

Full Paper

Regioselectivity and Tautomerism of Novel Five-Membered Ring Nitrogen Heterocycles Formed via Cyclocondensation of Acylthiosemicarbazides

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Abstract: A series of 1-acyl-4-phenyl/(acridin-9-yl)thiosemicarbazides **3**, including four new compounds, were prepared in order to study substituent effects on cyclization reactions with oxalyl chloride (producing imidazolidine-4,5-diones **4**), dimethyl acetylenedicarboxylate (to give thiazolidin-4-ones **7** and **8**) and autocondensation under alkaline conditions (to yield 1,2,4-triazoles **9**). A positional isomer, **10** of compound **3f** was also prepared. Altogether, twenty new compounds characterized and identified by IR, UV, ¹H, ¹³C and 2D NMR and quantum chemical calculations are described. The tautomerism of the products and regioselectivity of the reactions were evaluated. Compounds **3f-h**, **3h·2HCl**, **7b,d** and **10** were screened for cytotoxic activity against the L1210 leukemia cell line and all compounds, except for **3f**, exhibited promising inhibitions of cell growth.

Keywords: Acylthiosemicarbazides, imidazolidine-4,5-diones, thiazolidin-4-ones, 1,2,4-triazoles, acridines, cytotoxic activity.

Introduction

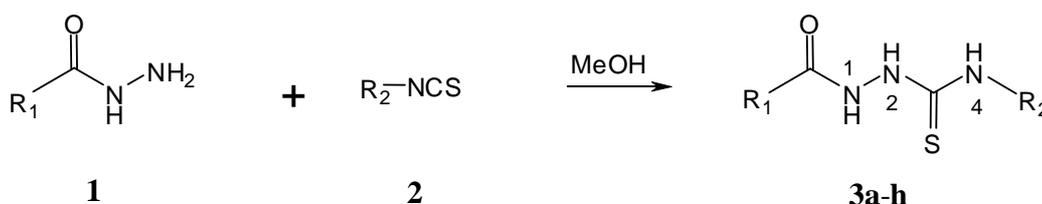
Acylthiosemicarbazides represent versatile synthons for various syntheses of nitrogen heterocycles. The acylthiosemicarbazide moiety provides an opportunity to perform cyclocondensations as well as addition–cyclization reactions. The products of these reactions, triazoles [1,2], thiazolidinones [3], imidazolidinediones [4], etc., all possess substantial pharmaceutical potential [1–7].

Acylthiosemicarbazides react with halocarbonyl compounds (e.g. ethyl bromoacetate, chloroacetic acid) to yield thiazolidinone derivatives [8,9]. Alternatively, reactions with oxalyl chloride afford imidazolidinediones [10,11]. Recently, we reported the courses of the reactions of alkyl- and aryl-substituted thiosemicarbazides with dimethyl acetylenedicarboxylate (DMAD) [12]. In the present work, we have explored the effect of the acyl group in acylthiosemicarbazides on the nucleophilicity of the nitrogen atoms by examining the reactions of selected acylthiosemicarbazides with oxalyl chloride, DMAD and, in addition, by intramolecular cyclocondensation under alkaline conditions. To elucidate the tautomeric states and structures of the products, as well as to characterize and confirm the identities of the new compounds, IR, UV, ^1H -, ^{13}C - and 2D-NMR spectroscopy and quantum chemical calculations were all variously applied as appropriate. The purities of the new compounds were confirmed by elemental (C, H and N) analysis and were within 0.4% of the calculated values in all cases. Taking into account the known pharmacological properties of acridine derivatives in anticancer chemotherapy [13], the potential cytotoxic activities of selected compounds were also investigated by testing them against the L1210 leukemia cell line.

Results and Discussion

The reactions of a series of acylhydrazides **1** ($\text{R}_1 = \text{CH}_3, \text{Ph}, 3\text{-Pyr}, 4\text{-Pyr}$) with isothiocyanates **2** ($\text{R}_2 = \text{Ph}, \text{acridin-9-yl}$) were used to prepare the starting acylthiosemicarbazides **3a–h** (see Scheme 1). In contrast to alkyl- and arylhydrazines, where both 1,4- and 2,4-disubstituted thiosemicarbazides were obtained [12], the acylhydrazides **1** afforded only 1,4-disubstituted acylthiosemicarbazides **3a–h** due to the diminished nucleophilicity of the N-1 nitrogen atom arising from the attached acyl group.

Scheme 1. The reaction of acylhydrazides **1** with isothiocyanates **2** to prepare the starting acylthiosemicarbazides **3a–h**. The reaction proceeded regioselectively *via* the reaction of N-2 of acylhydrazides **1**.



3a–h	a	b	c	d	e	f	g	h
R_1	CH_3	Ph	3-Py	4-Py	CH_3	Ph	3-Py	4-Py
R_2	Ph	Ph	Ph	Ph	Acr	Acr	Acr	Acr

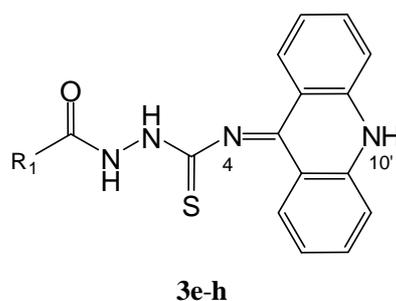
Legend: Py, pyridyl; Ph, phenyl; Acr, acridin-9-yl.

The structures and identities of the four new acylthiosemicarbazides **3e–h** were proven by ^1H - and ^{13}C -NMR and quantum chemical calculations (**3a–d** have been previously reported [14–16]). A linear correlation (1) between the experimental (δ_{exp}) and calculated (δ_{calc} , details provided further on) ^{13}C -NMR chemical shifts was obtained [12]:

$$\delta_{\text{exp}}^{13}\text{C} = 1.12 \times \delta_{\text{calc}}^{13}\text{C} - 20.58 \quad R = 0.99 \quad (1)$$

However, for the exchangeable NH protons, divergence from the correlation line was noticeable; in concert with our previous findings the N-10' atom of the acridinyl moiety present in **3e–h** is well known [17–24] to have a strong propensity to retain a proton, i.e. the HN-10' tautomer (a 9',10'-dihydroacridine structure, see Figure 1) is expected to dominate the tautomeric equilibrium in the subseries. This case is no exception as borne out by the diagnostically [17,18] shielded signals of C-4' and C-5' (ca. 119 ppm). The intriguing aspect in this example though, is the fact that dynamic exchange is fast—rendering the acridinyl side rings equivalent on the NMR timescale.

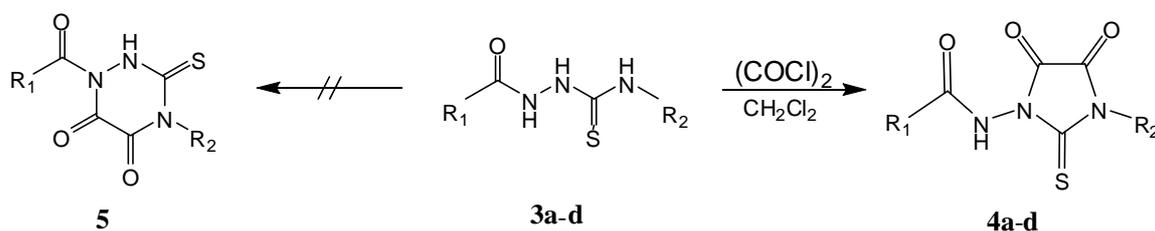
Figure 1. The predominant HN-10' tautomer for compounds **3e–h**.



This observation is presumably a result of rapid proton exchange, for whilst the labile proton signals are usually NMR observable in DMSO solution for these types of compounds [17–23], uncharacteristically, with one exception (one proton in the spectrum of **3g**), they were not observed at all in this subseries. This is in contrast to all the other compounds reported herein, where all the exchangeable protons were observed including, of note, all three exchangeable protons of a positional isomer of **3f** (*vide infra*).

Reports in the literature on the condensation of thiosemicarbazides with oxalyl chloride [10,11] prompted us to examine the reaction with these acyl analogs. Because the basicity of the thiosemicarbazide nitrogen atoms can be significantly influenced by the adjacent acyl group, a change of reaction course could be anticipated and indeed, consequently we found that only five-membered ring imidazole derivatives **4a–d** were formed from **3a–d** (Scheme 2). The structures of these four new imidazole derivatives **4a–d** were confirmed by ^{13}C -NMR based on the C=S signals at 179.1–179.6 ppm and three resonance signals of C=O carbons in the range 154–166 ppm, thereby discounting the presence of thiol and iminol tautomeric forms. Furthermore, the reaction products did not afford S-methylated isomers upon methylation with methyl iodide as would be expected in the case of six-membered ring triazine derivatives **5**, thereby confirming the non-participation of the nitrogen bearing the acyl group (N-1) in nucleophilic displacement.

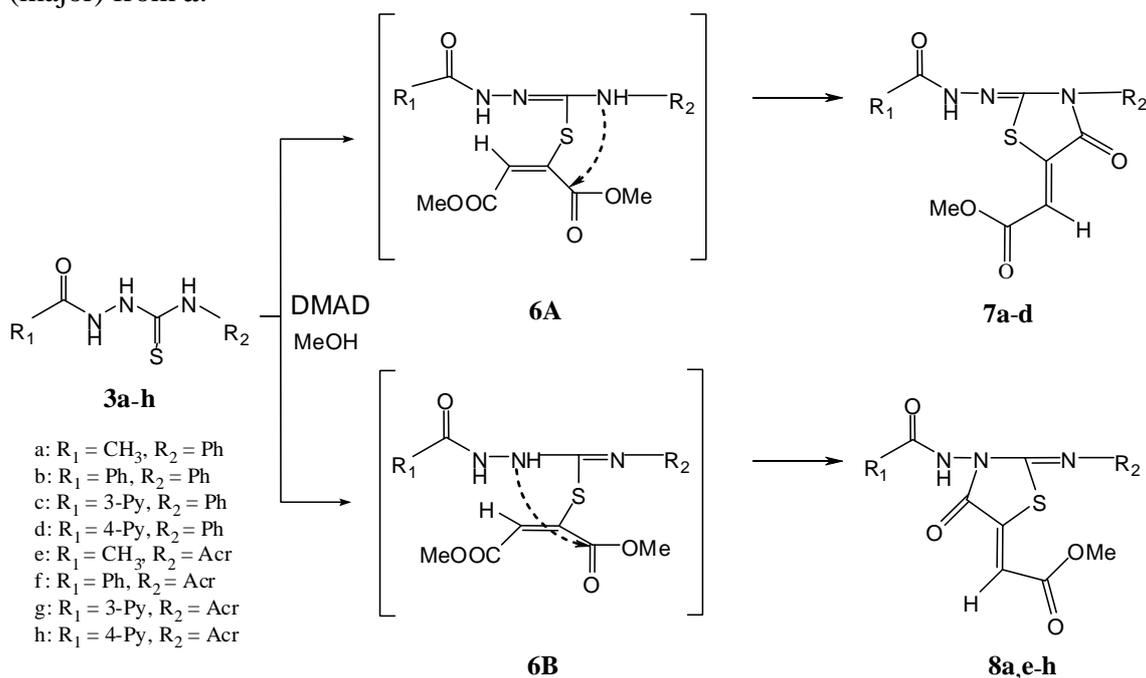
Scheme 2. The reaction of acylthiosemicarbazides **3a–d** with oxalyl chloride proceeded regioselectively to yield five-membered ring compounds **4a–d**.



The preparation of acridinyl derivatives **4e–h** was not successful however, despite repeated attempts applying various reaction conditions; only an inseparable mixture of products was detected by NMR in each instance. It is likely that due to the high reactivity of the oxalyl chloride, and bearing in mind the domination of the HN-10' tautomer, the formation of cyclic products competes with the reaction of oxalyl chloride with two molecules of **3** (reaction first at N-2 followed by reaction at N-2 or N-10' of the second molecule, or reaction at N-10' first etc.).

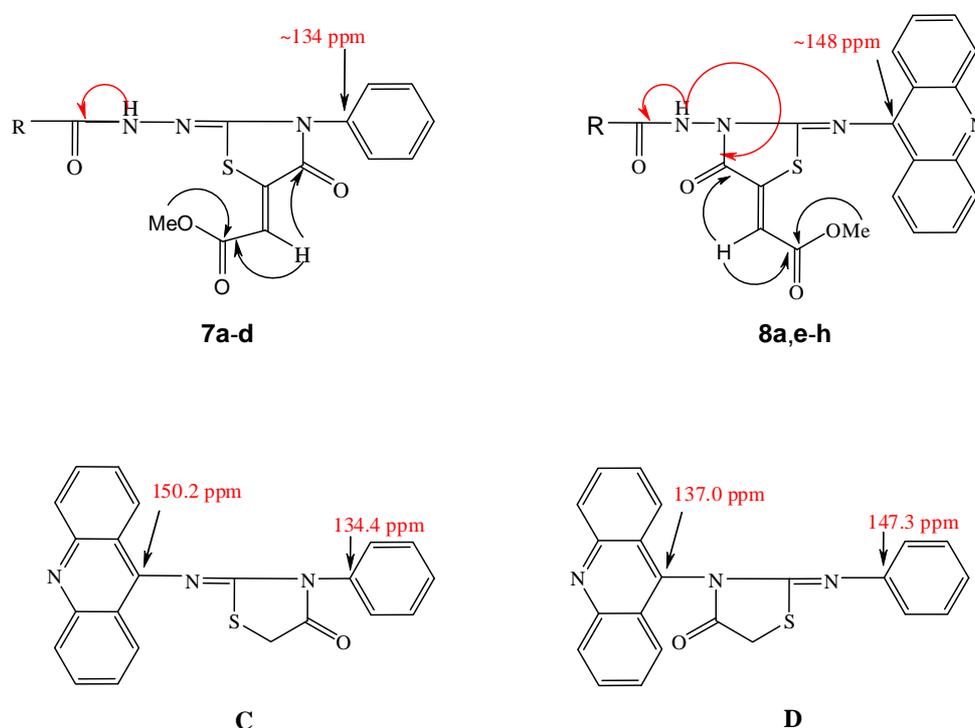
The next cyclization reaction of acylthiosemicarbazides **3** was conducted using DMAD in methanol. The reaction proceeds via nucleophilic attack by the thiosemicarbazide sulfur atom (after tautomeric shift to the thiol form with the hydrogen emanating from either N-2 or N-4) to the triple bond of DMAD. This first step may afford either of two possible tautomeric intermediates, **6A** or **6B** (Scheme 3), which are then able to undergo differing reaction routes to form either of the cyclic products **7** or **8**. (A third tautomeric intermediate, the HN-10' tautomer is also plausible and indeed likely in the case of the acridin-9-yl-substituted derivatives (**e–h**), but since it must transpose into either **6A** or **6B** for the final step of the cyclization reaction to occur, its presence is inconsequential.)

Scheme 3. Acylthiosemicarbazides **3a–h** reacted regioselectively with DMAD to yield compounds **7** or **8** depending on R_2 : **7** from **b–d**, **8** from **e–h** and both **7** (minor) and **8** (major) from **a**.



Consecutive intramolecular nucleophilic displacement may take place then *via* either by N-4 (**6A**) or N-2 (**6B**) to yield the five-membered ring products **7** or **8**, respectively. For acridin-9-yl-substituted acylthiosemicarbazides **3e–h**, the four new products **8e–h** were formed exclusively, whilst in the case of $R_2 = \text{phenyl}$, acylthiosemicarbazides **3b–d** formed exclusively the three new products **8b–d**, and, exceptionally in the case of **3a** ($R_1 = \text{methyl}$, $R_2 = \text{phenyl}$), both regioisomers **7a** (minor) and **8a** (major) were formed as two further new compounds in a 21:79 ratio. Both series **7** and **8** showed all the expected signals in the NMR spectra. The distinction between structures **7** and **8** was based on HMBC correlations between the NH and $-\text{CH}=\text{}$ protons with a central ring carbon and a comparison of the ^{13}C chemical shifts of the phenyl *ipso* carbon or the acridinyl C-9' carbon with appropriate model compounds **C** and **D** (see Figure 2) [25]. Whereas correlations from the NH proton to the carbonyl carbon of the acyl group were observed for **7a–d**, for derivatives **8a,e–h**, an additional correlation for the NH proton to the endocyclic carbonyl carbon C-4 of the thiazolidine ring was furthermore observed. (This carbon was identified by common correlations from H-6 and distinct from the correlations displayed by the methoxy methyl protons to carbonyl carbon C-7.) The correlation into the ring by the NH proton is unlikely for the structure of **7** since the respective atoms are separated by five bonds.

Figure 2. Pertinent HMBC correlations and determinative chemical shifts in comparison to model compounds **C** and **D** for distinguishing between structures **7** and **8**.



Further structural evidence comes from the similarity of the *ipso* phenyl carbon chemical shift at 134.0 ppm with the analogous phenyl signal at 134.4 ppm in model compound **C** (Figure 2) [25]. In the case of derivatives **8**, the signals of the C-9' acridine carbon resonate at higher values (~148 ppm) due to greater deshielding by an imino-type N-4 nitrogen bound to the acridine moiety and comparable with literature data [25]. An EI/MS spectrum of **8f** showed two intense fragment ions, in addition to

the molecular ion at 482 amu, identifiable as AcrNCS⁺ (236 amu) and PhCO⁺ (105 amu) which indicates scission of the C₂–N₃ and C₅–S bonds of the thiazolidine ring and debenzoylation as the two main fragmentation processes. The observation of the AcrNCS⁺ ion provides further confirmation of the structural assignment of this compound. The participation of N-1 in the cyclization process to yield a six-membered ring product was not observed at all, nor did N-2 or N-4 attack the other carbonyl carbon of DMAD to yield six-membered ring products.

The observed regioselectivity is intriguing and a plausible explanation lies in the supposition that there must be a delicate balance between the tautomeric forms **6A** and **6B**, i.e. a thermodynamic factor where the state of tautomerism is determined by the ability of the aryl groups to conjugate, and in addition, a kinetic factor where the reaction rate of the intermediate tautomer is determined by electron donation/withdrawal of the groups. In the case of **a** (R₁ = CH₃, R₂ = Ph), the electron donating ability of CH₃ should favor **6B** to react to give **8a**, but the effect is not strong (vide infra). The expectation that conjugation of double bond N₄=C₃ with the phenyl group in **6B** would overwhelm the tautomeric equilibrium is tempered by the realization that conjugation of double bond N₂=C₃ with the amide bond in **6A** must also be considerable, since a mixture of **7a** and **8a** was obtained. Replacement of CH₃ by an aryl group (Ph and Py, **b–d**) means that **6A** is now more favored thermodynamically due to stronger amide resonance conjugation by comparison and that the rate of **6B** is also slowed. The result is that the product distribution shifts exclusively to **7b–d** (and the appraisal that the electron donating ability of CH₃ is weak in combination with the results of **e**). For **e** (R₁ = CH₃, R₂ = Acr), in comparison to **a** (acridin-9-yl instead of phenyl) either **6B** is now thermodynamically more favored and/or **6A** is more disfavored kinetically, i.e. acridin-9-yl is either better at conjugation and/or electron withdrawal in comparison to phenyl positioned at N-4, and the result is that **8e** is produced exclusively. For the replacement of phenyl by acridin-9-yl, i.e. comparison of **f–h** to **b–d**, the same just-mentioned conjectures apply and the delicate balance is tipped wholly in favor of products **8f–h**, as observed, over products **7f–h**.

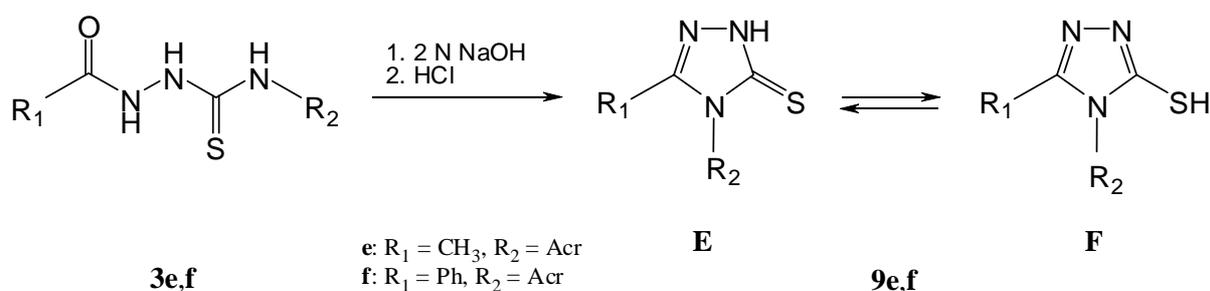
Finally, an autocondensation reaction of acylthiosemicarbazides **3e,f** was performed under reflux in NaOH solution affording two new 1,2,4-triazoles **9e,f** in moderate yields. The compounds precipitated from the solution upon acidification (Scheme 4). Lower yields of **9e,f** in comparison to analogous compounds where R₂ was not acridinyl [2,5,6] may be ascribed to two factors. The first is amino–imino tautomerism (HN-4 ↔ HN-10') in the acridinoamine part of **3** lowering the reactivity of N-4 when it is present as an imino-type nitrogen and deacetylation due to instability of the hydrazidic R₁CO–NH bond.

For **3g,h** with electron-withdrawing pyridyl substituents, deacetylation proceeded so fast that no product was able to be isolated despite numerous attempts using different reaction conditions by varying the base, temperature and solvent.

In the IR, triazoles **9** exhibit an NH absorption at 3424–3427 cm⁻¹ and a C=S absorption in the region 1239–1353 cm⁻¹; bands in the range 2550–2600 cm⁻¹, typical for S–H (tautomeric form **F**), were, however, not observed. The UV spectra of 4-amino-5-aryl-1,2,4-triazoles have been reported to display two absorption maxima or shoulders at 252–256 and 288–298 nm and are an indication that the thione form prevails in ethanol solution [5]. In triazoles **9e,f** measured in ethanol, the 252–298 nm range is overlapped by absorptions arising from the acridine skeleton [26] thereby precluding the

opportunity to discern whether the thione or thiol form is predominant. An additional absorption maximum, though, was present at 362 nm.

Scheme 4. Autocondensation cyclization proceeded effectively only in the case of **3e,f** to yield **9e,f** which can possibly exist in one of two tautomeric forms, thione (**E**) or thiol (**F**).



All of the ¹H-NMR signals of **9** were able to be interpreted and assigned thus leading to the full assignment of the ¹³C signals by the concerted application of HSQC and HMBC correlation spectra. The C-9' of the acridinyl moiety resonated at 134 ppm, typical for one bound to an sp³-hybridized N-4 nitrogen. The NH signals resonated in the region 12–14 ppm and correspond to those of similar structures [1,2,5], but cannot be used to distinguish between tautomeric forms **E** and **F** (Scheme 4). A ¹H–¹⁵N HSQC and HMBC spectra however, provided clear differentiation between the thione **E** or thiol **F** forms of **9e** as H-2, the labile proton, provided an appropriate correlation to a nitrogen signal, viz. N-2, at –176.8 ppm. The lower than usual value for the chemical shift of the C=S carbon (168 ppm, cf. 175–183 ppm for the C=S carbon in thiosemicarbazides [17,18]) suggests, however, that there might be some contribution of tautomeric structure **F** through fast exchange. The other carbon of the ring, C-5, resonates at 150 ppm, thereby discounting any contribution of the HC-5 tautomer.

DFT calculations provided energy differences (ΔE) between the tautomeric forms **E** and **F** that were heavily in favor of the thione form **E** by about 12 kcal mol^{–1} (Table 1).

Table 1. Selected experimental and calculated ¹H- and ¹³C-NMR chemical shifts and energy differences ($\Delta E_{\text{thione/thiol}}$) for the thione/thiol tautomerism of **9a–f**.

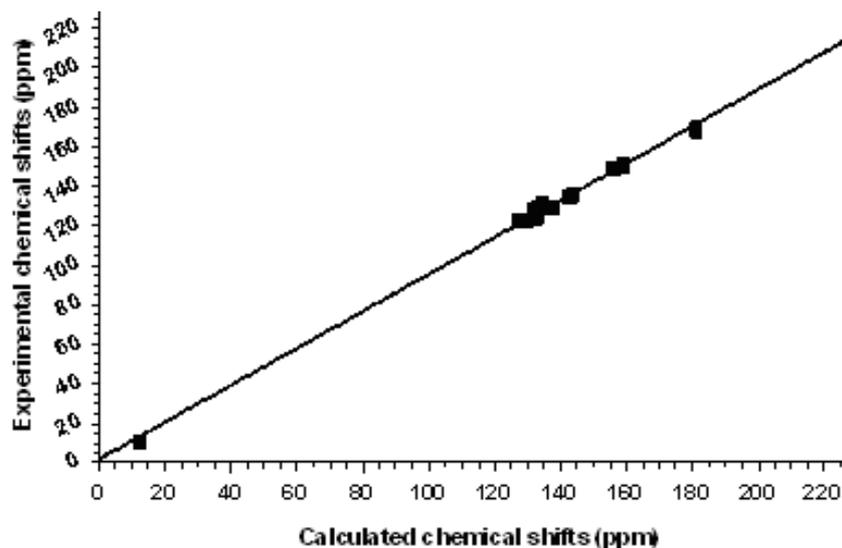
	9a	9b	9c	9d	9e	9f
δ ¹ H (NH) [ppm] ¹	13.66 8.79	14.12 9.15	14.23 9.19	14.34 9.21	14.13 8.96	14.65 9.30
δ ¹³ C (C=S) [ppm] ¹	167.3 180.7	168.5 180.9	168.8 180.9	169.1 181.3	168.4 181.3	169.2 181.6
$\Delta E_{\text{thione/thiol}}$ [kcal mol ^{–1}]	13.05	12.17	11.42	12.00	12.59	11.64

¹ Upper values experimental, lower values calculated at the B3LYP/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level of theory.

The calculated ¹H and ¹³C chemical shifts for **9e,f**, and also for the previously reported **9a–d** [15,27,28], were correlated with the experimental values. In all cases, linear correlations were found as

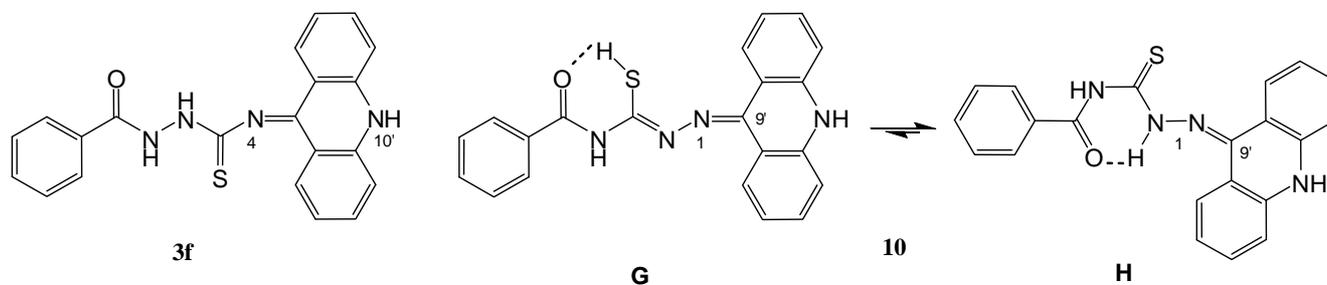
depicted in Figure 3 for the ^{13}C -NMR data. The ^1H chemical shifts of the labile NH protons again deviated substantially because the theoretical values did not take into account the effect of the solvent (Table 1). This problem is known and discussed in the literature and has recently been described for DMSO [29].

Figure 2. Correlation between the experimental and calculated ^{13}C -NMR chemical shifts (calculated at the B3LYP/6-311++G(2d,2p)//6-311+G(d,p) level of theory) for **9e,f** ($a = 0.94$, $b = 1.99$, $R = 0.998$).



For a direct comparison to **3f** in terms of its chemical and biological properties, a positional isomer hitherto unknown, 1-(acridin-9-yl)-4-benzoyl-thiosemicarbazide (**10**), was prepared from benzoyl isothiocyanate and (acridin-9-yl)hydrazine. The reaction proceeded regioselectively with only attack by N-2 of (acridin-9-yl)hydrazine observed. As anticipated [17–24], **10** also adopted a 9',10'-dihydro-acridine structure (the HN-10' tautomer, Scheme 5) as clearly evidenced by the diagnostically shielded signals of C-4' (116.8 ppm) and C-5' (118.3 ppm) [17,18].

Scheme 5. The structures of the positional isomers **3f** and **10**.



In contrast to **3**, restricted rotation about the $\text{C}_9=\text{N}_1$ double bond was slow thus rendering the side acridine rings in **10** observably non-equivalent. The formation of six-membered ring structures stabilized by hydrogen bonds $\text{C}=\text{O}\cdots\text{H}-\text{S}$ or $\text{C}=\text{O}\cdots\text{H}-\text{N}$, known for acylthioureas [30], is possible and is represented by tautomeric forms **G** and **H**, respectively. Such H-bonding may account for the

observation of the slightly smaller chemical shift of the thione carbon (172.8 ppm) and, in particular, for the extraordinarily deshielded signal of H-1' of the acridine ring (10.16 ppm).

Biological activity

Starting thiosemicarbazides **3f–h**, **3h·2HCl** as well as well-soluble final products, **7b,d** and **10**, were tested for biological activity wherein, except for **3f**, they were each found to have significant cytotoxic activity against the L1210 leukemia cell line. Mild antimicrobial inhibitions ($\text{MIC} > 125 \mu\text{g mL}^{-1}$) were also found against selected bacteria and fungi (not reported). The solubility of compound **3h** was so low in DMSO–RPMI that it was tested only at a concentration of 10^{-6} M and therefore it was also tested as the dihydrochloride **3h·2HCl**. The growth inhibitions by **3g** and **10** were 24 and 35%, respectively, at a concentration of 10^{-5} M and dilution to 10^{-6} M was not found to substantially reduce their cytotoxic effects. Compounds **3h·2HCl** and **7d** were highly effective at concentrations near to 10^{-4} M where they inhibited the growth of L1210 cells by 100 and 82%, respectively, although they were inferior at equal low concentrations (10^{-6} M) to **3g** and **10**. The most remarkable aspect of the results regards the two positional isomers **3f** and **10**, for whilst the latter expresses significant cytotoxic activity against the selected cell line, the former is completely devoid of activity altogether. The cytotoxic activities of the tested compounds are summarized in Table 2 with further analysis of the biological efficacy of all compounds planned.

Table 2. Cytotoxic activities of compounds **3f–h**, **3h·2HCl**, **7b,d** and **10**.

Compound	Conc. [$\times 10^{-5}$ M]	Cytotoxicity [%]	Conc. [$\times 10^{-6}$ M]	Cytotoxicity [%]
3f	1	0	1	0
3g	1	24	1	18
3h	not tested	–	1	15
3h·2HCl	7	100	1	8
7b	5	46	1	0
7d	8	82	1	0
10	1	35	1	31

Conclusions

In conclusion, new, five-membered ring nitrogen heterocyclic imidazolidine-4,5-diones **4**, thiazolidin-4-ones **7** and **8** and 1,2,4-triazoles **9** were synthesized via cyclization reactions of a series of 1-acyl-4-phenyl/(acridin-9-yl)thiosemicarbazides **3** using oxalyl chloride, DMAD and intramolecular cyclization under alkaline conditions, respectively. Their chemistry is characterized by a complexity of regioselectivity (**3**, **4**, **7**, **8** and **10**) and tautomerism (**3**, **4**, **9** and **10**). Altogether, twenty new compounds are described and have been variously characterized and identified by IR, UV, ^1H , ^{13}C and 2D NMR and quantum chemical calculations. The effect of the acyl group on N-1 was clearly evident with heightened regioselectivity, in particular the lack of formation of six-membered

rings, a result. Compounds **3f–h**, **3h·2HCl**, **7b,d** and **10** were screened for their cytotoxic activity against the L1210 leukemia cell line and all compounds, except for **3f**, exhibited promising inhibitions of cell growth.

Experimental

General

Melting points were determined on a Boetius block and are uncorrected. NMR spectra were obtained at room temperature in hexadeuteriodimethyl sulfoxide using a Varian Mercury Plus NMR spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C and a Varian Unity Inova NMR spectrometer operating at 60.8 MHz for ^{15}N . Tetramethylsilane was used as an internal standard for both ^1H and ^{13}C nuclei ($\delta_{\text{TMS}} = 0.00$ ppm for both) whilst nitromethane was used as an external standard for ^{15}N measurements ($\delta_{\text{CH}_3\text{NO}_2} = 0.00$ ppm). Heteronuclear 2D (HSQC and HMBC) experiments were optimized on 145 Hz (one-bond) and 8 Hz (long-range) for $J_{\text{H,C}}$ couplings and on 90 Hz for $J_{\text{H,N}}$ couplings. Elemental analyses were performed on a Perkin–Elmer CHN 2400 analyzer. UV-vis spectra were measured on a Varian Cary 100 UV-vis spectrophotometer in ethanol. Quantum chemical calculations were carried out within the framework of the DFT method according to the original proposal [31] using the Gaussian 03 program [32]. The absolute shielding constants were calculated at the B3LYP/6-311++G(2d,2p) level of theory using the GIAO method [33]. Acylthiosemicarbazides **3a–d** were prepared according to the literature [14–16] by the reaction of hydrazides **1** with phenyl isothiocyanates **2**.

General procedure for the syntheses of 1,4-disubstituted acylthiosemicarbazides **3e–h**

To a solution of the appropriate substituted hydrazides (**1**, 0.85 mmol) in methanol (2 mL), 9-isothiocyanatoacridine [34] (0.20 g, 0.85 mmol) was added and the reaction mixture heated until the reactants had been consumed (monitored by TLC, chloroform–methanol 9:1). The precipitate that formed was filtered off, washed with a small amount of methanol and dried at room temperature. The crude product was purified by crystallization from methanol–DMF.

4-(Acridin-9-yl)-1-(acetyl)-thiosemicarbazide (3e). The crude product (approximate yield 77%; m.p. 168–170 °C) was used to prepare derivatives **8e** and **9e**, as repeated attempts to crystallize it were unsuccessful.

4-(9',10'-Dihydroacridin-9'-ylidene)-1-(benzoyl)-thiosemicarbazide (3f). Yield 91%; m.p. 190–193 °C; Anal. Calc. for $\text{C}_{21}\text{H}_{16}\text{N}_4\text{OS}$ (372.44): 67.72% C, 4.33% H, 15.04% N: found: 67.34% C, 4.21% H, 14.85% N; $^1\text{H-NMR}$ δ : 8.64 (d, $J = 8.2$ Hz, 2H, H-1',8'), 8.02 (dd, $J = 8.0, 7.4$ Hz, 2H, H-3',6'), 7.91 (d, $J = 8.0$ Hz, 2H, H-4',5'), 7.80 (dd, $J = 8.6, 1.4$ Hz, 2H, H-2'',6''), 7.60 (dd, $J = 8.2, 7.4$ Hz, 2H, H-2',7'), 7.49 (m, 2H, H-3'',5''), 7.45 (m, 1H, H-4''); $^{13}\text{C-NMR}$ δ : 179.7 (C=S), 160.7 (C=O), 157.4 (C-9'), 139.7 (C-4a',10a'), 135.3 (C-3',6'), 129.5 (C-4''), 128.9 (C-3'',5''), 125.3 (C-1''), 124.8 (C-2'',6''), 124.5 (C-1',8'), 123.7 (C-2',7'), 119.3 (C-4',5'), 111.6 (C-8a',9a').

4-(9',10'-Dihydroacridin-9'-ylidene)-1-(3-pyridylcarbonyl)-thiosemicarbazide (**3g**). Yield 79%; m.p. 274–276 °C; Anal. Calc. for C₂₀H₁₅N₅OS (373.43): 64.33% C, 4.05% H, 18.75% N; found: 64.05% C, 3.98% H, 18.47% N; ¹H-NMR δ: 12.36 (s, 1H, NH), 9.08 (s, 1H, H-2"), 8.74 (m, 1H, H-6"), 8.26 (d, *J* = 7.4 Hz, 1H, H-4"), 8.04 (d, *J* = 8.4 Hz, 2H, H-1',8"), 7.78 (dd, *J* = 8.2, 6.8 Hz, 2H, H-3',6"), 7.65 (d, *J* = 8.2 Hz, 2H, H-4',5"), 7.59 (dd, *J* = 7.4, 5.2 Hz, 1H, H-5"), 7.26 (dd, *J* = 8.4, 6.8 Hz, 2H, H-2',7").

4-(9',10'-Dihydroacridin-9'-ylidene)-1-(4-pyridylcarbonyl)-thiosemicarbazide (**3h**). Yield 87%; m.p. 261–264 °C; Anal. Calc. for C₂₀H₁₅N₅OS (373.43): 64.33% C, 4.05% H, 18.75% N; found: 64.16% C, 4.01% H, 18.51% N; ¹H-NMR δ: 8.68 (m, 2H, H-2",6"), 8.64 (dd, *J* = 8.8, 1.0 Hz, 2H, H-1',8"), 8.04 (ddd, *J* = 8.6, 6.8, 1.0 Hz, 2H, H-3',6"), 7.89 (d, *J* = 8.6 Hz, 2H, H-4',5"), 7.70 (m, 2H, H-3",5"), 7.61 (ddd, *J* = 8.8, 6.8, 1.0 Hz, 2H, H-2',7"); ¹³C-NMR δ: 181.3 (C=S), 159.1 (C=O), 157.7 (C-9'), 150.4 (C-2",6"), 139.3 (C-4a',10a'), 135.6 (C-3',6'), 131.7 (C-4"), 124.5 (C-1',8"), 123.8 (C-2',7'), 118.9 (C-4',5'), 118.5 (C-3",5"), 111.5 (C-8a',9a'). For the preparation of the corresponding dihydrochloride **3h·2HCl** compound **3h** was dissolved in acetone (0.5 mL) and an equimolar solution of HCl in methanol (1 mL of 35% hydrochloric acid in 9 mL of methanol) was added. The mixture was stirred for 1 h followed by the addition of diethyl ether. The resulting precipitate was filtered off and dried in vacuo. Yield 85%; m.p. 180–182 °C; Anal. Calc. for C₂₀H₁₇N₅Cl₂OS (446.36): 53.82% C, 3.84% H, 15.69% N; found: 53.58% C, 3.61% H, 15.36% N.

General procedure for the syntheses of 1,3-disubstituted imidazolidine-4,5-diones **4a–d**

To a solution of the appropriate acylthiosemicarbazide (**3a–d**, 1.1 mmol) in dichloromethane (3 mL), oxalyl chloride (0.097 mL, 1.1 mmol) was added dropwise with stirring and cooling. The reaction mixture was stirred for 30 min at –10 °C and then for 30 min at –20 °C. The reaction mixture was then evaporated to dryness and the crude product crystallized from methanol.

*N*1-(4,5-Dioxo-3-phenyl-2-thioxo-1-imidazolidinyl)acetamide (**4a**). Yield 92%, m.p. 74–76 °C; Anal. Calc. for C₁₁H₉N₃O₃S (263.28): 50.18% C, 3.45% H, 15.96% N; found: 49.75% C, 3.32% H, 15.75% N; ¹H-NMR δ: 11.18 (s, 1H, NH), 7.60–7.40 (m, 5H, Ph), 2.07 (s, 3H, CH₃); ¹³C-NMR δ: 179.5 (C=S), 168.1 (NHC=O), 154.0, 152.5 (2 × C=O), 132.1 (C-1'), 129.5 (C-3',5'), 129.2 (C-4'), 128.2 (C-2',6'), 20.1 (CH₃).

*N*1-(4,5-Dioxo-3-phenyl-2-thioxo-1-imidazolidinyl)benzamide (**4b**). Yield 84%, m.p. 70–73 °C; Anal. Calc. for C₁₆H₁₁N₃O₃S (325.35): 59.07% C, 3.41% H, 12.92% N; found: 58.79% C, 3.35% H, 12.81% N; ¹H-NMR δ: 11.89 (s, 1H, NH); 8.00 (d, *J* = 7.0 Hz, 2H, H-2",6"), 7.67 (m, 1H, H-4"), 7.60–7.45 (m, 7H, H-2'–6', 3",5"). ¹³C-NMR δ: 179.6 (C=S), 165.1 (NHC=O), 154.0, 152.7 (2 × C=O), 132.9 (C-4"), 132.1 (C-1'), 130.6 (C-1"), 129.6 (C-4'), 129.2, 128.7 (C-3',5', C-3",5"), 128.2 (C-2',6'), 127.9 (C-2",6").

*N*3-(4,5-Dioxo-3-phenyl-2-thioxo-1-imidazolidinyl)nicotinamide (**4c**). Yield 91%, m.p. 140–143 °C; Anal. Calc. for C₁₅H₁₀N₄O₃S (326.34): 55.21% C, 3.09% H, 17.17% N; found: 55.06% C, 3.04% H, 17.11% N; ¹H-NMR δ: 12.53 (s, 1H, NH), 9.32 (d, *J* = 1.2 Hz, 1H, H-2"), 9.00 (dd, *J* = 5.1, 1.7 Hz,

1H, H-6"), 8.70 (ddd, $J = 8.0, 1.7, 1.2$ Hz, 1H, H-4"), 7.91 (dd, $J = 8.0, 5.1$ Hz 1H, H-5"), 7.65–7.53 (m, 3H, H-3',5', H-4'), 7.52–7.48 (m, 2H, H-2',6"); $^{13}\text{C-NMR}$ δ : 179.1 (C=S), 162.8 (NHC=O), 153.9, 152.4 (2 \times C=O), 150.0 (C-6"), 145.9 (C-2"), 139.6 (C-4"), 132.0 (C-1'), 129.6, 129.2 (C-3',5', C-4'), 128.2 (C-2',6'), 127.6 (C-3"), 125.4 (C-5").

*N*4-(4,5-Dioxo-3-phenyl-2-thioxo-1-imidazolidinyl)isonicotinamide (**4d**). Yield 89%, m.p. 146–148 °C; Anal. Calc. for $\text{C}_{15}\text{H}_{10}\text{N}_4\text{O}_3\text{S}$ (326.34): 55.21% C, 3.09% H, 17.17% N; found: 55.06% C, 3.04% H, 17.11% N; $^1\text{H-NMR}$ δ : 12.26 (s, 1H, NH); 8.86 (m, 2H, H-2",6"), 7.89 (m, 2H, H-3",5"), 7.65–7.46 (m, 5H, Ph); $^{13}\text{C-NMR}$ δ : 179.1 (C=S), 164.0 (NHC=O), 153.9, 152.4 (2 \times C=O), 150.6 (C-2",6"), 137.6 (C-4"), 132.0 (C-1'), 129.6, 129.2 (C-3',5', C-4'), 128.2 (C-2',6'), 121.5 (C-3",5").

General procedure for the syntheses of 1,3-thiazolan-5-yliden acetates **7a–d** and **8a,e–h**

To a stirred solution of the appropriate acylthiosemicarbazide (**3a–h**, 0.30 mmol) in methanol (1–2 mL), DMAD (0.05 mL, 0.40 mmol) was added dropwise at room temperature. The mixture was stirred at room temperature for a further 24 h until the reaction was complete (monitored by TLC, chloroform–methanol 9:1). The precipitate that had formed was filtered off, washed with a small amount of methanol and diethyl ether and the product crystallized from methanol.

Methyl 2-[2-(2-acetylhydrazono)-4-oxo-3-phenyl-1,3-thiazolan-5-yliden]acetate (7a). Minor product; $^1\text{H-NMR}$ δ : 10.63 (s, 1H, NH), 7.60–7.40 (m, 5H, Ph), 6.83 (s, 1H, H-6), 3.82 (s, 3H, OCH₃), 1.93 (s, 3H, CH₃); $^{13}\text{C-NMR}$ δ : 165.9, 165.8 (C-7, NHC=O), 163.1 (C-4), 151.0 (C-2), 140.9 (C-5), 134.1 (C-1'), 129.0, 128.8, 128.1 (C-2',6', C-3',5', C-4'), 114.6 (C-6), 52.6 (OCH₃), 21.0 (CH₃).

Methyl 2-[2-(2-benzoylhydrazono)-4-oxo-3-phenyl-1,3-thiazolan-5-yliden]acetate (7b). Yield 71%; m.p. 270–271 °C; Anal. Calc. for $\text{C}_{19}\text{H}_{15}\text{N}_3\text{O}_4\text{S}$ (381.41): 59.83% C, 3.96% H, 11.02% N; found: 59.69% C, 3.85% H, 10.94% N; $^1\text{H-NMR}$ δ : 11.28 (s, 1H, NH), 7.83 (d, $J = 7.2$ Hz, 2H, H-2",6"), 7.60–7.45 (m, 8H, H-2'-6', 3"-5"), 6.87 (s, 1H, H-6), 3.89 (s, 3H, OCH₃); $^{13}\text{C-NMR}$ δ : 165.9 (C-7), 163.5 (NHC=O), 163.5 (C-4), 157.5 (C-2), 140.8 (C-5), 134.0 (C-1'), 132.8 (C-1"), 131.7 (C-4"), 129.1 (C-3',5'), 129.0 (C-4'), 128.4 (C-2',6'), 128.1 (C-3",5"), 127.4 (C-2",6"), 115.1 (C-6), 52.6 (OCH₃).

Methyl 2-[4-oxo-3-phenyl-2-[2-(3-pyridylcarbonyl)hydrazono]-1,3-thiazolan-5-yliden]acetate (7c). Yield 69%; m.p. 235–237 °C; Anal. Calc. for $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$ (382.39): 56.54% C, 3.69% H, 14.65% N; found: 56.29% C, 3.55% H, 14.47% N; $^1\text{H-NMR}$ δ : 11.50 (s, 1H, NH), 8.97 (s, 1H, H-2"), 8.75 (d, $J = 4.4$ Hz, 1H, H-6"), 8.17 (d, $J = 7.6$ Hz, 1H, H-4"), 7.59–7.46 (m, 6H, H-2'-6', 5"), 6.87 (s, 1H, H-6), 3.81 (s, 3H, OCH₃); $^{13}\text{C-NMR}$ δ : 165.9 (C-7), 163.4 (C-4), 162.0 (NHC=O), 157.1 (C-2), 152.2 (C-6"), 148.3 (C-2"), 140.7 (C-5), 135.2 (C-4"), 134.0 (C-1'), 129.1 (C-3',5'), 129.0 (C-4'), 128.6 (C-3"), 128.1 (C-2',6'), 123.5 (C-5'), 115.2 (C-6), 52.6 (OCH₃).

Methyl 2-[4-oxo-3-phenyl-2-[2-(4-pyridylcarbonyl)hydrazono]-1,3-thiazolan-5-yliden]acetate (7d). Yield 63%; m.p. 251–253 °C; Anal. Calc. for $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$ (382.39): 56.54% C, 3.69% H, 14.65% N; found: 56.29% C, 3.55% H, 14.47% N; $^1\text{H-NMR}$ δ : 11.59 (s, 1H, NH), 8.76 (m, 2H, H-2",6"), 7.73 (m,

2H, H-3",5"), 7.62–7.46 (m, 5H, Ph), 3.81 (s, 3H, OCH₃); ¹³C-NMR δ: 165.9 (C-7), 163.5 (C-4), 161.9 (NHC=O), 157.9 (C-2), 150.3 (C-2",6"), 140.6 (C-4"), 139.9 (C-5), 133.9 (C-1'), 129.1 (C-3',5'), 128.1 (C-2',6'), 128.1 (C-4'), 121.3 (C-3",5"), 115.3 (C-6), 52.6 (OCH₃).

Methyl 2-[2-(2-acetylhydrazono)-4-oxo-3-phenyl-1,3-thiazolan-5-yliden]acetate (8a). Yield 76%; m.p. 176–178 °C; Anal. Calc. for C₁₄H₁₃N₃O₄S (319.34): 52.66% C, 4.10% H, 13.16% N; found: 52.31% C, 4.02% H, 13.08% N; ¹H-NMR δ: 11.04 (s, 1H, NH), 7.43 (m, 2H, H-3',5'), 7.23 (m, 1H, H-4'), 6.95 (m, 2H, H-2',6'), 6.91 (s, 1H, H-6), 3.75 (s, 3H, OCH₃), 2.08 (s, 3H, CH₃); ¹³C-NMR δ: 167.8 (NHC=O), 165.5 (C-7), 161.1 (C-4), 148.1 (C-2), 146.6 (C-1'), 137.9 (C-5), 129.5 (C-3v,5'), 125.2 (C-4'), 120.5 (C-2',6'), 116.9 (C-6), 52.6 (OCH₃), 20.2 (CH₃).

Methyl[2-(acridin-9-ylimino)-3-(acetylamino)-4-oxothiazolidin-5-ylidene]acetate (8e). Yield 75%; m.p. 265–267 °C; Anal. Calc. for C₂₁H₁₆N₄O₄S (420.45): 59.99% C, 3.84% H, 13.33% N; found: 59.70% C, 3.66% H, 13.18% N; ¹H-NMR δ: 11.37 (s, 1H, NH), 8.18, 8.17 (d, *J* = 8.4 Hz, 1H and d, *J* = 8.4 Hz, 1H, H-4' and H-5'), 8.03 (d, *J* = 8.4 Hz, H-1'), 7.91–7.83 (m, 3H, H-3',6',8'), 7.61, 7.59 (m, m, 2H, H-2' and H-7'), 6.99 (s, 1H, H-6), 3.63 (s, 3H, OCH₃), 2.21 (s, 3H, CH₃); ¹³C-NMR δ: 168.8 (NHC=O), 165.3 (C-7), 161.2 (C-4), 151.1 (C-2), 148.6 (C-4a',10a', C-9'), 137.1 (C-5), 130.8 (C-3',6'), 129.2 (C-4',5'), 125.8 (C-2',7'), 123.5 (C-1',8'), 118.1 (C-6), 116.8 (C-8a',9a'), 52.7 (OCH₃), 20.3 (CH₃).

Methyl[2-(acridin-9-ylimino)-3-(benzoylamino)-4-oxothiazolidin-5-ylidene]acetate (8f). Yield 93%; m.p. 172–175 °C; Anal. Calc. for C₂₆H₁₈N₄O₄S (482.51): 64.72% C, 3.76% H, 11.61% N; found: 64.56% C, 3.68% H, 11.37% N; ¹H-NMR δ: 12.04 (s, 1H, NH), 8.18, 8.17 (d, *J* = 8.4 Hz, 1H and d, *J* = 7.6 Hz, 1H, H-4' and H-5'), 8.10 (d, *J* = 7.6 Hz, 2H, H-2",6"), 8.03 (d, *J* = 8.4 Hz, 1H, H-1'), 7.94–7.84 (m, 3H, H-3',6',8'), 7.71 (m, 1H, H-4"), 7.67–7.58 (m, 4H, H-2', 7', 3",5"), 7.06 (s, 1H, H-6), 3.64 (s, 3H, OCH₃); ¹³C-NMR δ: 165.7 (C-7), 165.3 (NHC=O), 161.3 (C-4), 151.1 (C-2), 148.7 (C-4a',10a'), 148.5 (C-9'), 136.7 (C-5), 133.0 (C-4"), 130.8 (C-4',5'), 130.6 (C-1"), 129.3 (C-3',6'), 128.8 (C-3",5"), 127.9 (C-2",6"), 125.8 (C-2',7'), 123.2 (C-1',8'), 118.7 (C-6), 116.7 (8a',9a'), 52.7 (OCH₃).

Methyl[2-(acridin-9-ylimino)-4-oxo-3-(3-pyridylcarbonyl)thiazolidin-5-ylidene]acetate (8g). Yield 78%; m.p. 178–180 °C; Anal. Calc. for C₂₅H₁₇N₅O₄S (483.51): 62.10% C, 3.54% H, 14.48% N; found: 61.83% C, 3.47% H, 14.23% N; ¹H-NMR δ: 12.28 (s, 1H, NH), 9.22 (d, *J* = 2.4 Hz, 1H, H-2"), 8.85 (dd, *J* = 4.7, 1.4 Hz, 1H, H-6"), 8.42 (dd, *J* = 8.0, 1.4 Hz, 1H, H-4"), 8.15, 8.14 (d, *J* = 8.8 Hz, 1H and d, *J* = 8.0 Hz, 1H, H-4' and H-5'), 8.01 (d, *J* = 8.4 Hz, 1H, H-1'), 7.89–7.80 (m, 3H, H-3',6',8'), 7.64 (dd, *J* = 8.0, 4.7 Hz, 1H, H-5"), 7.60 (m, 2H, H-2',7'), 7.04 (s, 1H, H-6), 3.63 (s, 3H, OCH₃); ¹³C-NMR δ: 166.1 (C-7), 165.3 (NHC=O), 162.0 (C-4), 154.3 (C-4"), 151.8 (C-2), 149.5 (C-2"), 149.4 (C-4a',10a'), 149.2 (C-9'), 137.4 (C-5), 136.6 (C-6"), 131.6 (C-3',6'), 130.1 (C-4',5'), 127.3 (C-3"), 126.7 (C-2',7'), 124.7 (C-5"), 124.1 (C-1',8'), 119.5 (C-6), 117.5 (C-8a',9a'), 53.5 (OCH₃).

Methyl[2-(acridin-9-ylimino)-4-oxo-3-(4-pyridylcarbonyl)-thiazolidin-5-ylidene]acetate (8h). Yield 63%; m.p. 180–182 °C; Anal. Calc. for C₂₅H₁₇N₅O₄S (483.51): 62.10% C, 3.54% H, 14.48% N; found: 61.83% C, 3.47% H, 14.23% N; ¹H-NMR δ: 12.45 (s, 1H, NH), 8.86 (m, 2H, H-2",6"), 8.17 (m, 2H,

H-4',5'), 8.02 (d, $J = 8.0$ Hz, 1H, H-1'), 7.97 (m, 2H, H-3'',5''), 7.89–7.84 (m, 3H, H-3',6',8'), 7.62 (m, 2H, H-2',7'), 7.05 (s, 1H, H-6), 3.63 (s, 3H, OCH₃); ¹³C-NMR δ : 165.4 (C-7), 164.6 (NHC=O), 161.2 (C-4), 151.0 (C-2), 150.9 (C-2'',6''), 150.8 (C-4a',10a'), 148.3 (C-9'), 137.7 (C-4''), 136.7 (C-5), 131.3 (C-3',6'), 128.9 (C-4',5'), 126.1 (C-2',7'), 123.4 (C-1',8'), 121.6 (C-3'',5''), 118.9 (C-6), 116.8 (C-8a',9a'), 52.9 (OCH₃).

General procedure for the syntheses of 1,2,4-triazoles **9e,f**

The appropriate acylthiosemicarbazide (**3e,f**, 0.4 mmol) and 2 N aqueous sodium hydroxide (2 mL) were refluxed for 2–4 hours. After cooling, the precipitate was filtered off and the filtrate acidified by hydrochloric acid to a pH of 5–6. The precipitate that formed was also filtered off and combined with the previous collection. The product washed with water, dried and crystallized from methanol.

4-(Acridin-9-yl)-5-methyl-2,4-dihydro[1,2,4]triazole-3-thione (9e). Yield 64%; m.p. 259–261 °C; Anal. Calc. for C₁₆H₁₂N₄S (292.36): 65.73% C, 4.14% H, 19.16% N; found: 65.44% C, 4.06% H, 18.87% N; ¹H-NMR δ : 14.16 (s, 1H, NH), 8.35 (dd, $J = 8.6, 1.2$ Hz, 2H, H-4',5'), 7.98 (ddd, $J = 8.6, 6.5, 1.2$ Hz, 2H, H-3',6'), 7.75 (ddd, $J = 8.8, 6.5, 1.2$ Hz, 2H, H-2',7'), 7.59 (dd, $J = 8.8, 1.2$ Hz, 2H, H-1',8'), 1.92 (s, 3H, CH₃); ¹³C-NMR δ : 168.3 (C=S), 149.3 (C-5), 148.9 (C-4a',10a'), 134.8 (C-9'), 131.1 (C-3',6'), 129.6 (C-4',5'), 128.4 (C-2',7'), 122.7 (C-1',8'), 122.4 (C-8a',9a'), 10.9 (CH₃); ¹⁵N-NMR δ : –65.7, –104.3, –176.8 (N-2), –204.5.

4-(Acridin-9-yl)-5-phenyl-2,4-dihydro[1,2,4]triazole-3-thione (9f). Yield 71%; m.p. 255–258 °C; Anal. Calc. for C₂₁H₁₄N₄S (354.43): 71.16% C, 3.98% H, 15.81% N; found: 70.78% C, 3.84% H, 15.68% N; ¹H-NMR δ : 14.65 (s, 1H, NH), 8.30 (d, $J = 8.8$ Hz, 2H, H-4',5'), 7.94 (ddd, $J = 8.8, 6.0, 1.6$ Hz, 2H, H-3',6'), 7.75–7.68 (m, 4H, H-1',2',7',8'), 7.25 (m, 1H, H-4''), 7.15–7.12 (m, 4H, H-2'',3'',5'',6''); ¹³C-NMR δ : 169.2 (C=S), 151.0 (C-5), 148.8 (C-4a',10a'), 135.8 (C-9'), 131.1 (C-3',6'), 130.8 (C-4''), 129.5 (C-4',5'), 128.7 (C-3'',5''), 128.5 (C-2',7'), 126.8 (C-2'',6''), 125.0 (C-1''), 123.0 (C-1',8'), 122.7 (C-8a',9a').

1-(9',10'-Dihydroacridin-9'-ylidene)-4-(benzoyl)-thiosemicarbazide (10)

To a solution of benzoyl isothiocyanate (0.2 g, 1.22 mmol) in methanol (2 mL), acridin-9-yl hydrazine [35] (0.26 g, 1.22 mmol) was added. The mixture was stirred at room temperature until the reactants had been consumed (monitored by TLC, toluene–acetone 5:2). The precipitate that formed was filtered off, washed with a small amount of methanol and diethyl ether and the crude product crystallized from methanol. Yield 83%, m.p. 212–214 °C; Anal. Calc. for C₂₁H₁₆N₄OS (372.45): 67.72% C, 4.33% H, 15.04% N; found: 67.51% C, 4.26% H, 14.75% N; ¹H-NMR δ : 13.03 (s, 1H, H-2), 12.55 (s, 1H, H-10'), 10.14–10.03 (m, 2H, H-4, H-1'), 8.22 (d, $J = 7.6$ Hz, 1H, H-8'), 7.96 (d, $J = 7.2$ Hz, 2H, H-2'',6''), 7.84 (m, 2H, H-3',6'), 7.72–7.42 (m, 6H, H-3''–5'',4', 5',7'), 7.35 (m, 1H, H-2'); ¹³C-NMR δ : 172.8 (C=S), 163.7 (C=O), 143.1 (C-9'), 140.3 (C-4a'), 138.2 (C-10a'), 134.8 (C-1''), 134.5 (C-3'), 133.5 (C-6'), 132.0 (C-1'), 131.5 (C-4''), 128.2 (C-3'',5''), 128.0 (C-2'',6''), 124.0 (C-7'), 122.2 (C-8'), 122.0 (C-2'), 118.3 (C-5'), 116.8 (C-4'), 111.9 (C-8a'), 111.8 (C-9a').

Biological activity tests

The cytotoxic effects of compounds **3f–h**, **3h·2HCl**, **7b,d** and **10** were tested against the L1210 leukemia cell line using the MTT test at two concentrations for each compound within the range 10^{-4} – 10^{-6} M [36,37]. The compounds were first dissolved in DMSO and diluted in RPMI 1640 medium supplemented with antibiotics (penicillin 100 IU mL^{-1} and streptomycin $100 \mu\text{g mL}^{-1}$). The final DMSO concentration was below 0.1% at which level it did not inhibit the growth of the L1210 cells; the complete medium contains 10% fetal calf serum. L1210 leukemia cells were then cultured with the compounds for 3 days. Measurements were performed in triplicate and the cytotoxic activity was expressed as a percentage of growth control.

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References

1. Smicius, R.; Jakubkiene, V.; Burbuliene, M. M.; Mikalainyte, A.; Vainilavicius, P. Synthesis of 1-(6-methyl-2,4-dioxo-1,2,3,4-tetrahydro-3-pyrimidinyl)acetyl-4-alkyl(aryl)thiosemicarbazides and their heterocyclisation to 1,2,4-triazoles and 1,3,4-thiadiazoles. *J. Chem. Res.-S* **2002**, 170–172.
2. Gülerman, N. N.; Doğan, H. N.; Rollas, S.; Johansson, C.; Çelik, C. Synthesis and structure elucidation of some new thioether derivatives of 1,2,4-triazoline-3-thiones and their antimicrobial activities. *Farmaco* **2001**, 56, 953–958.
3. Singh, S. P.; Parmar, S. S.; Raman, K.; Stenberg, V. I. Chemistry and biological activity of thiazolidinones. *Chem. Rev.* **1981**, 81, 175–203.
4. Argentieri, T. M.; Butera, J. A. An overview of potassium channel activators for the treatment of overactive bladder: a survey of new structures 2000–2005. *Expert Opin. Ther. Patents* **2006**, 16, 573–585.
5. Colanceska-Ragenovic, K.; Dimova, V.; Kakurinov, V.; Molnar, D. G.; Buzarovska, A. Synthesis, antibacterial and antifungal activity of 4-substituted-5-aryl-1,2,4-triazoles. *Molecules* **2001**, 6, 815–824.
6. Zamani, K.; Faghihi, K.; Tofighi, T.; Shariatzadeh, M. R. Synthesis and antimicrobial activity of some pyridyl and naphthyl substituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives. *Turk. J. Chem.* **2004**, 28, 95–100.
7. Gažová, Z.; Bellová, A.; Daxnerová, Z.; Imrich, J.; Kristian, P.; Tomaščíková, J.; Bagelová, J.; Fedunová, D.; Antalík, M. Acridine derivatives inhibit lysozyme aggregation. *Eur. Biophys. J.* **2008**, in press.

8. Ergenç, N.; Çapan, G. Synthesis and anticonvulsant activity of new 4-thiazolidone and 4-thiazoline derivatives. *Farmaco* **1994**, *49*, 133–135.
9. Kelarev, V. I.; Silin, M. A.; Kotova, I. G.; Kobrakov, K. I.; Rybina, I. I.; Korolev, V. K. Synthesis of N-substituted 3-aminothiazolidin-4-ones containing heteroaryl fragments. *Chem. Heterocycl. Comp.* **2003**, *39*, 213–222.
10. Ulrich, H.; Sayigh, A. A. R. The reaction of oxalyl chloride with substituted ureas and thioureas. *J. Org. Chem.* **1965**, *30*, 2781–2783.
11. Özkýrýmly, S.; Hamalý, O. Synthesis and hypnotic activity of some 2-thioxo-4,5-imidazolidinedione derivatives. *Farmaco* **1995**, *50*, 65–67.
12. Tomaščíková, J.; Imrich, J.; Danihel, I.; Böhm, S.; Kristian, P. Heterocyclization of (acridin-9-yl)thiosemicarbazides with dimethyl acetylenedicarboxylate. *Coll. Czech. Chem. Commun.* **2007**, *72*, 347–362.
13. Demeunynck, M.; Charmantray, F.; Martelli, A. Interest of acridine derivatives in the anticancer chemotherapy. *Curr. Pharm. Des.* **2001**, *7*, 1703–1724.
14. Baxendale, Ian R.; Ley, S. V.; Martinelli, M. The rapid preparation of 2-aminosulfonamide-1,3,4-oxadiazoles using polymer-supported reagents and microwave heating. *Tetrahedron* **2005**, *61*, 5323–5349.
15. Foks, H.; Janowiec M.; Zwolska Z.; Augustynowicz-Kopec E. Synthesis and tuberculostatic activity of some piperazinylmethyl derivatives of 1,2,4-triazole-3-thiones. *Phosphorus Sulfur Silicon Relat. Elem.* **2005**, *180*, 537–543.
16. Kalyoncuoglu, N.; Rollas, S.; Sür-Altmer, D.; Yegenoglu, Y.; Ang, Ö. 1-[p-(Benzoylamino)benzoyl]-4-substituted thiosemicarbazides: synthesis and antibacterial and antifungal activities. *Pharmazie* **1992**, *47*, 796–797.
17. Klika K. D.; Balentová E.; Bernát J.; Imrich J.; Vavrušová M.; Kleinpeter E.; Pihlaja K.; Koch A. Tautomerism, regioisomerism, and cyclization reactions of acridinyl thiosemicarbazides. *J. Heterocycl. Chem.* **2006**, *43*, 633–643.
18. Balentová, E.; Imrich, J.; Bernát, J.; Suchá, L.; Vilková, M.; Prónayová, N.; Kristian, P.; Pihlaja, K.; Klika, K. D. Stereochemistry, tautomerism, and reactions of acridinyl thiosemicarbazides in the synthesis of 1,3-thiazolidines. *J. Heterocycl. Chem.* **2006**, *43*, 645–656.
19. Klika, K. D.; Balentová, E.; Bernát, J.; Imrich, J.; Vavrušová, M.; Pihlaja, K.; Koch, A.; Kleinpeter, E.; Kelling, A.; Schilde, U. Structural revision of products resulting from the reaction of methylhydrazine with acridin-9-yl isothiocyanate due to unexpected acridinyl migration and further reactions. *ARKIVOC* **2006**, (xvi), 93–108.
20. Bernát, J.; Balentová, E.; Kristian, P.; Imrich, J.; Sedlák, E.; Danihel, I.; Böhm, S.; Prónayová, N.; Pihlaja, K.; Klika, K. D. Methylation of acridin-9-ylthioureas. Structure, fluorescence and biological properties of products. *Coll. Czech. Chem. Commun.* **2004**, *69*, 833–849.
21. Klika, K. D.; Imrich, J.; Vilková, M.; Bernát, J.; Pihlaja, K. Unusual structures derived from N-acridin-9-yl methyl N'-acridin-9-yl thiourea based on the propensity of N-10 to retain H. *J. Heterocycl. Chem.* **2006**, *43*, 739–743.
22. Klika, K. D.; Bernát, J.; Imrich, J.; Chomča, I.; Sillanpää, R.; Pihlaja, K. Unexpected formation of a spiro acridine and fused ring system from the reaction between an N-acridinylmethyl substituted thiourea and bromoacetonitrile under basic conditions. *J. Org. Chem.* **2001**, *66*, 4416–4418.

23. Kristian, P.; Balentová, E.; Bernát, J.; Imrich, J.; Sedlák, E.; Danihel, I.; Böhm, S.; Prónayová, N.; Klika, K. D.; Pihlaja, K.; Baranová, J. Fluorescence and structure of methylated acridin-9-ylthioureas. *Chem. Pap.* **2004**, *58*, 268–275.
24. Adcock, B. *Aminoacridines* In *Acridines*, Acheson R. M., Ed. (from *The Chemistry of Heterocyclic Compounds*, Weissberger, A.; Taylor, E. C., Eds.); J. Wiley: New York, 1973, p 109.
25. Géci, I.; Valtamo, P.; Imrich, J.; Kivelä, H.; Kristian, P.; Pihlaja, K. Regiospecific synthesis, structure and electron ionization mass spectra of 1,3-thiazolidin-4-ones containing the acridine skeleton. *J. Heterocycl. Chem.* **2005**, *42*, 907–918.
26. Kristian, P. Isothiocyanates and their synthetic formers V. Synthesis of substituted 9-isothiocyanatoacridines and study of the relation between their physicochemical properties and structure. *Chem. Pap.* **1969**, *23*, 371–376.
27. Rostamizadeh, S.; Mollahoseini, K.; Moghadasi, S. A One-pot synthesis of 4,5-disubstituted-1,2,4-triazole-3-thiones on solid support under microwave irradiation. *Phosphorus Sulfur Silicon Relat. Elem.* **2006**, *181*, 1839–1845.
28. Hazzaa, A. A. B.; Ashour, F.; Shafik, R. M. Synthesis of 1,2,4-triazolylthiopropyl-amines as potential cardiovascular agents. *Pharmazie* **1980**, *35*, 324–325.
29. Vailikhit, V.; Treesuwan, W.; Hannongbua, S. A combined MD-ONIOM2 approach for ¹H NMR chemical shift calculations including a polar solvent. *Theochem-J. Mol. Struct.* **2007**, *806*, 99–104.
30. Hritzová, O.; Černák, J.; Šafař, P.; Fröhlichová, Z.; Csöreg, I. Furan derivatives of substituted phenylthiourea: spectral studies, semi-empirical quantum-chemical calculations and X-ray structure analyses. *J. Mol. Struct.* **2005**, *743*, 29–48.
31. Becke, A. D. Density-functional thermochemistry III. The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
32. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A. Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hagesawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzales, C.; Pople, J. A. *Gaussian 03, Revision B.05*, Gaussian, Inc., Pittsburgh PA **2003**.
33. Wolinski, K.; Hinton, J. F.; Pulay, P. Efficient implementation of the gauge-independent atomic orbital method for NMR chemical shift calculations. *J. Am. Chem. Soc.* **1990**, *112*, 8251–8260.
34. Kristian, P. Isothiocyanates VII. Synthesis of 9-isothiocyanatoacridine and some of its derivatives. *Chem. Pap.* **1961**, *15*, 641–646.

35. Albert, A.; Ritchie, B. 9-Aminoacridine. *Org. Synth.* **1942**, 22, 5–8.
36. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunolog. Meth.* **1983**, 65, 55–63.
37. Sabol, M.; Kutschy, P.; Siegfried, L.; Miroššay, A.; Suchý, M.; Hrbková, H.; Dzurilla, M.; Marušková, R.; Starková, J.; Paulíková, E. Cytotoxic effect of cruciferous phytoalexins against murine L1210 leukemia and B16 melanoma. *Biologia* **2000**, 55, 701–707.

Sample Availability: Samples of compounds **4a**, **7a–d**, **8a,e–h** **9e** and **10** are available from the authors.

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