HETEROCYCLIZATION OF (ACRIDIN-9-YL)THIOSEMICARBAZIDES WITH DIMETHYL ACETYLENEDICARBOXYLATE

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Received November 8, 2006
Accepted January 26, 2007

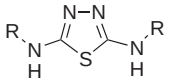
Two types of (acridin-9-yl)thiosemicarbazides with the acridine moiety in the thiourea part (Acr-NHCS, **10a**, **10b**) and hydrazine part (Acr-NHNHCS, **12a**–**12c**) were prepared to investigate their reactions with dimethyl acetylenedicarboxylate. Five-membered thiazolidinone derivatives **15a**, **15b**, **19a**–**19c** were formed; some aspects of corresponding reaction mechanisms are discussed. 1D and 2D ^{1}H and ^{13}C NMR spectroscopy and DFT quantum chemical calculations were used to elucidate the structure of the compounds.

Keywords: Thiosemicarbazides; Thiazolidinones; Acridines; Acetylenedicarboxylate; Quantum chemical calculations; Reaction mechanisms.

Thiosemicarbazides are versatile compounds that have been extensively used in the preparation of heterocyclic ring systems[1–7]. Some of them are of interest due to their antibacterial[8,9], antifungal[10], antiviral[11] and antitumor[8,12,13] activities. The variability of thiosemicarbazide reactions depends on the nucleophilic reactivity of either the sulfur or nitrogen atoms. Most of the studied heterocyclization reactions of thiosemicarbazides involved their reactions with carboxylic esters and halocarbonyl compounds. The primary interest was focused on the reactions of 4-substituted and 2,4-disubstituted thiosemicarbazides with carbonate and phosgene, respectively. By refluxing 4-phenyl- and 4-(ethoxyphenyl)thiosemicarbazide with diethyl carbonate in diethylene glycol monomethyl ether, 2,5-dianilino-1,3,4-thiadiazoles **1** were obtained[1]. When the 2-methyl-4-phenylthiosemicarbazide reacted with phosgene, 2,5-diphenylimino-3-methyl-1,3,4-thiadiazolidine **2**, as its hydrochloride, was isolated from the reaction mixture[1].

Collect. Czech. Chem. Commun. 2007, Vol. 72, No. 3, pp. 347–362

doi:10.1135/cccc20070347



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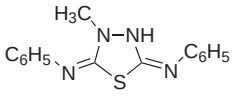
R = Ph, $C_2H_5OC_6H_4$



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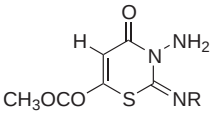
In previous papers[14,15], our attention was paid to stereochemistry, tautomerism and reactions of acridinylthiosemicarbazides. The reaction of 9-isothiocyanatoacridine with methylhydrazine yielded 4-(9,10-dihydroacridin-9-ylidene)-2-methylthiosemicarbazide spirocyclizing in small yield (4%) to 1′-methyl-10*H*-spiro[acridine-9,3′-[1,2,4]triazolidine]-5′-thione. With phenylhydrazine, the analogous reaction led to a mixture of 1′-phenyl- and 2′-phenyl-10*H*-spiro[acridine-9,3′-[1,2,4]triazolidine]-5′-thiones. A number of heterocyclic systems[16–20] has been prepared from dimethyl acetylenedicarboxylate (DMAD) and a variety of nucleophiles, including also thiosemicarbazides. An addition-cyclization reaction of DMAD and unsubstituted thiosemicarbazide proceeded through the thiourea moiety whereas the hydrazine part was not involved in the formation of the thiazolidinone ring[16]. The structure of a six-membered 1,3-thiazinone ring was proposed for the compound **3** from the spectral analysis of its hydrogenated adduct **4** (ref.[17]). However, the arguments for the structure determination, based on high-resolution MS data and non-equivalency of CH_2 protons in the ^{1}H NMR spectra, were shown to be misinterpreted[18]. Later, the structure of five-membered thiazolidinone ring **5** was unambiguously proved by synthesis, ^{1}H/^{13}C NMR spectroscopy and comparison of the obtained results with X-ray data for analogous derivatives[18–20].



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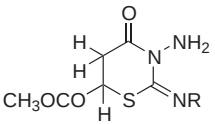

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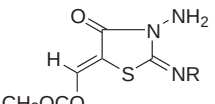

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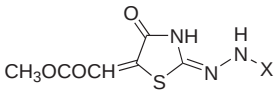
A solvent dependence was found for thienopyrimidine thiosemicarbazide derivatives in the addition to DMAD[21]. Protic methanol favored an attack of N-4 nitrogen atom to an α-(methoxycarbonyl) group in the intermediate similar to **18a** (Scheme 2) as well as elimination of the methoxy group to give thiazolidinone derivatives **6**. In a more basic aprotic dioxane, the intramolecular cyclization proceeded via the N-1 nitrogen atom on the

ethylene C–C bond and the final products were 1,3,4-thiadiazole derivatives **7** (ref.[21]).



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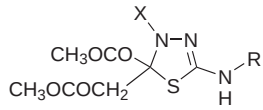

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Thiazolidinones possess a broad spectrum of biological activity[22]. Acridine derivatives[23], exhibiting significant biological and fluorescent properties, inspired us to use the acridine moiety in combination with analogous thiazolidine heterocycles to obtain new biologically interesting compounds. Our attention was devoted, for example, to the synthesis, structure and reaction mechanism of S_N substitution reactions of acridine thiosemicarbazides with methyl bromoacetate and bromoacetonitrile[14,15]. We studied also intercalation properties of some acridinylthioureas[24] and acridinylthiazolidinone derivatives[25] to determine a binding affinity with plasmid DNA (pUC19).

In this paper, we have prepared two different acridinylthiosemicarbazides with the acridine moiety in the thiourea part (AcrNHCS) or hydrazine part (AcrNHNHCS) of the thiosemicarbazides, with the aim to influence the cyclization course after the Ad_N addition reactions with dimethyl acetylenedicarboxylate. Due to the attached acridine, the thiosemicarbazide–thiosemicarbazone tautomerism determines the cyclization reaction pathway in a specific manner. We have tried to elucidate this peculiarity of the acridine skeleton in the compounds under study by means of complex NMR experiments and quantum chemical calculations.

RESULTS AND DISCUSSION

Two types of thiosemicarbazides were prepared by reactions of selected isothiocyanates and hydrazines, one with the AcrNHCS grouping (**10**; Eq. (*1*)) and the other with the AcrNHNHCS moiety (**12**; Eq. (*2*)):

$$\underset{\mathbf{8}}{\text{Acr–NCS}} + \underset{\mathbf{9}}{\text{NH}_2\text{–NR}^1\text{R}^2} \rightarrow \underset{\mathbf{10}}{\text{Acr–NH–C(=S)–NH–NR}^1\text{R}^2} \qquad (1)$$

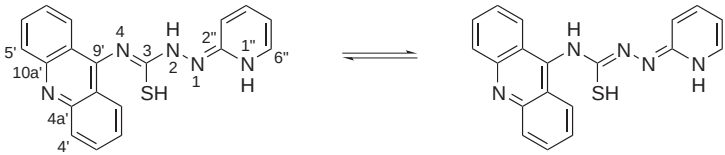
a: R^1 = H, R^2 = 2-pyridyl; **b**: R^1 = R^2 = Me

$$\underset{\mathbf{11}}{\text{Acr–NH–NH}_2} + \text{R–NCS} \rightarrow \underset{\mathbf{12}}{\text{Acr–NH–NH–C(=S)–NHR}} \qquad (2)$$

a: R = Me; **b**: R = 4-MeOC$_6$H$_4$; **c**: R = acridin-9-yl (9,10-dihydroacridin-9-ylidene)

*Thiosemicarbazides **10** and **12***

The starting thiosemicarbazides **10a**, **10b** were obtained by the reaction of 9-isothiocyanatoacridine[26] (**8**) with substituted hydrazines **9a**, **9b** (Scheme 1), whereas thiosemicarbazides **12a**–**12c** resulted from the treatment of (acridin-9-yl)hydrazine[27] with corresponding isothiocyanates (Scheme 2). Both the reactions were carried out at room temperature in methanol. The formation of products **10** and **12** was dependent on the nucleophilicity of nitrogen atoms and steric effects of hydrazine reagents. The electron-withdrawing 2-pyridyl substituent of hydrazine **9a** decreased the nucleophilicity of α-nitrogen to such extent that only β-nitrogen could attack the N=C=S group in the Ad_N reaction with 9-isothiocyanatoacridine (**8**). 4-(Acridin-9-yl)-1-(2-pyridyl)thiosemicarbazide (**10a**) exists in the DMSO-d_6 solution in a tautomeric SH forms A or B as proved by characteristic N=^{13}C(-SH)-NH signal (C-3) at 159.00 ppm instead of typical thiosemicarbazide C=S carbon signal at ~180 ppm [15]. Noteworthy with **10a** is also an evident tautomeric preference of the 1″,2″-dihydropyridine structure, with an exocyclic C=N double bond localized between pyridine C-2″ and thiosemicarbazide N-1 atoms, over the aromatized pyridine.



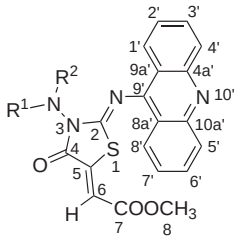
Unequivocal proof in this respect is lack of two ^{13}C NMR signals near 150 ppm, obligatory for pyridine C-2″ and C-6″ carbons in the case of the aromatic pyridine skeleton. Just one low-field pyridine signal for **10a** at 146.7 ppm (C-2″) was present and that of C-6″ was strongly shielded (125.1 ppm), analogously, for example, to 2-pyridone (136.1 ppm). It is interesting that no spirocyclization of **10a** involving an attack of N-1 to acridine C-9′ carbon, similar to that of 4-(acridin-9-yl)-1-phenylthiosemi-

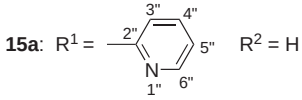
carbazide to its spiro isomer[14], described in the introduction, has been observed here, probably due to the distinct electron-withdrawing character of the neighboring pyridyl substituent. The acridine skeleton of **10a** was further characterized by ^{13}C NMR resonance signals of C-4a′,10a′ and C-4′,5′ carbons at 152.4 and 126.0 ppm, respectively, which are typical of the aromatic central acridine ring. On the other hand, another acridine tautomeric form, 9,10-dihydroacridin-9-ylidene **12**C, was present in 1-acridinylthiosemicarbazides **12a**, **12b** (Scheme 2) as proved by a significant decrease in the aforesaid values to 140 and 115 ppm, respectively[15].

*Thiazolidinones **15** and **19***

The reaction of 4-(acridin-9-yl)-1-(2-pyridyl)thiosemicarbazide (**10a**) and 4-(acridin-9-yl)-1,1-dimethylthiosemicarbazide (**10b**) with dimethyl acetylenedicarboxylate is illustrated in Scheme 1. The initial reaction is a nucleophilic addition of a soft sulfur atom of thiosemicarbazide **10a**, **10b** to a triple bond of DMAD that could afford two possible tautomeric intermediate isothiosemicarbazides **13**, **14**. They were not isolated but in subsequent intramolecular cyclization, the nucleophilic attack of one of the isothiosemicarbazide nitrogen atoms on ester carbonyl took place. In the case of pyridyl intermediate, theoretically any of nitrogen atoms N-1, N-2 or N-4 could attack any of two carbonyl groups, whereas analogous reaction with the other intermediate (**13b**, **14b**) is excluded for N-1 due to its dimethyl substitution (Scheme 1).

As final products of this reaction, methyl [2-(acridin-9-ylimino)-3-(2-pyridylamino or dimethylamino)-4-oxothiazolidin-5-ylidene]acetates **15a**, **15b** were revealed by ^{1}H and ^{13}C NMR spectroscopy (Tables I, II). The structure of the 2-imino-4-oxothiazolidin-5-ylidene ring of **15a**, **15b** was confirmed by a similarity of its characteristic ^{13}C NMR chemical shifts (C-2 (C=N) at 151 ppm, C-4 (C=O) at 162 ppm, and C-5 (C=C) at 139 ppm) to the values of analogous five-membered ring[20] as well as the spectra of

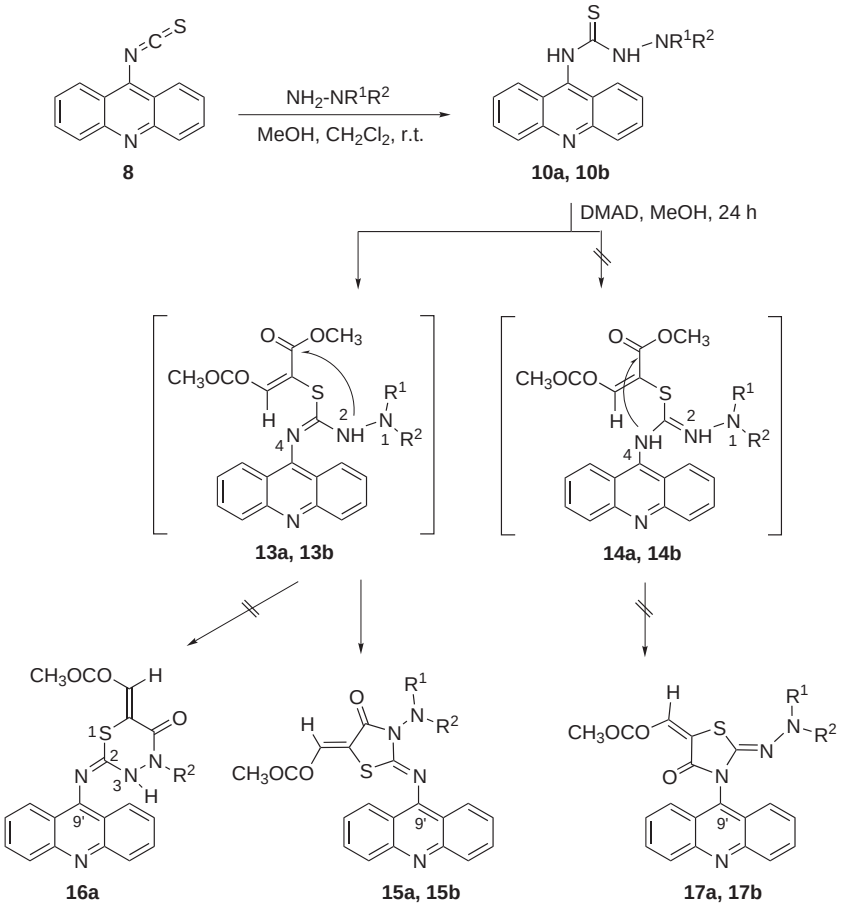




15b: $R^1 = R^2 = CH_3$

methyl [2-(acridin-9-ylimino)-3-(*tert*-butylamino)-4-oxothiazolidin-5-ylidene]-acetate, whose structure was unequivocally determined by X-ray crystallography (not shown here).

Another evidence of the structure of **15a** and **15b** follows from the acridine C-9′ chemical shift at 147.6 and 148.7 ppm, respectively, which is a typical value when acridine is attached to the exocyclic nitrogen of 2-iminothiazolidin-4-one[28]. The central ring in the acridine moiety is aro-



a: R^1 = H, R^2 = 2-pyridyl; **b**: R^1= R^2 = CH_3

SCHEME 1

matic as follows from the acridine C-4a′ and C-10a′ chemical shifts located at about 149 ppm. The close spectral resemblance of both compounds excludes thus a six-membered structure **16a** that has no **16b** alternative. By these arguments it is possible to denote the N-2 of the intermediate isothiosemicarbazides **13** as the atom mediating cyclization and to exclude the cyclization through the N-4 nitrogen of isothiosemicarbazides **14**. Such alternative regiomeric cyclization products, [3-(acridin-9-yl)-2-substituted hydrazono-4-oxothiazolidin-5-ylidene]acetates **17a**, **17b**, which would show the C-9′ signal shifted to the higher field, ca. 138 ppm [28], were not observed here. After cyclization of **10a** to **15a**, the usual pyridine chemical shifts of C-2″ (155.9 ppm) and C-6″ (148.7 ppm) have been restored, signalizing full aromatization of pyridine in the product.

A set of coupling constants for compound **15a** was calculated by the B3LYP/6-311+G(d,p)//B3LYP/6-311++G(2d,2p) calculations and compared with the experimental ones (Table III). The calculation of coupling constants $^{3}J_{C4–H6}$, corresponding to interaction of the exocyclic vinyl proton with the lactam carbonyl, gave the values 10.6 Hz for *E*-configuration and 6.3 Hz for *Z*-configuration. The latter value, which is in close correspondence to the experimental one, $^{3}J_{C4–H6}$ = 5.3 Hz, enabled us to prove the *Z*-configuration of the vinyl double bond, in agreement with lit.[20]. The prediction that the *Z*-configuration of compounds **15a**, **15b** is also energetically more favorable has been proved by quantum chemical calculations. The energy difference between *Z* and *E* isomers of **15a** is about 7 kcal/mol, in favor of the former one.

The above mentioned observations concerning reactions of 4-(acridin-9-yl)thiosemicarbazides **10a**, **10b** with DMAD revealed that the N-2 nitrogen of intermediates **13a**, **13b** is the reacting species rendering the intramolecular cyclization whereas the other nitrogens with diminished nucleophilicity due to the electron-withdrawing effect of attached acridine or pyridine do not take part in the ring formation. Such a reaction course favors intermediates **13a**, **13b** over their tautomers **14a**, **14b**.

Further, we have explored an analogous reaction of DMAD with reversely substituted 1-(acridin-9-yl)-4-R-thiosemicarbazides **12a**–**12c** (R = Me, 4-$MeOC_6H_4$ and acridin-9-yl), which adopted preferred 1-(9,10-dihydroacridin-9-ylidene) tautomeric form (Scheme 2). Thiosemicarbazides **12a**–**12c** may afford with DMAD in principle two tautomeric isothiosemicarbazides **18a**–**18c** and **20a**–**20c** from which theoretically two regioisomeric products **19a**–**19c** and **21a**–**21c**, respectively, could arise. Isothiosemicarbazides again could not be isolated as they spontaneously cyclized through a N-4 nitrogen atom to give final products, methyl {[2-(9,10-dihydroacridin-9-yliden)-

TABLE I
^{1}H NMR chemical shifts (in ppm) for substituted (thiazolidin-5-ylidene)acetates **15a**, **15b**, **19a**–**19c**

		H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-7′	H-8′	H-9	H-10′	H-10″	Other
15a	(exp)	7.84	7.50	7.74	8.14	8.14	7.74	7.50	7.84	10.02		-	3.64 (H-8), 6.99 (H-5″), 6.99 (H-6), 7.09 (H-3″), 7.58 (H-4″), 8.34 (H-6″)
	(theor)	8.09[a]	7.61[a]	7.94[a]	8.50[a]	8.50[a]	7.94[a]	7.61[a]	8.09[a]	6.50		–	3.42[a] (H-8), 7.13 (H-6), 7.03 (H-5″), 6.88 (H-3″), 7.77 (H-4″), 8.68 (H-6)
15b	(exp)	8.06	7.61	7.89	8.18	8.18	7.89	7.61	8.06	–		–	2.10 (H-1″), 3.62 (H-8), 6.85 (H-6)
	(theor)	8.21[a]	7.61[a]	7.92[a]	8.45[a]	8.45[a]	7.92[a]	7.61[a]	8.21[a]	–		–	1.84[a] (H-1″), 3.87[a] (H-8), 6.97 (H-6)
19a	(exp)	9.34	7.08	7.51	7.30	7.26	7.51	7.17	8.31	–	10.89	–	3.43 (H-1″), 3.80 (H-8), 6.72 (H-6)
	(theor)	9.84	7.24	7.59	7.01	6.92	7.57	7.25	8.73	–	6.86	–	3.92[a] (H-1″), 3.74[a] (H-8), 7.00 (H-6)
19b	(exp)	8.71	6.59	7.26	7.11	7.13	7.35	7.03	8.33	–	10.45	–	3.80 (H-8), 3.83 (H-7″), 6.79 (H-6), 7.04 (H-3″/5″), 7.37 (H-2″/6″)
	(theor)	9.80	6.76	7.41	6.84	6.91	7.58	7.33	9.15	–	6.67	–	3.77[a] (H-8), 3.88[a] (H-7″), 7.01 (H-6), 7.22[a] (H-3″/5″), 7.41[a] (H-2″/6″)
19c	(exp)	7.42	5.68	7.16	7.05	7.19	7.49	7.18	8.32	–	10.83	–	3.91 (H-8), 7.00 (H-6), 7.42 (H-2″,7″), 8.00 (H-3″,6″), 8.20 (H-1″,8″), 8.39 (H-4″,5″)
	(theor)	8.65	7.44[a]	7.85	7.20	7.05	7.69	7.37	9.30	–	6.86	–	3.88[a] (H-8), 7.39 (H-2″,7″), 7.44 (H-6), 7.46 (H-1″,8″), 7.81 (H-3″,6″), 8.42 (H-4″,5″)

[a] Averaged value; theoretical B3LYP/6-311+G(d,p)//B3LYP/6-311++G(2d,2p) calculations (see Experimental).

TABLE II
^{13}C NMR chemical shifts (in ppm) for substituted (thiazolidin-5-ylidene)acetates **15a**, **15b**, **19a**–**19c**

		C-1′	C-2′	C-3′	C-4′	C-4a′	C-5′	C-6′	C-7′	C-8′	C-8a′	C-9′	C-9a′	C-10a′	Other
15a	(exp)	123.7	125.6	130.7	129.2	148.9	129.2	130.7	125.6	123.7	116.8	147.6	116.8	148.9	52.6 (C-8), 108.8 (C-3″), 116.8 (C-5″), 117.7 (C-6), 137.7 (C-5), 138.2 (C-4″), 148.7 (C-6″), 151.9 (C-2), 155.9 (C-2″), 162.2 (C-4), 165.4 (C-7)
	(theor)	128.9[a]	129.6[a]	134.0[a]	137.3[a]	155.2[a]	137.3[a]	134.0[a]	129.6[a]	128.9[a]	121.9[a]	155.6[a]	121.9[a]	155.2[a]	53.3 (C-8), 112.1 (C-3″), 121.4 (C-5″), 126.5 (C-6), 142.8 (C-4″), 148.6 (C-5), 154.9 (C-6″), 161.1 (C-2), 162.8 (C-2″), 167.4 (C-4), 169.9 (C-7)
15b	(exp)	123.9	125.7	130.8	129.1	149.2	129.1	130.8	125.7	123.9	116.9	148.7	116.9	149.2	43.0 (C-1″), 52.5 (C-8), 116.0 (C-6), 139.3 (C-5), 151.2 (C-2), 162.0 (C-4), 165.5 (C-7)
	(theor)	129.4[a]	128.1[a]	133.8[a]	137.1[a]	155.6[a]	137.1[a]	133.8[a]	128.1[a]	129.4[a]	120.9[a]	158.9	120.9[a]	155.6[a]	41.8 (C-1″), 53.7 (C-8), 124.9 (C-6), 149.7 (C-5), 154.8 (C-2), 171.8 (C-4), 170.1 (C-7)
19a	(exp)	131.6	119.8	131.6	116.2	140.4	115.7	131.0	121.0	125.0	119.0	149.2	115.2	138.3	29.7 (C-1″), 52.3 (C-8), 113.7 (C-6), 142.3 (C-5), 155.3 (C-2), 164.1 (C-4), 166.0 (C-7)
	(theor)	140.2	124.6	136.2	117.4	144.3	117.4	135.3	125.2	132.4	128.4	154.8	123.5	143.0	35.2 (C-1″), 53.7 (C-8), 117.4 (C-6), 157.8 (C-5), 157.4 (C-2), 170.1 (C-4), 173.8 (C-7)
19b	(exp)	132.0	119.3	131.1	116.1	140.5	115.7	130.8	120.9	125.5	119.3	150.2	115.3	138.5	52.2 (C-8), 55.5 (C-7″), 114.2 (C-3″/5″), 114.5 (C-6), 127.1 (C-1″), 128.9 (C-2″/6″), 142.3 (C-5), 154.7 (C-2), 159.6 (C-4″), 164.1 (C-4), 166.2 (C-7)
	(theor)	140.8	123.8	135.8	117.7	145.3	117.0	135.3	126.3	133.7	129.1	154.8	124.3	142.1	53.6 (C-8), 55.6 (C-7″), 116.9 (C-3″/5″), 117.7 (C-6), 135.7 (C-1″), 135.8 (C-2″/6″), 156.1 (C-5), 168.3 (C-2), 167.3 (C-4″), 170.7 (C-4), 173.4 (C-7)
19c	(exp)	130.8	118.3	131.3	115.9	140.1	115.8	131.2	121.1	124.9	118.5	150.1	114.2	138.1	52.6 (C-8), 115.1 (C-6), 122.6 (C-8″,9a″), 123.2 (C-1″,8″), 128.1 (C-2″,7″), 129.6 (C-4″,5″), 131.1 C-3″,6″), 136.7 (C-9″), 141.8 (C-5), 149.0 (C-4a″,10a″), 153.3 (C-2), 163.7 (C-4), 166.1 (C-7)
	(theor)	134.0	126.0	137.3	119.8	145.2	117.2	137.1	126.4	134.3	125.7	169.3	121.7	142.8	55.8 (C-8), 111.2 (C-6), 121.9 (C-8a″,9a″), 128.5 (C-2″,7″), 129.1 (C-1″,8″), 133.6 (C-3″,6″), 137.0 (C-4″,5″), 155.0 (C-4a″,10a″), 157.1 (C-2), 160.1 (C-5), 168.2 (C-4), 170.8 (C-7)

[a] Averaged value; theoretical B3LYP/6-311+G(d,p)//B3LYP/6-311++G(2d,2p) calculations (see Experimental).

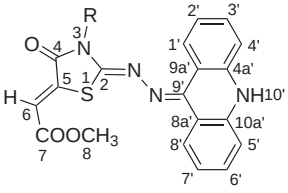
TABLE III
Selected C-H coupling constants ^3J (in Hz) of methyl [2-(acridin-9-ylimino)-4-oxo-3-(2-pyridylamino)thiazolidin-5-ylidene]acetate (**15a**)

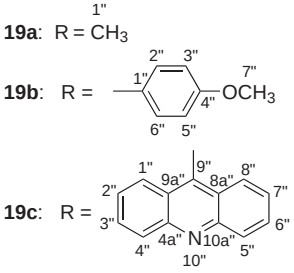
	$^3J_{C8a',9a'-H2',7'}$	$^3J_{C1',8'-H3',6'}$	$^3J_{C2',7'-H4',5'}$	$^3J_{C4',5'-H2',7'}$	$^3J_{C3',6'-H1',8'}$
exp	8.0	6.2	8.4	6.1	8.7
theor *Z*	**7.8**[a]	**6.8**[a]	**8.3**[a]	**6.2**[a]	**8.6**[a]
theor *E*	7.8[a]	6.8[a]	8.4[a]	6.2[a]	8.6[a]
	$^3J_{C4a',10a'-H1',8'}$	$^3J_{C9'-H1',8'}$	$^3J_{C4-H6}$	$^3J_{C7-H8}$	
exp	5.8	3.4	5.3	3.9	
theor *Z*	**5.4**[a]	**4.0**[a]	**6.3**	**3.7**	
theor *E*	5.4[a]	4.0[a]	10.6	4.0	

[a] Averaged value; theoretical B3LYP/6-311+G(d,p)//B3LYP/6-311++G(2d,2p) calculations (see Experimental).

hydrazono]-3-substituted-4-oxothiazolidin-5-ylidene}acetates **19a**–**19c**. No 3-(acridinylimino)thiazolidinones **21** analogous to **15**, which could be formed by cyclization through the N-2 nitrogen, were observed.

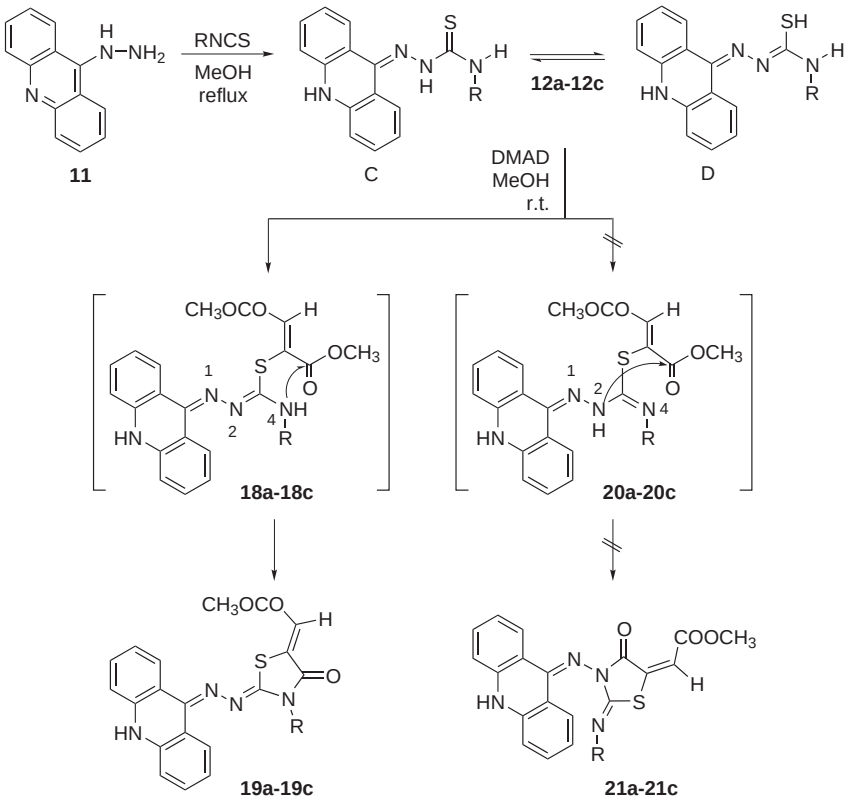
The structure of thiazolidinones **19a**–**19c** was established using a set of advanced NMR methods. ^{13}C chemical shifts of thiazolidinone skeleton in three derivatives **19a**–**19c** were very similar (C-2 (C=N) at ca. 154 ppm, C-4 (C=O) at 164 ppm, and C-5 (C=C) at 142 ppm) and differed, though little, from those of **15a**, **15b** (vide supra).





Crucial evidence was therefore based on the observed three-bond coupling between the 3-methyl protons and ring carbonyl in an inversion gHMBC spectra of methyl derivative **19a**. Such transfer of polarization might not be possible for the regioisomer **21a** where both groups are too

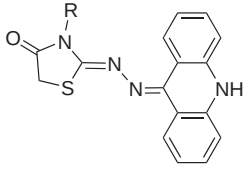
distant (5 bonds) to yield any cross-peak. Observed was also NMR non-equivalence of the acridine peripheral rings due to hindered rotation about the planar C=N–N=C system in position 2 of **19a**–**19c** as a consequence of a high barrier to inversion known for such systems[15]. Such rigidity contrasted with a freely rotating 2-(acridin-9-yl)imino group in **15a**, **15b** showing the equivalence of peripheral benzene rings in acridine, thus confirming dissimilarity of molecules **15** and **19**. Supporting evidence was also similarity of chemical shifts of α-carbons to N-3 (**19a** – CH_3 (C-1″) ≈ 29 ppm, **19b** – C_{ipso} (C-1″, in 4-$CH_3OC_6H_4$) ≈ 127 ppm) to assigned models **22** (ref.[15]) (**22a** – CH_3 ≈ 29 ppm, **22b** – C_{ipso} ≈ 128 ppm) and those of acridine C-9′ (**19a**/**22a** – 149/147 ppm, **19b**/**22b** – 150/148 ppm). The definitive structure of regioisomer **19c** has been established by the agreement of the above values as



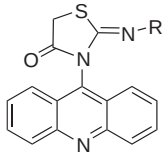
a: R = Me, **b**: R = 4-$CH_3OC_6H_4$; **c**: R = acridin-9-yl

SCHEME 2

well as ^{13}C chemical shift of C-9″ of other acridine ring (**19c** – 136.7 ppm) with a model 2-(alkyl(aryl)imino)-3-(acridin-9-yl)thiazolidin-4-ones **23** (C-9″ ≈ 138 ppm)[28]. It is also noteworthy that the acridine on the hydrazine side of compounds **12c**, **19c** is present in the 9,10-dihydro form, whereas the other acridine on the thiourea side is aromatic.



22



23

R = Me (a), 4-$CH_3OC_6H_4$ (b)

Very interesting is a dramatic drop of chemical shifts of acridine H-1′ and especially of H-2′ in **19b**, **19c** (6.59 and 5.68 ppm, respectively) which is caused by a magnetic shielding of these protons by the 3-(4-methoxyphenyl) or 3-(acridin-9-yl) substituent in fixed positions of both proximal aromatics. In both series we have ascertained that the attack of nucleophilic nitrogen in intermediates **13**, **18** was always aimed at that DMAD carbonyl group which was closer to the sulfur atom, probably due to a shorter distance, better accessibility and higher stability of the five-membered compared with the six-membered heterocycle. The different reaction courses might be explained by a lowered energy of molecules **19** due to the formation of the large conjugated (9,10-dihydroacridin-9-ylidene)hydrazone system in position 2 of the thiazolidinone product. Such extended planarity could enhance the well-known intercalation ability of acridine in unforeseeable manner and as such is to be examined later.

Our conclusions regarding the structure of the compounds under study obtained from NMR data were also confirmed by quantum chemical calculations. Theoretical ^1H and ^{13}C NMR chemical shifts agreed with experimental ones (see also ref.[29]), the noteworthy differences were observed only for the ^{13}C chemical shifts of C-9′/C-9″. Experimental and theoretical B3LYP/6-311++G(2d,2p) calculations of ^1H and ^{13}C chemical shifts are in close relation and linearly correlate: $\delta\ ^1H_{exp}/^{13}C_{exp} = a\delta\ ^1H_{theor}/^{13}C_{theor} + b$ (corresponding data for methyl [2-(acridin-9-ylimino)-3-(*tert*-butylamino)-4-oxothiazolidin-5-ylidene]acetate were also included). The significant correlation with parameters a = 0.939, b = 2.492, correlation coefficient r = 0.993, number of points n = 128 for carbon atoms of all compounds **15** and

19 was found. An analogous correlation for hydrogen atoms is complicated by the fact that the correlation field is split into two correlations: the one for the labile N-H protons (a = 1.282, b = 1.936, r = 0.997, n = 5) and the other for remaining values (a = 0.901, b = 0.564, r = 0.976, n = 78).

EXPERIMENTAL

Melting points were determined on a Boetius block and are uncorrected. NMR spectra were obtained using a Varian Mercury Plus NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C at room temperature in hexadeuteriodimethyl sulfoxide (**10**, **12**, **15**, **19**). Tetramethylsilane was used as an internal standard for both nuclei (δ_{TMS} 0.00 ppm). The heteronuclear 2D experiments were optimized to 145 Hz (one-bond) and 8 Hz (long-range) J(H,C) couplings. Elemental analysis was performed on a Perkin–Elmer CHN 2400 analyzer. Quantum chemical calculations were carried out within the framework of the DFT method according to the original proposal[30] using the Gaussian 03 program[31]. The absolute shielding constants were calculated at the B3LYP/6-311++G(2d,2p) level using the GIAO method[32]. Planarity or any symmetry was never anticipated. All reasonable conformations were taken into consideration and calculations started from an appropriate closely related structure. All structures checked by vibrational analysis behaved as energy minima. The coupling constants were calculated according to recent suggestions[33–35].

Preparation of Thiosemicarbazides **10a**, **12c**. General Procedure

To a solution of 9-isothiocyanatoacridine[26] (0.3 g, 1.27 mmol) in dichloromethane (2 ml), substituted hydrazine (1.27 mmol) was added. The mixture was stirred at room temperature until the reactants disappeared (monitored by TLC, toluene–acetone 5:2). The precipitate formed was filtered off, washed with a small amount of dichloromethane and diethyl ether, and the crude product was crystallized from methanol.

4-(Acridin-9-yl)-1-(2-pyridyl)thiosemicarbazide (**10a**). Yield 81%, m.p. 240–244 °C. For $C_{19}H_{15}N_5S$ (345.4) calculated: 66.07% C, 4.38% H, 20.27% N; found: 65.87% C, 4.21% H, 20.18% N. ^{1}H NMR (400 MHz, DMSO-d_6): 6.89 dd, 1 H, J(4″,5″) = 6.6, J(5″,6″) = 7.0 (H-5″); 7.33 ddd, 1 H, J(3″,4″) = 9.6, J(4″,5″) = 6.6, J(4″,6″) = 1.2 (H-4″); 7.40 ddd, 2 H, J(1′,2′) = 8.4, J(2′,3′) = 6.8, J(2′,4′) = 1.2 (H-2′,7′); 7.54 d, 1 H, J(3″,4″) = 9.6 (H-3″); 7.76 ddd, 2 H, J(2′,3′) = 6.8, J(3′,4′) = 8.4, J(1′,3′) = 1.2 (H-3′,6′); 7.86 dd, 2 H, J(3′,4′) = 8.4, J(2′,4′) = 1.2 (H-4′,5′); 8.23 dd, 1 H, J(5″,6″) = 7.0, J(4″,6″) = 1.2 (H-6″); 8.47 dd, 2 H, J(1′,2′) = 8.4, J(1′,3′) = 1.2 (H-1′,8′). ^{13}C NMR (100 MHz, DMSO-d_6): 112.6 (C-8a′,9a′); 112.7 (C-5″); 115.3 (C-3″); 122.4 (C-2′,7′); 123.7 (C-1′,8′); 125.1 (C-6″); 126.0 (C-4′,5′); 129.0 (C-4″); 131.6 (C-3′,6′); 146.3 (C-9′); 146.7 (C-2″); 152.4 (C-4a′,10a′); 159.0 (C-3, C-SH).

4-(Acridin-9-yl)-1-(9,10-dihydroacridin-9-ylidene)thiosemicarbazide (**12c**). Yield 82%, m.p. 208–210 °C. For $C_{27}H_{19}N_5S$ (445.5) calculated: 72.79% C, 4.30% H, 15.72% N; found: 72.35% C, 4.12% H, 15.61% N. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): 6.26 dd, 1 H, J(6″,7″) = 7.2, J(7″,8″) = 7.8 (H-7″); 7.24 d, 1 H, J(5″,6″) = 8.4 (H-5″); 7.32 dd, 2 H, J(1′,2′) = 9.2, J(2′,3′) = 7.6 (H-2′,7′); 7.43–7.50 m, 4 H (H-2″, H-4″, H-6″, H-8″); 7.59 dd, 1 H, J(2″,3″) = 7.6, J(3″,4″) = 8.0 (H-3″); 7.78 dd, 2 H, J(2′,3′) = 7.6, J(3′,4′) = 8.2 (H-3′,6′); 8.24 d, 2 H, J(1′,2′) = 9.2 (H-1′,8′); 8.26 m, 1 H (H-1″); 8.44 d, 2 H, J(3′,4′) = 8.2 (H-4′,5′); 8.25 s, 1 H; 11.48 s, 1 H; 12.84 s, 1 H (H-2, H-4, H-10″).

Preparation and NMR spectral data of 4-(acridin-9-yl)-1,1-dimethylthiosemicarbazide[36] (**10b**), 1-(9,10-dihydroacridin-9-ylidene)-4-methylthiosemicarbazide[15] (**12a**) and 1-(9,10-dihydroacridin-9-ylidene)-4-(4-methoxyphenyl)thiosemicarbazide[15] (**12b**) were described elsewhere.

Reaction of Thiosemicarbazides **10a**, **10b**, **12a**–**12c** with DMAD. General Procedure

To a stirred solution of appropriate thiosemicarbazide (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 24 h until the reaction was complete (monitored by TLC, toluene–acetone 5:2). The precipitate was filtered off, washed on a filter with a small amount of methanol and diethyl ether and the crude product was crystallized from methanol.

Methyl [2-(acridin-9-ylimino)-4-oxo-3-(2-pyridylamino)thiazolidin-5-ylidene]acetate (**15a**). Yield 73%, m.p. 154–156 °C. For $C_{24}H_{17}N_5O_3S$ (455.5) calculated: 63.29% C, 3.76% H, 15.38% N; found: 62.82% C, 3.74% H, 15.13% N.

Methyl [2-(acridin-9-ylimino)-3-(dimethylamino)-4-oxothiazolidin-5-ylidene]acetate (**15b**). Yield 43%, m.p. 192–194 °C. For $C_{21}H_{18}N_4O_3S$ (406.5) calculated: 62.06% C, 4.46% H, 13.78% N; found: 61.98% C, 4.35% H, 13.65% N.

Methyl {2-[(9,10-dihydroacridin-9-ylidene)hydrazono]-3-methyl-4-oxothiazolidin-5-ylidene}acetate (**19a**). Yield 81%, m.p. 225–227 °C. For $C_{20}H_{16}N_4O_3S$ (392.4) calculated: 61.21% C, 4.11% H, 14.28% N; found: 60.91% C, 3.99% H, 13.91% N.

Methyl {2-[(9,10-dihydroacridin-9-ylidene)hydrazono]-3-(4-methoxyphenyl)-4-oxothiazolidin-5-ylidene}acetate (**19b**). Yield 77%, m.p. 333–335 °C. For $C_{26}H_{20}N_4O_4S$ (484.5) calculated: 64.45% C, 4.16% H, 11.56% N; found: 64.29% C, 4.02% H, 11.37% N.

Methyl {3-(acridin-9-yl)-2-[(9,10-dihydroacridin-9-ylidene)hydrazono]-4-oxothiazolidin-5-ylidene}acetate (**19c**). Yield 83%, m.p. 332–334 °C. For $C_{32}H_{21}N_5O_3S$ (555.6) calculated: 69.18% C, 3.81% H, 12.60% N; found: 68.85% C, 3.77% H, 12.56% N.

Financial support of this work by the Slovak Grant Agency VEGA (Grant No. 1/2471/05) and the NMR Program (Grant No. 2003SP200280203) is gratefully acknowledged.

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