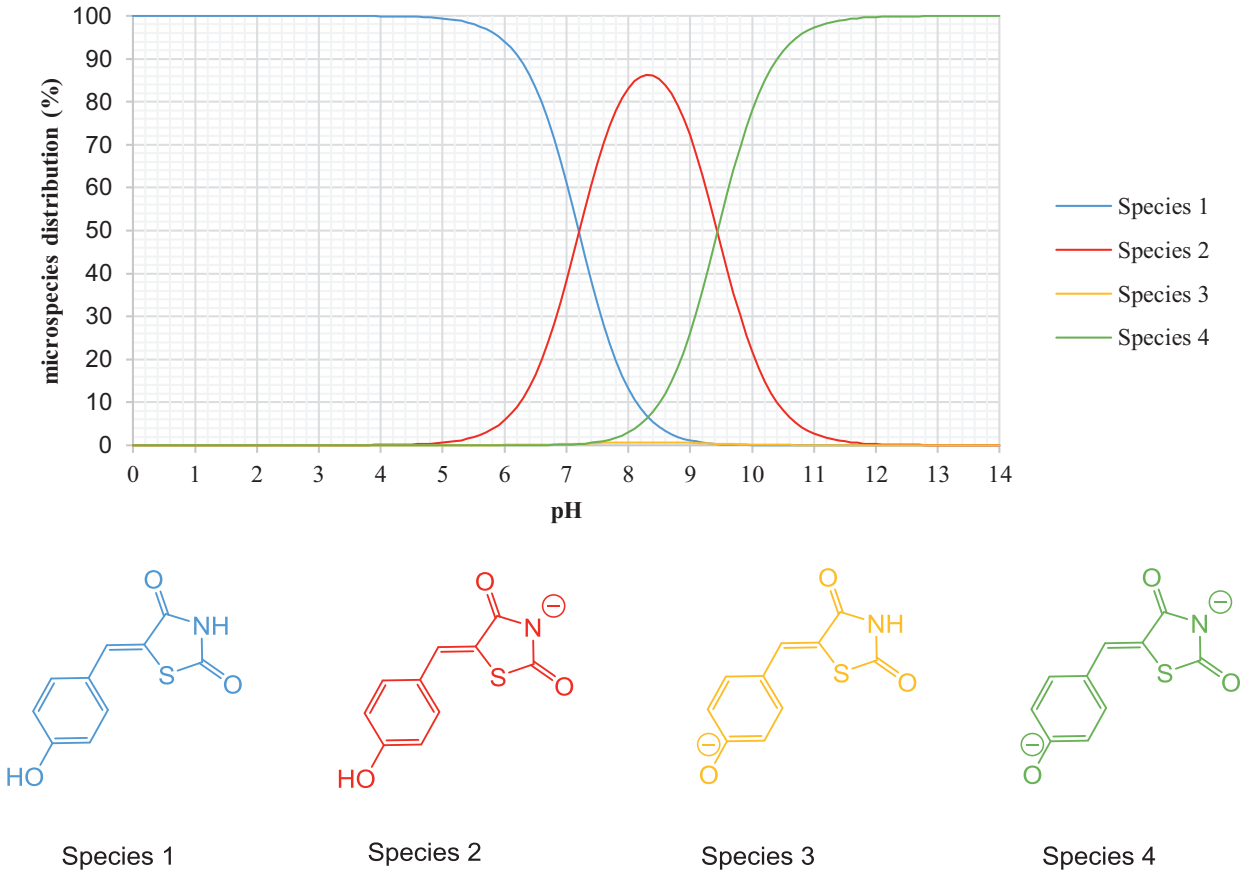


Fig. 3. The four species computed and their relative abundance of (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4-dione in the pH range of 0–14.

of regioselectivity. In all final compounds **5a-f**, independent on the type of alkylating agent used, the broad signal of the phenolic proton was found ranging between 10.310–10.689 ppm, proving that the substitution took place just at the nitrogen atom of compound **2**.

Moreover, significant changes in shifting was identified for the benzylidene protons and small differences in shifting were identified for the benzyl protons signals from the final compounds **5a-f**, compared with the signals of the corresponding protons from compound **2**. Using an O-alkylated derivative (compound **6**) of compound **2** as spectral reference, a cross-validation of the N-alkylation hypothesis of compound **2**, when obtaining the compounds **5a-f** in alkaline environment was made.

By correlating the results of the analysis of the spectral data with those of the acidic character of the two acid groups of the (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione (compound **2**), results are congruent in concluding that the alkylation of (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione (compound **2**) in alkaline medium is regioselective, resulting only N-substituted reaction products when using the reagents in a 1:1 molar ratio.

4. Experimental section

All chemicals used for synthesis, purification and analysis of the compounds were of analytical reagent grade purity and have been purchased from local suppliers. The melting points of the synthesized compounds were obtained by the open glass capillary method, using an MPM-H1 melting point apparatus (Schorpp Gerätetechnik, Überlingen, Germany) and are uncorrected.

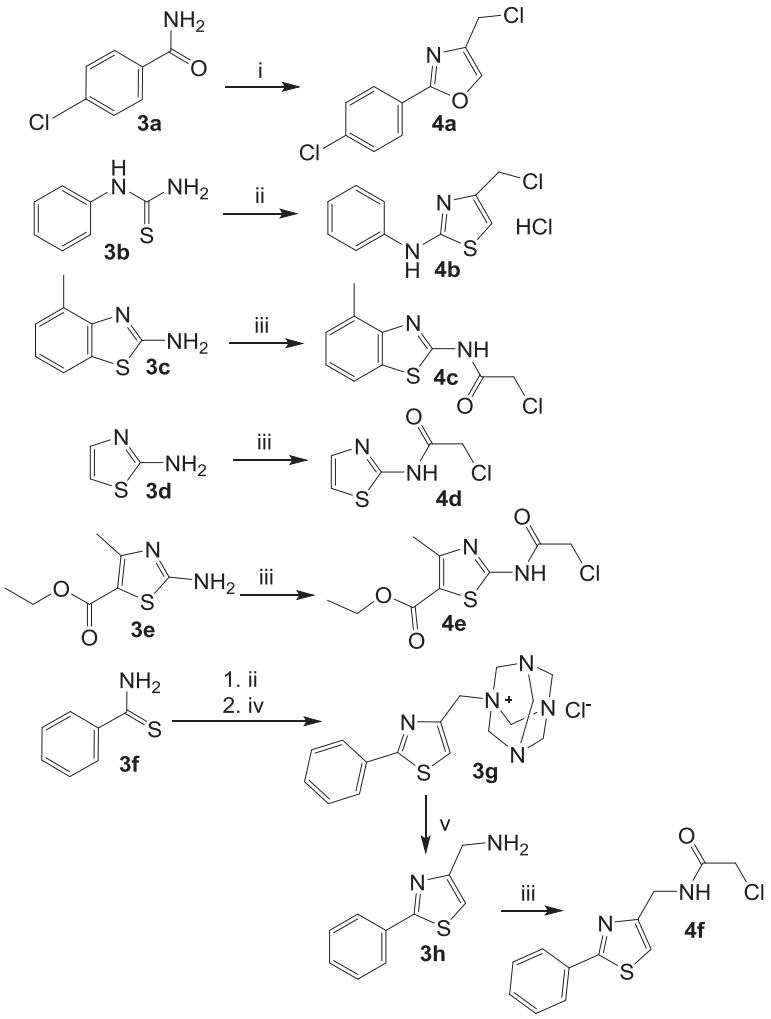
MS spectra were obtained in positive ionization with an Agilent 1100 series with an Agilent Ion Trap SL mass spectrometer (70 eV) instrument (Agilent Technologies, CA, USA). IR spectra were recorded after compression of the solid powder samples in anhydrous KBr pellets under vacuum, using a FT/IR 6100 spectrometer (Jasco, Cremella, Italy).

The ^{1}H and ^{13}C NMR spectra were recorded on an Avance NMR spectrometer (Bruker, Karlsruhe, Germany) using DMSO–d_6 as solvent at 300 K. Chemical shift values are reported in δ units as ppm (parts per million), relative to TMS as internal standard. The pKa and the derived microspecies concentration calculations were performed on www.chemicalize.com, a web application which provides structure-based calculations and predictions for molecules (ChemAxon, Budapest, Hungary) [24–26].

4.1. Chemical synthesis

The parent compound (Z)−5-(4-hydroxybenzylidene) thiazolidine-2,4–dione (compound **2**) was obtained using our previously reported protocol, starting from thiazolidine-2,4–dione (compound **1**) [22].

In order to validate one of the possible synthetic chemical ways and to eliminate the other, (Z)−5-(4-hydroxybenzylidene)-thiazolidine-2,4–dione (**2**) was subjected to alkylation in DMF (dimethylformamide) in alkaline environment provided by K_2CO_3 using the alkylation agents **4a-f** (Scheme 1). The reaction mixture was stirred overnight at room temperature, using a previously reported protocol [27]. In the synthesis of the compound **5c**, the amount of K_2CO_3 used was doubled, because the compound **4c** was used as hydrochloride, as isolated. Because the aim of the



Scheme 1. Reaction scheme used for obtaining the intermediate compounds **4a-f**.

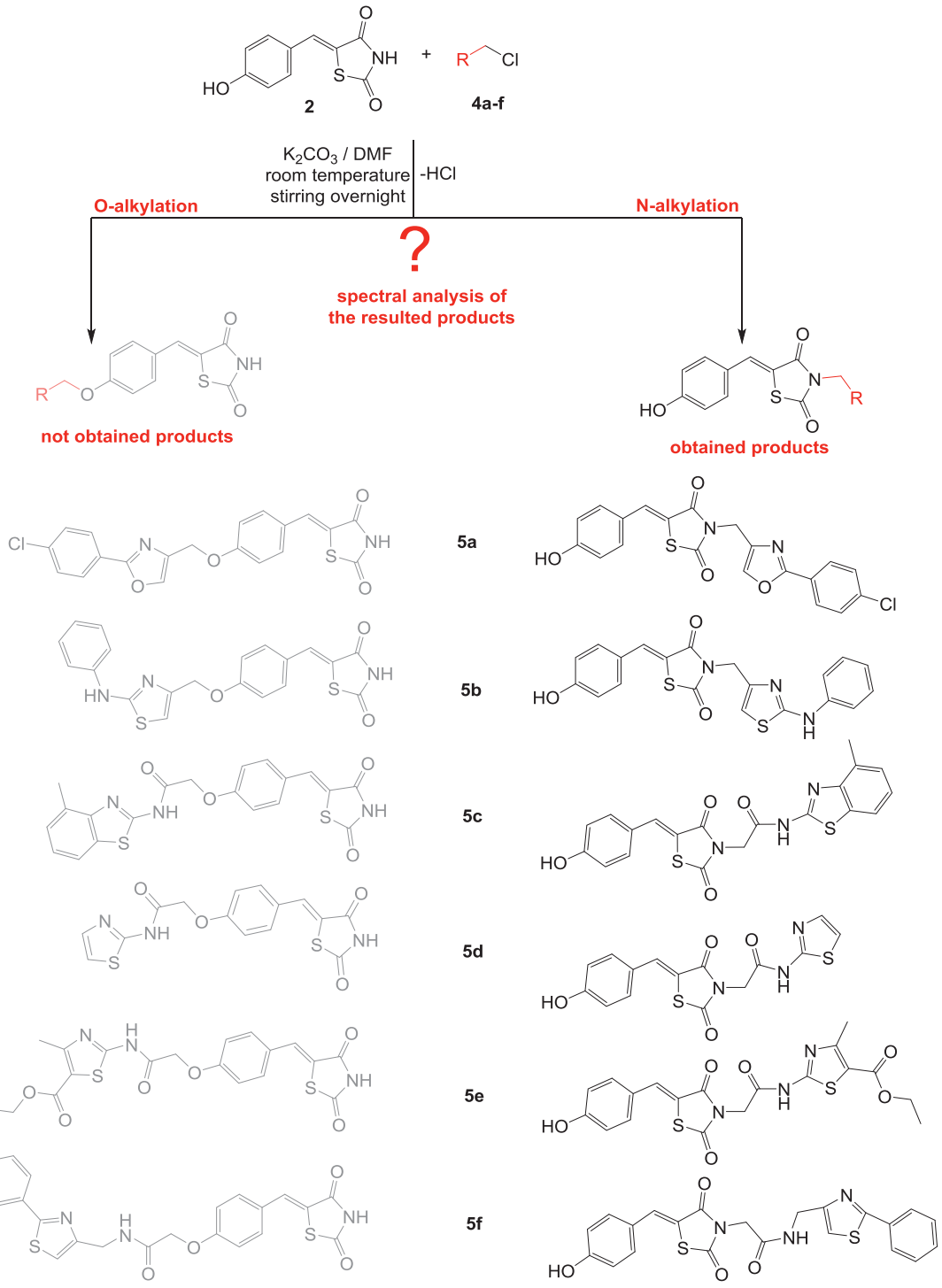
present work was to evaluate the possible products of the reaction (O or N alkyl derivatives), the reaction conditions were not changed in order to obtain better yields in final products. General view of the potential final compounds' structures presented in the discussion of the two synthesis hypothesis (N or O alkylation) is presented in Scheme 2.

The synthesis of the aliphatic chlorine intermediates **4a-f** is shown in Scheme 1. Intermediate compounds **4a-e** and the final compound **5a** were obtained using previously reported protocols [14,27,28]. The characterization data of the intermediate compounds **4b-e**, previously reported in the literature, is consistent with the reported data [28–31].

The intermediate compound 3 g was synthesized using a previously reported protocol [32], involving in the first step a Hantzsch cyclisation, in order to obtain the thiazole ring and next, the formation of the urotropinium chloride salt in the presence of the urotropine and the previously obtained 4-(chloromethyl)−2-phenylthiazole. Later, the formation of the intermediate compound 3 h ((2-phenylthiazol-4-yl)methanamine) was based on the experimental protocol used previously in the synthesis of (2-phenyloxazol-4-yl)methanamine, based on the Delépine reaction on intermediate compound 3 g [33]. The obtained compound 3 h was isolated as yellow oily liquid and was used without further purification.

Synthesis of intermediate compound **4f** was performed by adapting a previously reported protocol [14]. In a glass flask equipped with a magnetic stirrer, immersed in an ice bath, 2.85 g (15 mmol) of intermediate 3 h, 15 mL of dichloromethane and 3.03 g (30 mmol) of triethylamine were added to the reaction mixture and 2.26 g (20 mmol) of chloroacetyl chloride were added dropwise, for about 15 min. The reaction mixture was left stirring in a closed vessel for 2 h, waiting to reach the room temperature. The solvent was evaporated under reduced pressure. Water was added over the resulted solid and the resulted precipitate was filtered under reduced pressure. The impure solid was recrystallized from a mixture of methanol-water and activated charcoal, in order to obtain the pure white solid.

Copies of MS, IR, ^{1}H NMR and ^{13}C NMR spectra for the synthesized compounds **1, 2**, 3 h, **4c-f, 5a-f** and **6** are provided in the Supplementary Material. ^{1}H–^{1}H COSY NMR and ^{1}H–^{13}C HSQC NMR spectra were recorded for compounds **2, 5d, 5e** and **6** and are included in the Supplementary Material.



Scheme 2. Reaction scheme for the synthesis of the final compounds **5a-f**.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Gabriel Marc: Conceptualization, Funding acquisition, Formal analysis, Investigation, Methodology, Project administration, Resources, Writing - original draft, Writing - review & editing. **Anca Stana:** Funding acquisition, Methodology, Writing - original draft. **Adrian Pîrnău:** Funding acquisition, Investigation, Resources, Validation. **Laurian Vlase:** Investigation, Validation. **Smaranda Oniga:** Funding acquisition, Resources, Validation, Writing - review & editing. **Ovidiu Oniga:** Formal analysis, Project administration, Supervision.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.130629.

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