METHYLATION OF ACRIDIN-9-YLTHIOUREAS. STRUCTURE, FLUORESCENCE AND BIOLOGICAL PROPERTIES OF PRODUCTS

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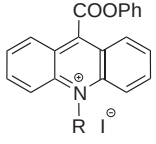
The structure, fluorescence, biological properties and S(N)-methylation reactions of ten 1,1-alkyl/aryl-disubstituted 3-(acridin-9-yl)thioureas **4** have been studied. Various reaction conditions allowed to obtain corresponding *S*-methyl **5** and *S*,*N*-dimethyl derivatives **6** in good yields. Structure and stereochemistry of the synthesized products are demonstrated by ab initio quantum chemical calculations and NMR techniques including PDQF-COSY, selective INEPT and NOE-difference experiments. Remarkable upfield ^{13}C shifts of resonance signals of carbons C-4a, C-10a adjacent to acridine N-10 are characteristic of hydroiodides in contrast to free bases. *Z* configuration in isothioureas **7** with secondary amino rest in relation to *E* configuration of isothioureas with primary amino rest is discussed. Of the obtained products, some isothiourea salts **6** exhibit more than 2 orders of magnitude higher intensity of fluorescence, using 9-isothiocyanatoacridine as a standard. The obtained isothiourea hydroiodides **5** and dimethylisothiourea iodides **6** show remarkable biological activity against *Mycobacterium tuberculosis*.

Keywords: Acridines; Isothioureas; Fluorescence; PDQF COSY; INEPT; NOE; DFT; Ab initio calculations; Antimycobacterial activity; NMR spectroscopy.

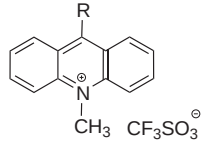
Acridine derivatives have been long known to interact with nucleic acids[1] and showing a large variety of biological properties[2–4]. Due to high fluorescence many of them have been utilized as sensitive biomarkers for investigation of specific behavior of biomolecules and natural products[5]. Continuing our recent research dealing with fluorescence of 9-acridinyl deriva-

tives[6,7], it was interesting to study quaternary acridinium salts as a new interesting class of fluorogenes. In the literature, only several examples of this type of compounds were described[8]. For example, 10-carboxymethylacridinium derivatives **1** [9,10], derivatives of 10-methylacridinium triflate **2a**–**2d** [11,12] and 9-(hydroxyimino)acridinium **3** [5] exhibit high chemiluminescence, which was used for the direct detection of basic sites in DNA.



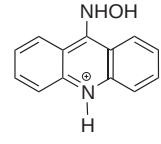
1a-1b

1a, R = CH_2COOH
1b, R = CH_2CH_2COOH



2a-2d

2a, R = H
2b, R = CH_3
2c, R = NCS
2d, R = NH(CS)OR



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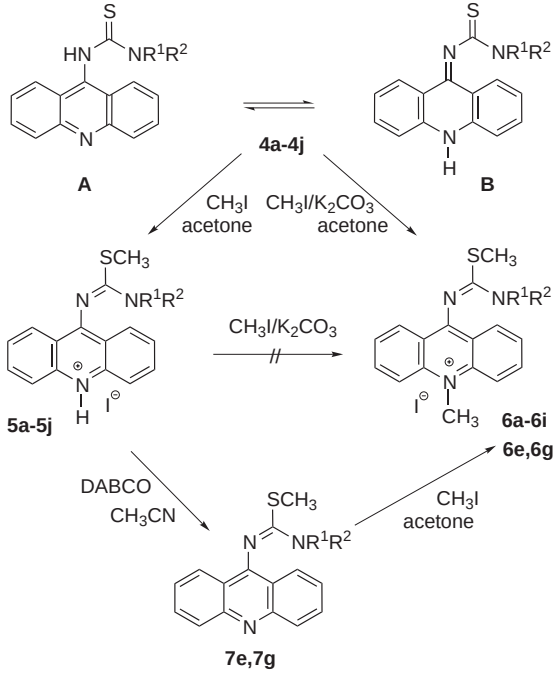
In our recent paper, we have found interesting fluorescence properties of *S*-methylisothiourea hydroiodides containing primary amino rests[13]. In the frame of this research we were interested in different S(N)-methylation reactions and structure of acridin-9-ylthioureas **4a**–**4j** with secondary amino rests (Scheme 1) owing to the influence of their substituents on the fluorescence and biological activity of the obtained products.

RESULTS AND DISCUSION

The starting 1,1-disubstituted 3-(acridin-9-yl)thioureas **4a**–**4j** were obtained by the reaction of secondary amines with 9-isothiocyanatoacridine[14]. By subsequent methylation with methyl iodide in acetone, the corresponding *S*-methylisothiourea hydroiodides **5a**–**5j** were formed. From the synthetic point of view, the most interesting was the different behavior of thioureas with primary[13] or secondary amino rests **4a**–**4i** when attempting to prepare *S*,*N*-dimethyl products. The acridinyl thioureas with secondary amino rests **4a**–**4i** were alkylated with an excess of methyl iodide in alkaline medium to give *S*,*N*-dimethylated products **6a**–**6i**, however, thioureas with primary amino rests surprisingly did not react under the same conditions. The direct N-methylation of all corresponding acridinium hydroiodides **5a**–**5i** in alkaline medium (K_2CO_3) with additional amount of methyl iodide to **6a**–**6i** was unsuccessful. The *S*,*N*-dimethyl derivatives (e.g. **6e**, **6g**) could be obtained also by methylation of the free bases of corresponding

isothioureas (e.g. **7e**, **7g**). The free bases **7e**, **7g** were obtained only by using a stronger base (1,4-diazabicyclo[2.2.2]octane, DABCO). This multiple step synthesis of *S*,*N*-dimethylacridinylisothioureas (**4** → **5** → **7** → **6**) is less convenient than the simple methylation of acridinylthioureas **4a**–**4i** with excess of methyl iodide in the presence of K_2CO_3. The course of these reactions is demonstrated in Scheme 1.

The obtained values of NMR spectra that confirm the structure of the synthesized compounds are given in the Experimental. The structure elucidation of compound **5g** was accomplished by application of conventional



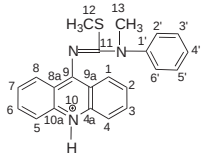
4-7	a	b	c	d	e	f	g
R^1	Me	Et	iPr	Pr	iPr	iBu	Me
R^2	cyclohexyl	cyclohexyl	cyclohexyl	Pr	iPr	iBu	Ph

4-7	h	i	j
R^1, R^2	-$CH(CH_3)(CH_2)_4$-	-$CH(CH_3)(CH_2)_3CH(CH_3)$-	-$(CH_2)_2O(CH_2)_2$-

SCHEME 1

1D and 2D NMR experiments (^{1}H and ^{13}C {^{1}H}, DEPT, selective INEPT, NOE difference, 2D DQF-COSY, CHDEC). ^{1}H chemical shifts and coupling constants were extracted from the 1D spectra using PERCH NMR software[15]. The ^{1}H NMR spectrum of this compound was characterized by the presence of singlets (δ 2.00 ppm, SCH_3; δ 3.55 ppm, NCH_3) and the AA′BB′C coupling system for the five protons of phenyl ring. The presence of the carbon atom of the C–SCH_3 group was evident in ^{13}C NMR spectra. The chemical shifts and coupling constants for compound **5g** are presented in Table I. Due to the equivalence of the corresponding protons and carbons of the two outer rings of the acridine skeleton, seven sp^2 hybridized carbons – four methines (CH) and three quaternary (C) carbons – were observed in ^{13}C NMR spectra. The presence of the proton on nitrogen atom of acridine was supported by a NOE difference experiment that showed dipolar couplings between the NH (δ 13.74 ppm) and H-4,5 (δ 7.92 ppm) protons with

TABLE I
NMR data (400 MHz, DMSO-d_6) for **5g**

Position	δ ^{1}H, ppm (multiplicity, J in Hz)[a]	
H-1,8	8.28 (ddd, $^3J_{\text{H-1(8) H-2(7)}}$ = 8.5, $^4J_{\text{H-1(8) H-3(6)}}$ = 1.4, $^5J_{\text{H-1(8) H-4(5)}}$ = 0.6)	
H-2,7	7.68 (ddd, $^3J_{\text{H-2(7) H-1(8)}}$ = 8.5, $^3J_{\text{H-2(7) H-3(6)}}$ = 6.8, $^4J_{\text{H-2(7) H-4(5)}}$ = 1.1)	
H-3,6	8.07 (ddd, $^3J_{\text{H-3(6) H-4(5)}}$ = 8.6, $^3J_{\text{H-3(6) H-2(7)}}$ = 6.8, $^4J_{\text{H-3(6) H-1(8)}}$ = 1.4)	
H-4,5	7.92 (ddd, $^3J_{\text{H-4(5) H-3(6)}}$ = 8.6, $^4J_{\text{H-4(5) H-2(7)}}$ = 1.1, $^5J_{\text{H-4(5) H-1(8)}}$ = 0.6)	
H-10	13.74 bs	
H-12	2.00 s	
H-13	3.55 s	
H-2′,6′	7.58 (dddd, AA′ part of AA′BB′C system, $^3J_{\text{H-2′ H-3′}}$ = $^3J_{\text{H-6′ H-5′}}$ = 7.9, $^4J_{\text{H-2′ H-6′}}$ = 2.2, $^4J_{\text{H-2′(6′) H-4′}}$ = 1.2, $^5J_{\text{H-2′ H-5′}}$ = $^5J_{\text{H-6′ H-3′}}$ = 0.5)	
H-3′,5′	7.45 (dddd, BB′ part of AA′BB′C system, $^3J_{\text{H-3′ H-2′}}$ = $^3J_{\text{H-5′ H-6′}}$ = 7.9, $^4J_{\text{H-3′ H-5′}}$ = 1.6, $^3J_{\text{H-3′(5′) H-4′}}$ = 7.5, $^5J_{\text{H-3′ H-6′}}$ = $^5J_{\text{H-5′ H-2′}}$ = 0.5)	
H-4′	7.40 (tt, C part of AA′BB′C system, $^3J_{\text{H-4′ H-3′(5′)}}$ = 7.5, $^4J_{\text{H-4′ H-2′(6′)}}$ = 1.2	

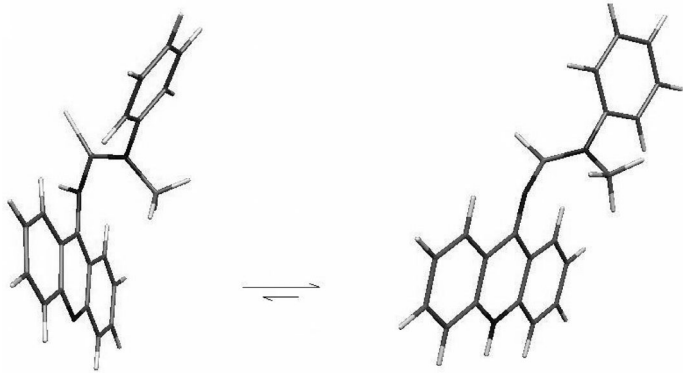
δ ^{13}C (ppm): 126.1 C-1,8; 127.8 C-2,7; 135.6 C-3,6; 118.7 C-4,5; 139.7 C-4a,10a; 115.4 C-8a,9a; 156.4 C-9; 167.2 C-11; 15.7 C-12; 42.1 C-13; 142.2 C-1′; 126.9 C-2′,6′; 129.6 C-3′,5′; 129.0 C-4′. [a] Spin analysis was performed using PERCH NMR software.

34% increase in the signal intensity. The chemical shifts observed for NH at 13.74 ppm, which is more downfield than usual, can be explained by the presence of positive charge on nitrogen atom (compound **5g** is an immonium salt). Irradiation of protons H-2′,6′ (δ 7.58 ppm) showed discernible enhancement of protons H-3′,5′ (δ 7.45 ppm) of the phenyl ring and of the methyl group on the nitrogen atom (δ 3.55 ppm). Selective INEPT and 2D (DQF-COSY, CHDEC) NMR experiments were necessary for complete assignment of chemical shifts of all carbons and protons of compound **5g**.

Some peculiar problems regarding the course of methylation reactions of acridinylthioureas **4** have been tried to explain by ab initio calculations of tautomeric forms, configuration, conformation and electronic structures of selected derivatives. Quantum chemical calculations B3LYP/6-31G(d,p)[16,17] were carried out for compounds **4g**, **7g** and two model compound of the type **7** (R^1 = H, R^2 = CH_3; R^1 = H, R^2 = CH_2CH_3), (Tables II, III). It follows from these results, that the most energetically stable rotamer for thiourea **4g** with secondary amino rest (R^1 = Me, R^2 = Ph) is the one with the sulfur atom of the thione group more distanced from the acridine skeleton. The acridine skeleton and the –NH(CS)NR^1R^2 grouping take uncrewed orientation of about 43 and 13° for aminoform **A** and iminoform **B**, respectively (Scheme 2, Table II). Iminoform **B** is more stable than aminoform **A** by 3.14 kcal mol^{-1} (Table III). Noteworthy, the iminoform was also confirmed by NMR experiments in similar 1-(acridin-9-yl)thiosemicarbazides[18].

Isothioureas with secondary amino rests (e.g. **7g**) occur in the *Z* configuration (Scheme 3), which is a little more stable than *E* configuration (0.64 kcal mol^{-1}, Table III).

This fact is in contrast to isothioureas with primary amino rests[13] for which the *E* configuration was found as optimal (R^1 = H, R^2 = Me, ΔE =

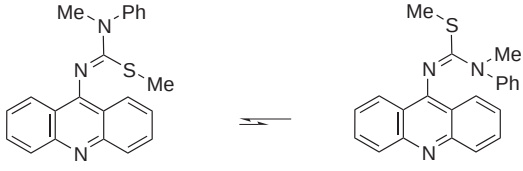


SCHEME 2

TABLE II
Selected structure parameters calculated by the B3LYP/6-31G(d,p) method (Schemes 2–4)

Structure	Valence angle, °				Dihedral angle, °		
	C9–N1–C	N1–C–N3	C–N3–Ph	C–N3–Me	C9a–C9–N1–C	C9–N1–C–N3	C9–N1–C–S
A	129.4	117.0	120.4	123.8	42.8	40	142.3
B	135.1	114.3	121.8	120.3	12.7	–108.1	78.0
C	131.6	119.3	123.2	118.1	124.4	159.3	–18.4
D	130.8	128.5	121.8	121.6	62.7	20.9	–162.6
E	122.1	125.9	121.8	126.1	62.7	–2.4	178.5
F	128.9	129.7	116.4[a]	125.9	66.2	15.0	–168.8
G	122.2	125.9	114.5[a]	126.6[b]	84.2	3.1	–178.1
H	128.7	129.8	116.4[a]	126.6[a]	65.0	15.7	–168.2

[a] Valence angle C–N3–H. [b] Valence angle C–N3–Et. Dihedral angles N1–C–N3–H for structures **E** and **G** are 6.4 and –9.37°, respectively (negative angle means reverse clockwise direction of rotation).



Z-configuration **C** ⇌ *E*-configuration **D**

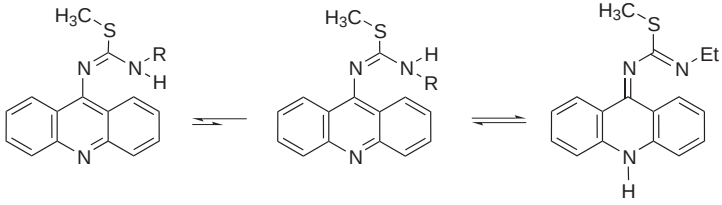
SCHEME 3

TABLE III
B3LYP/6-31 G(d,p) calculated energies of some molecular structures under study (Schemes 2–4)

Structure	X,Y			
	A, **B**	**C**, **D**	**E**, **F**	**G**, **H**
E_X, a.u.	–1373.1851225	–1412.2329351	–1181.2171185	–1220.5365802
E_Y, a.u.	–1373.1901221	–1412.2319157	–1181.2141526	–1220.533619
$\Delta E_{Y,X}$, a.u.	0.0049996	0.0010194	0.0029659	0.0029612
$\Delta E_{Y,X}$, kcal mol^{-1}	3.1372989	0.6396836	1.8611319	1.8581826

$\Delta E_{Y,X} = E_Y - E_X$.

3.49 kcal mol^{-1}) and can be ascribed to the stabilization of the isomers by intramolecular proton interaction of the N-3 with π-orbitals of the aromatic ring[19] (Scheme 4).



R = Me (**E**, **F**), Et (**G**, **H**)

SCHEME 4

The interaction for structures **E**, **F** and **G**, **H** is about 1.86 kcal mol^{-1} for both ones (Table III). We assume this is a reason for predominant *E* configuration of isothioureas with primary amino rest, which prevents the formation of *S*(*N*)-disubstituted products.

TABLE IV
Spectral characteristics of selected acridine thiourea derivatives **4b**, **4c**, **4e**, **4f**, **4g**, **4i**, **4j**

Compound	Absorbance		ε_{410nm}	Fluorescence[a]		Sensitivity	Efficiency
	λ_{max} nm	ε ml mol^{-1} cm^{-1}	ml mol^{-1} cm^{-1}	λ_{max}, nm (F/F_0)	*F*, a.u.	$F_{max} \times \varepsilon_{410\,nm}$	$F_{max}/\varepsilon_{410\,nm}$
4b	426.4	4.15	4.02	478 (7.43)	45.96	184.76	11.43
4c	438.8	13.44	9.33	505 (1.86)	11.5	107.30	1.23
4e	435.2	13.59	10.38	496 (1.44)	8.9	92.38	0.86
4f	434.0	12.31	9.99	510 (1.34)	8.3	82.92	0.83
4g	432.2	15.43	12.00	– (0)	0	–	–
4i	434.0	11.65	9.62	505 (1.97)	12.2	117.36	1.27
4j	432.2	17.07	14.02	518 (0.84)	5.20	72.9	0.37

[a] Fluorescence corresponds to emission of a compound at concentration 1.0×10^{-6} mol l^{-1} in acetonitrile. E_0 refers to maximum intensity fluorescence of 9-isothiocyanatoacridine at λ_{em} = 446 nm, at 1.0×10^{-6} mol l^{-1} in acetonitrile.

Electronic absorption spectra of the synthesized compounds **4**–**6** measured in acetonitrile exhibit a broad absorption band in the range 410–438 nm for **4**, 425–430 nm for **5** and 436–441 nm for **6** (Tables IV–VI, Figs 1, 2).

A significant increase in fluorescence has been observed for compounds **5**, **6** compared with starting thioureas **4** (Tables IV–VI). Acridinium salts of *S*(*N*)-dimethyl-isothioureas **6a**–**6i** exhibited the highest intensity of fluorescence (Table VI, Fig. 2) with the maximal value more than 2 orders of magnitude higher than 9-isothiocyanatoacridine used as a standard for relative fluorescence. It is well known that iodide is an efficient quencher of fluorescence[20]. Therefore, the observed higher fluorescence quantum yield of *S*(*N*)-dimethylisothiourea analogs might be caused by steric hindrance of *N*-methyl affecting the interaction of cationic nitrogen of acridine skeleton with iodide anion. This is not the case in the *S*-methylisothiourea analogs where iodide anions have free access to the acridine skeleton, which results in the quenching of their fluorescence. All derivatives with the *N*-methylanilino rest (**4g**, **5g**, **6g**) exhibit extremely low fluorescence, whereas the highest fluorescence is observed for dipropyl and diisobutyl derivatives **6d**

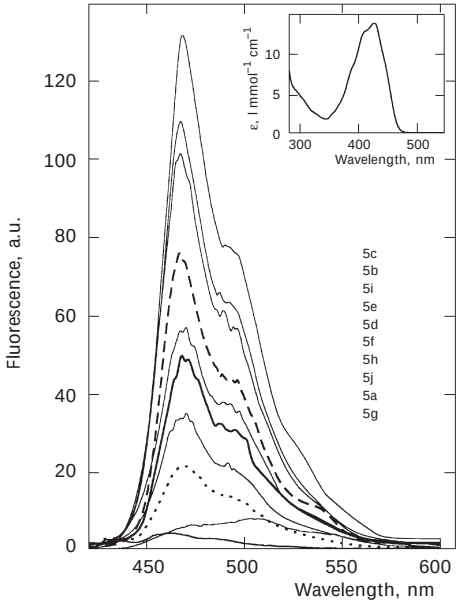


FIG. 1

Fluorescence emission spectra of *S*-methylisothiourea analogs **5a**–**5j** in concentration 1.0×10^{-6} mol l^{-1}. Inset: Representative electronic absorption spectrum of *S*-methylisothiourea analog **5d**

and **6f**. We assume that combinations of polar and steric effects are responsible for these differences.

The synthesized products were screened against *Mycobacterium tuberculosis*, eight of them (**5b**, **5c**, **5f**, **5i**, **6b**, **6c**, **6e**, **6f**) showing remarkable biological activity. The screening was carried out at the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF), Southern Research Institute, Birmingham (Alabama, U.S.A.).

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra (cm^{-1}) were measured on a Specord 75 IR spectrophotometer (Zeiss) in chloroform or KBr discs. NMR spectra of compound **5g** were acquired at 25 °C on a JEOL JNR-L-400 spectrometer operating at 399.78 MHz for ^1H NMR (internal tetramethylsilane, TMS_{int} = 0 ppm) and 100.54 MHz for ^{13}C NMR (TMS_{int} = 0 ppm) in hexadeuteriodimethyl sulfoxide. Spin analysis was performed using PERCH software[15] for the extraction of chemical shifts (ppm, δ-scale) and coupling constants (*J*, Hz). Spectra were produced by using standard pulse sequences. ^1H and ^{13}C NMR spectra of compounds **4e**, **5e**, **5h**, **5i**, **6b**, **6d**, **6f**, **6g**, **6h** were obtained on a Varian VXR spectrometer (300.131 MHz) at room temperature, other ^1H NMR spectra were taken on a Tesla BS 587A spectrometer (80 MHz). Quantum chemical calcula-

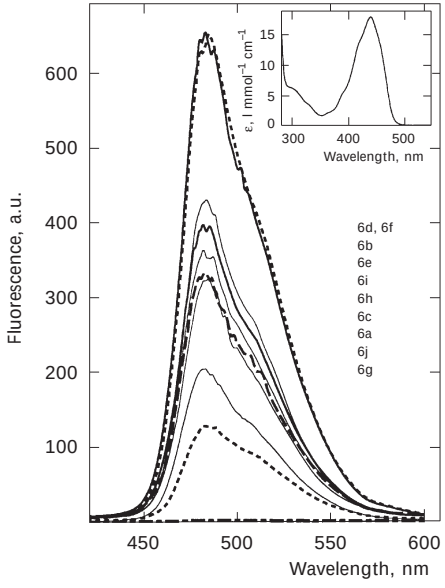


FIG. 2

Fluorescence emission spectra of *S,N*-dimethylisothiourea analogs **6a**–**6i** in concentration 1.0×10^{-6} mol l^{-1}. Inset: Representative electronic absoption spectrum of *S,N*-dimethylisothiourea analog **6d**

tions of totally optimized structures under study were carried out by non-empirical RHF method (using the 6-31G(d,p)[16] basis sets) and density functional theory[17]. Elemental analysis was performed on a Perkin–Elmer analyser CHN 2400. The reaction course was monitored by TLC Silufol plates (Kavalier®, Czech Republic). The preparative column chromatography (flash chromatography) was performed on the Kieselgel Merck Typ 9385, 230–400 mesh. Absorption spectra of acridin-9-yl derivatives were obtained using a UV-3000 Shimadzu spectrophotometer at concentrations 1.5–2.0 × 10^{-5} mol l^{-1} in acetonitrile. Absorption coefficients are expressed in $mmol^{-1}$ cm^{-1} (Figs 1, 2, Tables IV, V). Fluorescence measurements were performed on a Shimadzu RF-5000 spectrofluorometer. Emission spectra were recorded in the region 420–600 nm at the excitation wavelength. The obtained fluorescence spectra are averages of 3–6 successive scans at the excitation wavelength (410 nm). Fluorescence intensities expressed in arbitrary units [a.u.] (Figs 1, 2, Tables IV, V) correspond to emission of compounds at concentration 1.0 × 10^{-6} mol l^{-1} in acetonitrile. All measure-

TABLE V
Spectral characteristics of *S*-methylisothiourea analogs **5a**–**5j**

Compound	Absorbance		ε_{410nm}	Fluorescence[a]		Sensitivity	Efficiency
	λ_{max} nm	ε ml mol^{-1} cm^{-1}	ml mol^{-1} cm^{-1}	λ_{max}, nm (F/F_0)	F, a.u.	$F_{max} \times \varepsilon_{410\,nm}$	$F_{max}/\varepsilon_{410\,nm}$
5a	425.0	11.89	11.34	506 (1.30)	8.06	91.400	0.711
5b	429.5	16.01	14.18	468.8 (17.75)	109.7	1555.5	7.736
5c	430.5	16.27	13.43	470.4 (21.36)	132.0	1772.8	9.829
5d	428.7	13.70	12.48	470.0 (9.40)	58.1	725.1	4.655
5e	428.6	13.47	11.83	468.4 (12.40)	76.6	906.2	6.475
5f	428.6	14.69	13.42	470.4 (7.78)	48.1	645.5	3.584
5g	425.2	13.62	12.14	461.2 (0.74)	4.56	55.35	0.376
5h	429.4	14.27	13.36	469.2 (5.75)	35.5	474.2	2.657
5i	429.6	14.21	12.78	468.4 (16.41)	101.4	1295.9	7.934
5j	426.8	11.05	10.85	469.4 (3.43)	21.2	230.0	1.954

[a] Fluorescence corresponds to emission of a compound at concentration 1.0 × 10^{-6} mol l^{-1} in acetonitrile. E_0 refers to maximum intensity fluorescence of 9-isothiocyanatoacridine at λ_{em} = 446 nm, at 1.0 × 10^{-6} mol l^{-1} in acetonitrile.

ments were performed at 25 °C. F_0 refers to maximum intensity fluorescence of 9-isothiocyanatoacridine at λ_{em} = 446 nm under the same conditions.

The synthesis of 3-(acridin-9-yl)-1-alkyl/arylthioureas **4a**, **4d**, **4g**, **4j** was described in our previous paper[21]. The same method utilizing the reaction of 9-isothiocyanatoacridine[14] with secondary amines was used also for the synthesis of thioureas **4b**, **4c**, **4e**, **4f**, **4h**, **4i**, **4j** in this work.

3-(Acridin-9-yl)-1-cyclohexyl-1-ethylthiourea (**4b**): Yield 71%, m.p. 191–193 °C. For $C_{22}H_{25}N_3S$ (363.5) calculated: 72.69% C, 6.93% H, 11.56% N; found: 72.41% C, 6.81% H, 11.19% N. IR (KBr): 1620 (N=C); 1588 (NCS). ^{1}H NMR ($CDCl_3$ and $(CD_3)_2SO$, 3:1): 11.25 s, 1 H (NH); 8.20 d, 2 H, *J* = 8.0, 7.70–7.29 m, 4 H, 7.13 m, 2 H (Acr); 4.02 m, 1 H (CH (cyclohexyl)); 3.45 m, 2 H (CH_2 (Et)); 2.25–1.20 m, 10 H (5 × CH_2 (cyclohexyl)); 1.10 m, 3 H (CH_3 (Et)).

3-(Acridin-9-yl)-1-cyclohexyl-1-isopropylthiourea (**4c**): Yield 83%, m.p. 190–192 °C. For $C_{23}H_{27}N_3S$ (377.5) calculated: 73.17% C, 7.21% H, 11.13% N; found: 73.45% C, 7.33% H, 11.34% N. IR (KBr): 1620 (N=C); 1575 (NCS). ^{1}H NMR ($CDCl_3$ and $(CD_3)_2SO$, 5:1): 11.32 s,

TABLE VI
Spectral characteristics of *S*(*N*)-dimethylisothiourea analogs **6a**–**6j**

Compound	Absorbance		ε_{410nm} ml mol^{-1} cm^{-1}	Fluorescence[a]		Sensitivity $F_{max} \times \varepsilon_{410\,nm}$	Efficiency $F_{max}/\varepsilon_{410\,nm}$
	λ_{max} nm	ε ml mol^{-1} cm^{-1}		λ_{max}, nm (F/F_0)	*F*, a.u.		
6a	441.0	13.77	8.15	482.8 (32.98)	203.8	1661.0	25.006
6b	441.0	17.86	10.66	483.6 (69.92)	432.0	4605.1	40.525
6c	441.0	18.67	10.04	484.4 (52.41)	323.8	3250.9	32.251
6d	441.0	17.80	10.11	484.0 (106.4)	657.2	6644.3	65.005
6e	441.0	15.76	8.85	483.6 (64.6)	399.3	3533.8	45.119
6f	441.0	17.68	9.66	485.6 (105.7)	653.1	6308.9	67.609
6g	436.6	14.74	9.68	483.6 (0.24)	1.50	14.52	0.155
6h	441.0	18.49	10.85	482.8 (53.65)	331.5	3596.8	30.553
6i	441.0	17.58	10.66	483.6 (58.85)	363.6	3876.0	34.109

[a] Fluorescence corresponds to emission of a compound at concentration 1.0 × 10^{-6} mol l^{-1} in acetonitrile. E_0 refers to maximum intensity fluorescence of 9-isothiocyanatoacridine at λ_{em} = 446 nm, at 1.0 × 10^{-6} mol l^{-1} in acetonitrile.

1 H (NH); 8.22 m, 2 H, 7.52–7.30 m, 4 H, 7.17 m, 2 H (Acr); 6.10–5.30 m, 1 H (CH (iPr)); 3.90 m, 1 H (CH (cyclohexyl)); 2.25–1.00 m, 16 H (5 × CH_2 (cyclohexyl), 2 × CH_3 (iPr)).

3-(Acridin-9-yl)-1,1-diisopropylthiourea (**4e**): Yield 91%, m.p. 180–182 °C. For $C_{20}H_{23}N_3S$ (337.5) calculated: 71.18% C, 6.87% H, 12.45% N; found: 71.41% C, 6.64% H, 12.30% N. IR ($CHCl_3$): 1625 (N=C); 1587 (NCS). ^{1}H NMR ($CDCl_3$): 10.83 s, 1 H (NH); 7.91 d, 2 H, *J* = 7.2, 7.18–6.72 m, 6 H (Acr); 6.03 m, 1 H (CH (iPr)); 3.94 m, 1 H (CH (iPr)); 1.45 d, 6 H, *J* = 7.0 (2 × CH_3 (iPr)); 1.43 d, 6 H, *J* = 7.0 (2 × CH_3 (iPr)).

3-(Acridin-9-yl)-1,1-diisobutylthiourea (**4f**): Yield 88%, m.p. 206–211 °C. For $C_{22}H_{27}N_3S$ (365.5) calculated: 72.29% C, 7.44% H, 11.50% N; found: 72.57% C, 7.72% H, 11.13% N. IR ($CHCl_3$): 1625 (N=C); 1588 (NCS). ^{1}H NMR ($CDCl_3$): 10.80 s, 1 H (NH); 7.85 d, 2 H, *J* = 7.8, 7.10–6.70 m, 6 H (Acr); 3.95 d, 2 H, *J* = 7.4 (NCH_2 (iBu)); 3.43 d, 2 H, *J* = 7.4 (NCH_2 (iBu)); 2.70 m, 1 H (CH (iBu)); 2.08 m, 1 H (CH (iBu)); 1.15 d, 6 H, *J* = 6.6 (2 × CH_3 (iBu)); 0.90 d, 6 H, *J* = 6.6 (2 × CH_3 (iBu)).

3-(Acridin-9-yl)-1-(2-methylpiperidino)thiourea (**4h**): Yield 82%, m.p. 200–205 °C. For $C_{20}H_{21}N_3S$ (335.5) calculated: 71.61% C, 6.31% H, 12.53% N; found: 70.78% C, 6.14% H, 11.84% N. IR ($CHCl_3$): 1625 (N=C); 1581 (NCS). ^{1}H NMR ($CDCl_3$): 10.83 s, 1 H (NH); 7.90 m, 2 H, 7.20–6.55 m, 6 H (Acr); 6.12–5.24 m, 1 H, 5.00–4.00 m, 1 H, 3.63–2.85 m, 1 H (NCH, NCH_2 (piperidine)); 2.30–1.40 m, 6 H (3 × CH_2 (piperidine)); 1.40 m, 3 H (CH_3).

3-(Acridin-9-yl)-1-(2,6-dimethylpiperidino)thiourea (**4i**): Yield 91%, m.p. 195–198 °C. For $C_{21}H_{23}N_3S$ (349.5) calculated: 72.17% C, 6.63% H, 12.02% N; found: 72.39% C, 6.85% H, 12.37% N. IR ($CHCl_3$): 1623 (N=C); 1584 (NCS). ^{1}H NMR ($CDCl_3$ and $(CD_3)_2SO$, 1:1): 11.19 s, 1 H (NH); 8.23 d, 2 H, *J* = 8.2, 7.73–7.30 m, 4 H, 7.15 m, 2 H (Acr); 5.80 m, 1 H (NCH (piperidine)); 4.55 m, 1 H (NCH (piperidine)); 2.18–1.40 m, 6 H (3 × CH_2 (piperidine)); 1.43 d, 3 H, *J* = 7.4 (CH_3); 1.23 d, 3 H, *J* = 7.4 (CH_3).

3-(Acridin-9-yl)-1,1-morpholinothiourea (**4j**)[21]: Yield 92%, m.p. 230–235 °C. IR ($CHCl_3$): 1633 (N=C); 1567 (NCS). ^{1}H NMR ($CDCl_3$ and $(CD_3)_2SO$, 1:1): 11.45 bs, 1 H (NH); 8.20 d, 2 H, *J* = 8.2, 7.77–7.33 m, 4 H, 7.17 m, 2 H (Acr); 4.50–3.50 m, 8 H (4 × CH_2 (morpholine)).

Preparation of 3-(Acridin-9-yl)-1,1-alkyl/aryl-2-methylisothiourea Hydroiodides **5a**–**5j**. General Method

The suspension of thioureas **4a**–**4j** (1 mmol) and methyl iodide (1.5 mmol) in acetone (15 ml) was stired at room temperature. After stirring 30 min, the reaction mixture became clear indicating the end of the reaction. The reaction course was followed by TLC (benzene/acetone, 5:2). The obtained solution was concentrated in vacuo, the product was precipitated by addition of ether, filtered off, washed with ether and dried.

3-(Acridin-9-yl)-1-cyclohexyl-1,2-dimethylisothiourea hydroiodide (**5a**): Yield 84%, m.p. 217–219 °C. For $C_{22}H_{26}IN_3S$ (491.4) calculated: 53.77% C, 5.33% H, 8.55% N; found: 53.49% C, 5.10% H, 8.38% N. IR (KBr): 1620 (C=N); 1565 (NCS). ^{1}H NMR ($CDCl_3$): 13.50 bs, 1 H (NH); 8.69 d, 2 H, *J* = 8.4, 8.13–7.60 m, 4 H, 7.40 m, 2 H (Acr); 4.16 m, 1 H (CH (cyclohexyl)); 3.20 s, 3 H (NCH_3); 2.15–0.88 m, 10 H (5 × CH_2 (cyclohexyl)); 1.98 s, 3 H (SCH_3).

3-(Acridin-9-yl)-1-cyclohexyl-1-ethyl-2-methylisothiourea hydroiodide (**5b**): Yield 87%, m.p. 197–200 °C. For $C_{23}H_{28}IN_3S$ (505.4) calculated: 54.65% C, 5.58% H, 8.31% N; found: 54.88% C, 5.81% H, 8.54% N. IR (KBr): 1617 (C=N); 1540 (NCS). ^{1}H NMR ($CDCl_3$): 8.65 d, 2 H, *J* = 8.5, 8.00 d, 2 H, *J* = 8.8, 7.80 m, 2 H, 7.44 m, 2 H (Acr); 4.12 m, 1 H (NCH (cyclohexyl)); 3.70 q, 2 H, *J* = 7.7 (NCH_2 (Et)); 2.30–1.20 m, 10 H (5 × CH_2 (cyclohexyl)); 1.92 s, 3 H (SCH_3); 1.35 t, 3 H, *J* = 7.7 (CH_3 (Et)).

3-(Acridin-9-yl)-1-cyclohexyl-1-isopropyl-2-methylisothiourea hydroiodide (**5c**): Yield 85%, m.p. 210–214 °C. For $C_{24}H_{30}IN_3S$ (519.5) calculated: 55.49% C, 5.82% H, 8.09% N; found: 55.79% C, 6.11% H, 8.22% N. IR (KBr): 1622 (C=N); 1566 (NCS). ^{1}H NMR ($CDCl_3$): 8.69 d, 2 H, *J* = 8.5, 8.00 d, 2 H, *J* = 8.4, 7.80 m, 2 H, 7.42 m, 2 H (Acr); 4.63–3.32 m, 2 H (NCH (cyclohexyl), NCH (iPr)); 2.75–1.20 m, 10 H (5 × CH_2 (cyclohexyl)); 1.84 s, 3 H (SCH_3); 1.46 d, 6 H, *J* = 7.0 (2 × CH_3 (iPr)).

3-(Acridin-9-yl)-1,1-dipropyl-2-methylisothiourea hydroiodide (**5d**): Yield 82%, m.p. 178–184 °C. For $C_{21}H_{26}IN_3S$ (479.4) calculated: 52.61% C, 5.47% H, 8.76% N; found: 52.85% C, 5.63% H, 8.98% N. IR (KBr): 1620 (C=N); 1567 (NCS). ^{1}H NMR ($CDCl_3$): 13.60 bs, 1 H (NH); 8.68 d, 2 H, *J* = 8.7, 8.03 d, 2 H, *J* = 8.8, 7.82 m, 2 H, 7.44 m, 2 H (Acr); 3.65 t, 4 H, *J* = 7.7 (2 × NCH_2 (Pr)); 2.25–1.38 m, 4 H (2 × CH_2 (Pr)); 1.90 s, 3 H (SCH_3); 1.03 t, 6 H, *J* = 7.4 (2 × CH_3 (Pr)).

3-(Acridin-9-yl)-1,1-diisopropyl-2-methylisothiourea hydroiodide (**5e**): Yield 88%, m.p. 220–223 °C. For $C_{21}H_{26}IN_3S$ (479.4) calculated: 52.61% C, 5.47% H, 8.76% N; found: 52.85% C, 5.71% H, 8.93% N. IR (KBr): 1626 (C=N); 1565 (NCS). ^{1}H NMR ($CDCl_3$): 8.66 d, 2 H, *J* = 8.5, 8.00 d, 2 H, *J* = 8.8, 7.80 m, 2 H, 7.43 m, 2 H (Acr); 4.73–3.85 m, 2 H (2 × NCH (iPr)); 1.85 s, 3 H (SCH_3); 1.48 d, 12 H, *J* = 6.6 (4 × CH_3 (iPr)). ^{1}H NMR ((CD_3)$_2$SO): 13.34 s, 1 H (NH); 8.08 dd, 2 H, *J* = 8.7, 1.5 (H-1, H-8 (Acr)); 7.99 ddd, 2 H, *J* = 8.1, 6.6, 1.5 (H-3, H-6 (Acr)); 7.85 dd, 2 H, *J* = 8.1, 1.1 (H-4, H-5 (Acr)); 7.58 ddd, 2 H, *J* = 8.7, 6.6, 1.1 (H-2, H-7 (Acr)); 4.75–4.20 m, 2 H (2 × NCH (iPr)); 1.92 s, 3 H (SCH_3); 1.39 m, 12 H (4 × CH_3 (iPr)). ^{13}C NMR ((CD_3)$_2$SO): 165.6 (N=C); 152.7 (C-9); 140.0 (C-4a, C-10a); 135.2 (C-3, C-6); 126.3 (C-1, C-8); 124.2 (C-2, C-7); 118.7 (C-4, C-5); 114.9 (C-8a, C-9a); 55.0 (CH (iPr)); 51.0 (CH (iPr)); 19.7 (4 × CH_3 (iPr)); 15.4 (SCH_3).

3-(Acridin-9-yl)-1,1-diisobutyl-2-methylisothiourea hydroiodide (**5f**): Yield 79%, m.p. 192–197 °C. For $C_{23}H_{30}IN_3S$ (507.5) calculated: 54.44% C, 5.96% H, 8.28% N; found: 54.70% C, 6.22% H, 8.54% N. IR (KBr): 1630 (C=N); 1545 (NCS). ^{1}H NMR ($CDCl_3$): 8.75 d, 2 H, *J* = 8.3, 8.02 m, 2 H, 7.87 m, 2 H, 7.45 m, 2 H (Acr); 3.54 d, 4 H, *J* = 7.5 (2 × NCH_2 (iBu)); 2.25 m, 2 H (2 × CH (iBu)); 1.92 s, 3 H (SCH_3); 1.08 d, 12 H, *J* = 7.0 (4 × CH_3 (iBu)).

3-(Acridin-9-yl)-1,2-dimethyl-1-phenylisothiourea hydroiodide (**5g**): Yield 89%, m.p. 210–215 °C. For $C_{22}H_{20}IN_3S$ (485.4) calculated: 54.44% C, 4.15% H, 8.66% N; found: 54.75% C, 4.46% H, 8.91% N. IR (KBr): 1613 (C=N); 1547 (NCS). The values of ^{1}H and ^{13}C NMR are given in Table I.

1-(Acridin-9-yl)-2-methyl-3-(2-methylpiperidino)isothiourea hydroiodide (**5h**): Yield 81%, m.p. 210–216 °C. For $C_{21}H_{24}IN_3S$ (477.4) calculated: 52.83% C, 5.07% H, 8.80% N; found: 53.04% C, 5.38% H, 8.96% N. IR (KBr): 1625 (C=N); 1540 (NCS). ^{1}H NMR ($CDCl_3$): 13.45 s, 1 H (NH); 8.63 d, 2 H, *J* = 8.5, 8.17–7.60 m, 4 H, 7.42 m, 2 H (Acr); 4.65 m, 1 H, 4.15 m, 1 H, 3.40 m, 1 H (NCH, NCH_2 (piperidine)); 2.20–1.55 m, 6 H (3 × CH_2 (piperidine)); 2.00 s, 3 H (SCH_3); 1.44 d, 3 H, *J* = 6.4 (CH_3). ^{13}C NMR ((CD_3)$_2$SO): 166.6 (N=C); 153.4 (C-9); 138.8 (C-4a, C-10a); 133.6 (C-3, C-6); 124.6 and 124.4 (C-1, C-8); 122.8 (C-2, C-7); 117.5 (C-4, C-5); 114.0 (C-8a, C-9a); 51.5 (C-2 (piperidine)); 42.8 (C-6 (piperidine)); 28.6 and 23.9 and 16.6 (C-3, C-4, C-5 (piperidine)); 14.5 and 14.2 (CH_3, SCH_3).

1-(Acridin-9-yl)-3-(2,6-dimethylpiperidino)-2-methylisothiourea hydroiodide (**5i**): Yield 86%, m.p. 188–191 °C. For $C_{22}H_{26}IN_3S$ (491.4) calculated: 53.77% C, 5.33% H, 8.55% N; found: 53.98% C, 5.54% H, 8.71% N. IR (KBr): 1620 (C=N); 1545 (NCS). ^{1}H NMR ($CDCl_3$): 8.65 d, 2 H, *J* = 8.2, 8.03 d, 2 H, *J* = 8.3, 7.80 m, 2 H, 7.42 m, 2 H (Acr); 4.60 m, 2 H (2 × CH (piperidine)); 2.18 s, 3 H (SCH_3); 2.05–1.60 m, 6 H (3 × CH_2 (piperidine)); 1.50 d, 6 H, *J* = 7.0 (2 × CH_3). ^{1}H NMR ((CD_3)$_2$SO): 13.23 s, 1 H (NH); 8.09 d, 2 H, *J* = 8.7 (H-1, H-8);

7.97 m, 2 H (H-3, H-6); 7.82 d, 2 H, *J* = 8.1 (H-4, H-5); 7.55 m, 2 H (H-2, H-7); 4.51 m, 2 H (2 × CH (piperidine)); 2.01 s, 3 H (SCH_3); 2.00–1.50 m, 6 H (3 × CH_2 (piperidine)); 1.40 m, 6 H (2 × CH_3 (piperidine)). ^{13}C NMR ($(CD_3)_2SO$): 168.6 (N=C); 153.1 (C-9); 139.9 (C-4a, C-10a); 135.1 (C-3, C-6); 125.9 (C-1, C-8); 124.1 (C-2, C-7); 118.6 (C-4, C-5); 114.9 (C-8a, C-9a); 52.1 (2 × CH (piperidine)); 29.5 (2 × CH_2 (piperidine)); 19.4 (2 × CH_3); 15.4 (SCH_3); 12.9 (CH_2 (piperidine)).

1-(Acridin-9-yl)-2-methyl-3-morpholinoisothiourea hydroiodide (**5j**): Yield 75%, m.p. 200–203 °C. For $C_{19}H_{20}IN_3OS$ (465.4) calculated: 49.04% C, 4.33% H, 9.03% N; found: 49.38% C, 4.71% H, 9.36% N. IR (KBr): 1624 (C=N); 1543 (NCS). ^1H NMR ($CDCl_3$): 13.87 s, 1 H (NH); 8.67 d, 2 H, *J* = 8.6, 8.06 d, 2 H, *J* = 8.7, 7.81 m, 2 H, 7.50 m, 2 H (Acr); 3.87 bs, 8 H (4 × CH_2 (morpholine)); 1.93 s, 3 H (SCH_3).

Preparation of 1,1-Disubstituted 2-Methyl-3-(10-methylacridin-9-yl)isothiourea Iodides (**6a**–**6i**). General Method

Method A: To a suspension of thiourea **5a**–**5i** (0.4 mmol) in acetone (15 ml), methyl iodide (3 ml) and K_2CO_3 (110 mg, 0.8 mmol) were added and the reaction mixture was stirred at room temperature for 24 h. Acetone was evaporated to dryness, the crude residue was extracted with acetonitrile and the excess of K_2CO_3 was filtered off. After addition of ether to a concentrated solution of the crude compound in acetonitrile, the product precipitated in the form of yellow crystals.

Method B: To a solution of *S*-methylisothiourea **7e**, **7g** (1 mmol) in acetone (20 ml), methyl iodide (1.5 mmol) was added and stirred at room temperature for 12 h. The solvent with excess of methyl iodide was evaporated. By addition of ether to the evaporated residue the final products **6e**, **6g** were obtained in the crystalline form.

1-Cyclohexyl-1,2-dimethyl-3-(10-methylacridin-9-yl)isothiourea iodide (**6a**): Yield 81%, m.p. 185–190 °C. For $C_{23}H_{28}IN_3S$ (505.5) calculated: 54.65% C, 5.58% H, 8.31% N; found: 54.92% C, 5.85% H, 8.57% N. IR (KBr): 1625 (C=N); 1575 (NCS). ^1H NMR ($CDCl_3$): 8.30–7.85 m, 6 H, 7.56 ddd, 2 H, *J* = 8.1, 5.0, 2.9 (Acr); 4.36 s, 3 H (N^+CH_3); 4.12 m, 1 H (CH (cyclohexyl)); 3.25 bs, 3 H (NCH_3); 2.25–1.10 m, 10 H (5 × CH_2 (cyclohexyl)); 2.08 s, 3 H (SCH_3).

1-Cyclohexyl-1-ethyl-2-methyl-3-(10-methylacridin-9-yl)isothiourea iodide (**6b**): Yield 88%, m.p. 192–197 °C. For $C_{24}H_{30}IN_3S$ (519.5) calculated: 55.49% C, 5.82% H, 8.09% N; found: 55.21% C, 5.43% H, 7.87% N. IR (KBr): 1600 (C=N); 1540 (NCS). ^1H NMR ($(CD_3)_2SO$): 8.21 d, 2 H, *J* = 9.0 (H-4, H-5 (Acr)); 8.16 d, 2 H, *J* = 8.7 (H-1, H-8 (Acr)); 8.07 m, 2 H (H-3, H-6 (Acr)); 7.62 m, 2 H (H-2, H-7 (Acr)); 4.22 s, 3 H (N^+CH_3); 4.08 m, 1 H (NCH (cyclohexyl)); 3.90–3.50 m, 2 H (NCH_2 (Et)); 2.10–1.20 m, 10 H (5 × CH_2 (cyclohexyl)); 1.95 s, 3 H (SCH_3); 1.09 m, 3 H (CH_3 (Et)). ^{13}C NMR ($(CD_3)_2SO$): 168.4 (N=C); 153.4 (C-9); 141.4 (C-4a, C-10a); 135.7 (C-3, C-6); 126.7 (C-1, C-8); 124.0 (C-2, C-7); 117.7 (C-4, C-5); 116.1 (C-8a, C-9a); 61.3 (C-1 (cyclohexyl); 42.4 (NCH_2); 35.5 (N^+CH_3); 29.4 (C-2, C-6 (cyclohexyl)); 25.1 and 25.0 and 24.3 (C-3, C-4, C-5 (cyclohexyl)); 15.3 and 14.1 (SCH_3, CH_3).

1-Cyclohexyl-1-isopropyl-2-methyl-3-(10-methylacridin-9-yl)isothiourea iodide (**6c**): Yield 79%, m.p. 212–217 °C. For $C_{25}H_{32}IN_3S$ (533.5) calculated: 56.28% C, 6.05% H, 7.88% N; found: 56.62% C, 6.39% H, 7.49% N. IR (KBr): 1610 (C=N); 1547 (NCS). ^1H NMR ($CDCl_3$): 8.30–7.85 m, 6 H, 7.59 m, 2 H (Acr); 4.80–3.33 m, 2 H (NCH (cyclohexyl), NCH (iPr)); 4.37 s, 3 H (N^+CH_3); 1.91 s, 3 H (SCH_3); 1.45 d, 6 H, *J* = 6.6 (2 × CH_3 (iPr)); 2.45–0.75 m, 10 H (5 × CH_2 (cyclohexyl)).

2-Methyl-3-(10-methylacridin-9-yl)-1,1-dipropylisothiourea iodide (**6d**): Yield 84%, m.p. 169–172 °C. For $C_{22}H_{28}IN_3S$ (493.4) calculated: 53.55% C, 5.72% H, 8.52% N; found: 53.27% C, 5.51% H, 8.48% N. IR (KBr): 1610 (C=N); 1580 (NCS). ^{1}H NMR ($(CD_3)_2SO$): 8.22 d, 2 H, *J* = 8.7 (H-4, H-5 (Acr)); 8.16 d, 2 H, *J* = 8.4 (H-1, H-8 (Acr)); 8.07 m, 2 H (H-3, H-6 (Acr)); 7.62 m, 2 H (H-2, H-7 (Acr)); 4.23 s, 3 H (N^+CH_3); 3.71 m, 2 H (NCH_2 (Pr)); 3.57 m, 2 H (NCH_2 (Pr)); 1.94 s, 3 H (SCH_3); 1.90 m, 2 H (CH_2 (Pr)); 1.64 m, 2 H (CH_2 (Pr)); 1.02 m, 3 H (CH_3 (Pr)); 0.81 m, 3 H (CH_3 (Pr)). ^{1}H NMR ($CDCl_3$): 8.27–7.96 m, 6 H, 7.56 m, 2 H (Acr); 4.35 s, 3 H (N^+CH_3); 3.67 m, 4 H (2 × NCH_2 (Pr)); 1.96 s, 3 H (SCH_3); 1.84 m, 4 H (2 × CH_2 (Pr); 1.02 m, 6 H (2 × CH_3 (Pr)). ^{13}C NMR ($(CD_3)_2SO$): 168.8 (N=C); 154.2 (C-9); 141.4 (C-4a, C-10a); 135.7 (C-3, C-6); 126.7 (C-1, C-8); 124.00 (C-2, C-7); 117.7 (C-4, C-5); 116.1 (C-8a, C-9a); 53.7 (NCH_2 (Pr)); 52.9 (NCH_2 (Pr)); 35.6 (N^+CH_3); 20.5 (CH_2 (Pr)); 20.3 (CH_2 (Pr)); 15.3 (SCH_3); 11.0 (2 × CH_3 (Pr)). ^{13}C NMR ($CDCl_3$): 168.8 (N=C); 154.9 (C-9); 141.7 (C-4a, C-10a); 136.4 (C-3, C-6); 126.9 (C-1, C-8); 124.5 (C-2, C-7); 117.6 (C-4, C-5); 116.6 (C-8a, C-9a); 54.4 (NCH_2 (Pr)); 53.7 (NCH_2 (Pr)); 36.9 (N^+CH_3); 21.0 (2 × CH_2 (Pr)); 16.1 (SCH_3); 11.3 (2 × CH_3 (Pr)).

1,1-Diisopropyl-2-methyl-3-(10-methylacridin-9-yl)isothiourea iodide (**6e**): Yield 79%, m.p. 207–210 °C. For $C_{22}H_{28}IN_3S$ (493.4) calculated: 53.55% C, 5.72% H, 8.52% N; found: 53.12% C, 5.32% H, 8.39% N. IR ($CHCl_3$): 1620 (C=N); 1566 (NCS). ^{1}H NMR ($CDCl_3$): 8.30–7.90 m, 6 H, 7.59 m, 2 H (Acr); 4.75–4.00 m, 2 H (2 × NCH (iPr)); 4.42 s, 3 H (N^+CH_3); 1.98 s, 3 H (SCH_3); 1.49 d, 12 H, *J* = 7.0 (4 × CH_3 (iPr)).

1,1-Diisobutyl-2-methyl-3-(10-methylacridin-9-yl)isothiourea iodide (**6f**): Yield 80%, m.p. 190–196 °C. For $C_{24}H_{32}IN_3S$ (521.5) calculated: 55.28% C, 6.18% H, 8.06% N; found: 55.57% C, 6.43% H, 8.23% N. IR (KBr): 1605 (C=N); 1565 (NCS). ^{1}H NMR ($CDCl_3$): 8.30–7.85 m, 6 H, 7.53 m, 2 H (Acr); 4.46 s, 3 H (N^+CH_3); 3.51 m, 4 H (2 × NCH_2 (iBu)); 2.70–1.63 m, 2 H (2 × CH (iBu)); 2.01 s, 3 H (SCH_3); 1.05 m, 12 H (4 × CH_3 (iBu)). ^{13}C NMR ($CDCl_3$): 169.2 (N=C); 154.9 (C-9); 141.8 (C-4a, C-10a); 136.6 (C-3, C-6); 126.8 (C-1, C-8); 124.5 (C-2, C-7); 117.9 (C-4, C-5); 116.5 (C-8a, C-9a); 60.2 (NCH_2 (iBu)); 59.4 (NCH_2 (iBu)); 37.2 (N^+CH_3); 27.7 (CH (iBu)); 27.1 (CH (iBu)); 20.4 (4 × CH_3 (iBu)); 16.5 (SCH_3).

1,2-Dimethyl-3-(10-methylacridin-9-yl)-1-phenylisothiurea iodide (**6g**): Yield 88%, m.p. 195–200 °C. For $C_{23}H_{22}IN_3S$ (499.4) calculated: 55.31% C, 4.44% H, 8.41% N; found: 55.68% C, 4.75% H, 8.55% N. IR ($CHCl_3$): 1624 (C=N); 1567 (NCS). ^{1}H NMR ($CDCl_3$): 8.27 dd, 2 H, *J* = 8.4, 1.6 (H-1, H-8 (Acr)); 8.17 dd, 2 H, *J* = 8.1, 1.2 (H-4, H-5 (Acr)); 8.11 ddd, 2 H, *J* = 8.1, 6.6, 1.6 (H-3, H-6 (Acr)); 7.70 ddd, 2 H, *J* = 8.4, 6.6, 1.2 (H-2, H-7 (Acr)); 7.32 m, 5 H (Ph); 4.44 s, 3 H (N^+CH_3); 3.58 s, 3 H (NCH_3); 2.15 s, 3 H (SCH_3). ^{1}H NMR ($(CD_3)_2SO$): 8.33 m, 2 H (H-1, H-8 (Acr)); 8.27 m, 2 H (H-4, H-5 (Acr)); 8.14 m, 2 H (H-3, H-6 (Acr)); 7.73 m, 2 H (H-2, H-7 (Acr)); 7.57 m, 2 H (Ph); 7.44 m, 3 H (Ph); 4.28 s, 3 H (N^+CH_3); 3.54 s, 3 H (NCH_3); 1.97 s, 3 H (SCH_3). ^{13}C NMR ($CDCl_3$): 168.3 (N=C); 158.0 (C-9); 142.0 (C-1′ (Ph)); 141.5 (C-4a, C-10a); 136.9 (C-3, C-6); 130.0 (C-3′, C-5′ (Ph)); 129.4 (C-4′ (Ph)); 127.1 (C-1, C-8); 126.9 (C-2′, C-6′(Ph)); 125.2 (C-2, C-7); 117.8 (C-4, C-5); 117.1 (C-8a, C-9a); 42.8 (NCH_3); 37.4 (N^+CH_3); 16.6 (SCH_3). ^{13}C NMR ($(CD_3)_2SO$): 167.6 (N=C); 156.6 (C-9); 142.2 (C-1′ (Ph)); 141.3 (C-4a, C-10a); 136.3 (C-3, C-6); 129.8 (C-3′, C-5′ (Ph)); 129.2 (C-4′ (Ph)); 127.1 (C-1, C-8); 127.0 (C-2′, C-6′(Ph)); 124.7 (C-2, C-7); 117.8 (C-4, C-5); 116.4 (C-8a, C-9a); 42.2 (NCH_3); 35.9 (N^+CH_3); 15.8 (SCH_3).

2-Methyl-1-(10-methylacridin-9-yl)-3-(2-methylpiperidino)isothiourea iodide (**6h**): Yield 78%, m.p. 205–208 °C. For $C_{22}H_{26}IN_3S$ (491.4) calculated: 53.77% C, 5.33% H, 8.55% N; found: 53.46% C, 5.19% H, 8.45% N. IR ($CHCl_3$): 1603 (C=N); 1560 (NCS). ^{1}H NMR ($CDCl_3$): 8.27–7.80 m, 6 H, 7.60 m, 2 H (Acr); 4.60 m, 1 H and 4.12 m, 1 H and 3.48 m, 1 H (NCH,

NCH_2 (piperidine)); 4.35 s, 3 H (N^+CH_3); 2.10 s, 3 H (SCH_3); 2.00–1.50 m, 6 H (3 × CH_2 (piperidine)); 1.46 d, 3 H, J = 7.0 (CH_3 (piperidine)). ^{13}C NMR ($CDCl_3$): 168.4 (N=C); 154.7 (C-9); 141.5 (C-4a, C-10a); 136.1 (C-3, C-6); 126.8 and 126.6 (C-1, C-8); 124.3 and 124.2 (C-2, C-7); 117.3 (C-4, C-5); 116.4 (C-8a, C-9a); 53.0 (NCH (piperidine)); 44.4 (NCH_2 (piperidine)); 36.6 (N^+CH_3); 30.0 and 25.2 and 17.8 (3 × CH_2 (piperidine); 16.0 and 15.9 (SCH_3, CH_3 (piperidine)).

1-(2,6-Dimethylpiperidino)-2-methyl-3-(10-methylacridin-9-yl)isothiourea iodide (**6i**): Yield 85%, m.p. 185–189 °C. For $C_{23}H_{28}IN_3S$ (505.5) calculated: 54.65% C, 5.58% H, 8.31% N; found: 54.87% C, 5.78% H, 8.47% N. IR (KBr): 1600 (C=N); 1545 (NCS). ^1H NMR ($CDCl_3$): 8.34–7.87 m, 6 H, 7.58 m, 2 H (Acr); 4.58 m, 2 H (2 × CH (piperidine)); 4.37 s, 3 H (N^+CH_3); 2.06 s, 3 H (SCH_3); 2.25–1.50 m, 6 H (3 × CH_2 (piperidine)); 1.50 m, 6 H (2 × CH_3).

Preparation of 1,1-Disubstituted 3-(Acridin-9-yl)-*S*-methylisothioureas **7e**, **7g**. General Method

To a solution of corresponding *S*-methylisothiourea hydroiodides **5e**, **5g** (1 mmol) in acetonitrile (30–40 ml), 1,4-diazabicyclo[2.2.2]octane (1.5 mmol) was added and refluxed for 2.5 h. The reaction mixture was then poured into water (200 ml), extracted with ethyl acetate (3 × 25 ml) and dried over anhydrous $CaCl_2$. The residue obtained after evaporation of the solvent was subjected to the flash chromatography on silica gel (benzene/acetone, 5:2) affording the crystalline product after evaporation of the solvent.

3-(Acridin-9-yl)-1,1-diisopropyl-2-methyllisothiourea (**7e**): Yield 78%, m.p. 197–200 °C. For $C_{21}H_{25}N_3S$ (351.5) calculated: 71.76% C, 7.17% H, 11.95% N; found: 71.08% C, 7.28% H, 11.85% N. IR ($CHCl_3$): 1620 (C=N); 1553 (NCS). ^1H NMR ($CDCl_3$): 8.18 m, 2 H, 8.12 m, 2 H, 7.77 m, 2 H, 7.43 m, 2 H (Acr); 4.37 septet, 2 H, J = 6.6 (2 × CH (iPr)); 1.54 s, 3 H (SCH_3); 1.53 d, 12 H, J = 6.6 (4 × CH_3 (iPr)).

3-(Acridin-9-y)l-1,2-dimethylphenylisothiourea (**7g**): Yield 84%, m.p. 190–193 °C. For $C_{22}H_{19}N_3S$ (357.5) calculated: 73.92% C, 5.36% H, 11.75% N; found: 73.87% C, 5.32% H, 11.71% N. IR(KBr): 1610 (C=N); 1545 (NCS). ^1H NMR ($CDCl_3$): 8.19 m, 2 H, 8.16 m, 2 H, 7.78 m, 2 H, 7.48 m, 2 H (Acr); 7.31 bs, 5 H (Ph); 3.51 s, 3 H (NCH_3); 1.85 s, 3 H (SCH_3).

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