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NEW APPROACH TO SYNTHESIS OF *N*-SUBSTITUTED 9-AMINO/IMINO-ACRIDINES WITH IMPORTANT FLUORESCENCE PROPERTIES

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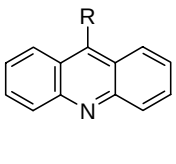
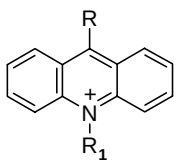
Abstract - In order to obtain new fluorogens simple versatile methods of synthesis of some *O*-alkyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethanesulfonates, *O*-alkyl [(10-methyl-l0*H*-acridin-9-ylidene)amino]methanethioates and *O*-alkyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylates were elaborated. The fluorescence properties of compounds under study were discussed.

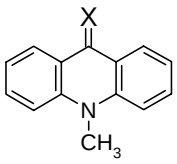
Recently, a great attention has been paid to compounds suitable for fluorescence labelling of biomolecules.[1-3] Acridine derivatives play an important role as such fluorescent reagents.[4-6] Derivatives based on 9-isothiocyanatoacridine (**1a**) represent a new potential source of fluorogens as it is shown in our previous works concerning the fluorescence properties of 48 9-acridinyl derivatives namely 9-isothiocyanatoacridines (**1a**), thioureas (**1b**) and iminothiocarbonates (**1c**).[7,8]



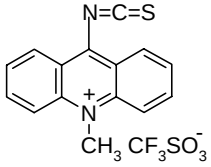
1	R
a	NCS
b	NH(CS)NHalkyl(aryl)
c	N=C(OCH$_3$)(Salkyl)



2	R	R_1
a	COOH	CH_2COOH
b	NHaryl	CH_3 (H)



3	X
a	=CH-alkyl (aryl)
b	=N-C(S)SH
c	=N-N=O
d	=N-NHCHO
e	=N-N=P(Ph)$_3$
f	=N-NH-C(S)-NH$_2$



4

As new chemiluminescent compounds of this type, 10-carboxymethylacridinium derivative (**2a**)[9] and 9-arylmethylene-10-methyl-9,10-dihydroacridine (**3a**)[10] were synthetized. Quarternary acridinium salts of *N*-substituted acridan systems are applied as chemiluminescent agents or DNA intercalators (**2b**).[11,12] Among them, 9-aminoacridinium salts attract still great attention. There is only a limited number of papers concerning 9-imino-10*H*-dihydroacridines (**3**), mostly *N*-aryl and alkyl substituted **3a** described in the literature so far.[13,14] Also, a little attention was devoted to other 9-substituted-10-methyl-10*H*-

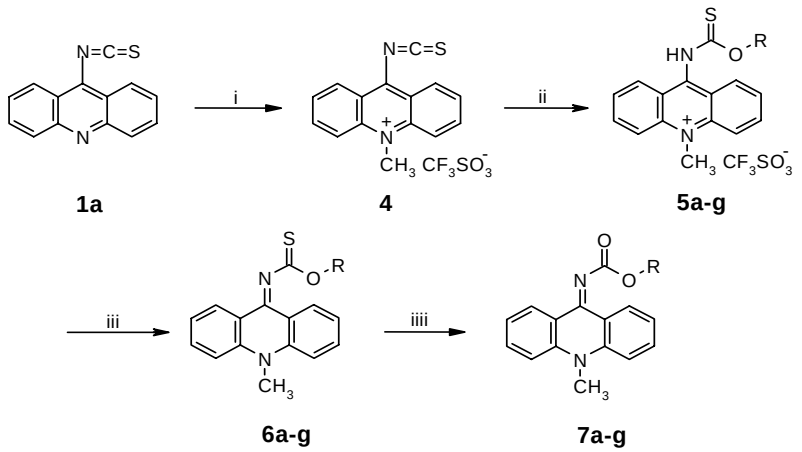
acridin-9-ylidene analogues derivatized with e.g. imino-*N*-carbothioate (**3b**),[15] nitrosoimine (**3c**),[16] formylhydrazone (**3d**), triphenylphosphorane hydrazone (**3e**)[17] and thiosemicarbazone (**3f**)[18] groups.

The aim of our work was the synthesis of novel types of acridine compounds with 10-methylacridinium-9-yl and 10-methyl-10*H*-acridin-9-ylidene skeleton derivatized with properly substituted amino and imino groups. As a synthone for this work we used highly reactive 10-methyl-9-isothiocyanatoacridinium triflate (**4**) that allowed us to prepare in the simple and mild conditions several types of new acridine compounds with interesting fluorescence properties.

To obtain a basic synthone (**4**),[19] we have elaborated a new convenient method consisting in direct methylation of 9-isothiocyanatoacridine (**1a**) with methyl trifluoromethanesulfonate in an excellent yield 90 %. This quarternization of acridine nitrogen cannot be realized with usual methylation agents as methyl iodide and methyl or tosyl sulfate.

The reactivity of 9-NCS group in 10-methyl-9-isothiocyanatoacridinium triflate (**4**) is increased so much that nucleophilic addition reactions with various types of alcohols take place at room temperature whereas the analogous reaction of phenyl isothiocyanate required increased temperature and longer reaction time.[20] An explanation can be rationalized by involving a highly preferred approach of calculation of frontier molecular orbital energies HOMO and LUMO of nucleophile and appropriate isothiocyanate, resp. We have modeled the reaction of phenyl isothiocyanate, 9-isothiocyanatoacridine (**1a**) and 10-methyl-9-isothiocyanatoacridium triflate (**4**) with methylamine using AM1 method. Energies of the LUMO-HOMO differences Δε are 9.39461 eV for phenyl isothiocyanate, 7.83420 eV for (**1a**), and 3.77800 eV for (**4**).

The first goal was to explore reactions of activated **4** in relation to well-known reactions of other simple isothiocyanates with alcohols which are weak nucleophiles in neutral conditions. The reaction with seven primary and secondary alcohols afforded, contrary to other well-investigated isothiocyanates (e.g. phenyl[20] or benzoyl[21]), appropriate *O*-alkyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethane sulfonates (**5a-g**) in yields above 80 % even at room temperature (Scheme 1).



Scheme 1. R= CH_3 (**a**), C_2H_5 (**b**), iso-C_3H_7 (**c**), cyclohexyl(**d**), n-C_4H_9 (**e**), sec- C_4H_9 (**f**), iso- C_4H_9 (**g**). i : $CF_3SO_3CH_3$, chloroform, ii: alcohol, iii: CH_3OH, CH_3ONa, iiii: mesityl nitriloxide, acetonitrile.

On the other hand, *tert*-butanol did not react under similar conditions, even in the presence of potassium *tert*-butoxide. Similarly, no reaction of **4** with phenols and phenoxides was observed. Compounds prepared were well soluble in water and acetonitrile and exhibited an intensive green-yellow fluorescence in the solution. Melting points varied considerably with the rate of heating and could not be used as the reliable constants for characterization purposes.

After addition of powder sodium methoxide to a methanolic solution of **5a**–**g** at room temperature trifluoromethanesulfonic acid was split off to give *O*-alkyl [(10-methyl-10*H*-acridin-9-ylidene)amino] methanethioates (**6a-g**) **.** This new simple method allows to synthetize in good yields dihydroacridin-9-yliden compounds with reactive iminothiocarbonate substituents, which can serve for further synthetic purposes. Straightforward synthesis of such dihydroacridines was uknown so far.

In continuation, we were interested how the replacement of the sulfur atom in compounds (**6**) by oxygen will affect the fluorescence properties of resulting products. For this purpose, **6a-g** were treated with mesityl nitriloxide in acetonitrile. The reaction proceeded smoothly at room temperature affording *O*-alkyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylates (**7a-g**) in the nearly quantitative yields.

Structure of synthetized compounds was confirmed by spectral methods and the elemental analysis. NMR spectra exhibited typical acridine proton and carbon signals, as well as, signals of *N*-methyl group and 9-amino/imino substituent. Particularly, 10-*N*-methyl and OCH_n signals in ^1H NMR spectra and C=O (C=S, C=N) signals in ^{13}C NMR spectra are a valuable tool evidencing the changes in the structure. Methylation on the acridine nitrogen with methyl triflate can be readily seen from deshielded 10-*N*-methylacridinium of **4** and **5** signal in the region 4.70 – 4.90 ppm in contrast with other 10-*N*-methylated derivatives **6** and **7** whose methyl signal is found between 3.75 – 3.95 ppm. Another proof of dihydroacridine skeleton are deshielded H-1 and H-8 protons due to a magnetic anisotropy of the exocyclic 9-C=N double bond. Transformation of **6** into **7** is represented by an apppearance of amidic C=O absorption bands in IR spectra (~1660 cm^{-1}), C=O resonance signals in ^{13}C NMR spectra (*ca.* 164 ppm) instead of C=S signals (*ca.* 195 ppm) and upfield shift of OCH_n protons (0.3-0.4 ppm) in ^1H NMR spectra.

Electronic absorption spectra of 9-substituted acridine derivatives under study measured in acetonitrile exhibited absorption with maxima in the region 360 – 480 nm. Quarternization of 10-nitrogen into triflate salt of isothiocyanate (**4**) causes a strong bathochromic shift *ca.* 60 nm of three absorption bands of **1** with maxima at 366, 388 and 410 nm. Similar shift was observed also with **5** and **6** where the shape of absorption band is simplified to one distinct maximum at 430 –

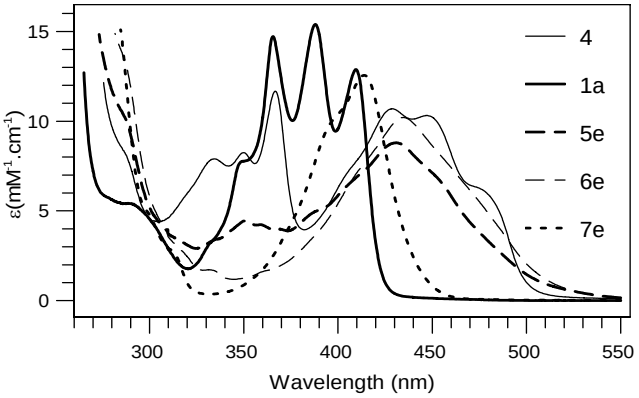


Figure 1. Electronic absorption spectra of acridine derivatives **(1a, 4, 5e, 6e** and **7e)**

440 nm, typical for dihydroacridines. Replacing of sulfur in **6** with oxygen is manifested by a hypsochromic shift about 20 nm as a result of higher excitation energy of carboxylates in **7** (Figure 1, Table 1). Some of the synthesized compounds exhibited unusually high intensity of fluorescence with the maximal value more than 2 orders of magnitude higher than 9-isothiocyanatoacridine (**1a**) known as a fluorescence marker for amino and thiol groups of biomolecules (Figure 2, Table 1). The highest intensity has been observed with triflate salts (**4**) and (**5**), whereas the formation of the dihydroacridine structure in **6** and **7** resulted in a drastic decrease of fluorescence intensity, mainly with sulfur containing methanethioates (**6**). In accord with the literature data[9] we assume that higher fluorescence of **5** is caused by an enhanced conjugation of 9-amino or imino group with the acridinium skeleton. On the other hand, a reversed polarization oriented from the 10-methyldihydroacridin-9-ylidene part to C=X group (X=S, O) in **6** and **7** can be taken as a reason of diminished electron density on the acridine skeleton and therefore of a lowered intensity of fluorescence at these compounds (Scheme 2).

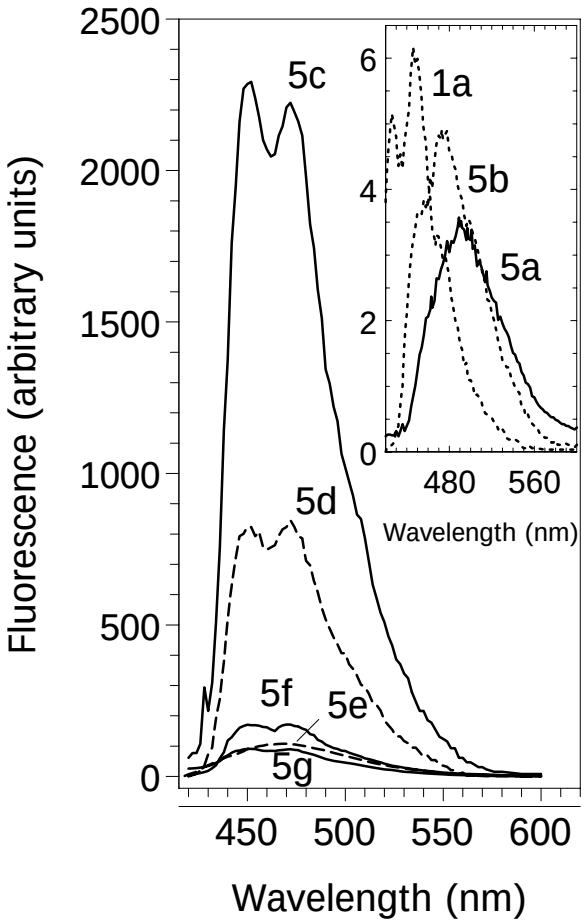


Figure 2. Fluorescence spectra of acridine derivatives **(1a** and **5a-g).**

5 **6, 7**

Scheme 2.

As follows from Table 1, the branching on the C-1 of alkyl rest causes a dramatic enhancement of the fluorescence intensity of isopropyl and cyclohexyl derivatives **5c** and **5d**, respectively. Similarly, the highest fluorescence is observed for 2-butyl derivative **5f** compared to other butyl substituted analogs **5e** and **5g**, though, less in the absolute values. We assume that combination of polar and steric effects is responsible for these differences.

From the given results follows that selected compounds (**5c,d**) exhibited remarkable fluorescence which exceeded that of 9-isothiocyanatoacridine (**1a)** from 1x 10^2 to 4 x 10^2 times. This observation allows to assume that the synthon (**4**) can be successfully utilized as a new marker for nucleophilic reactions of OH groups of biomolecules, as well as, for the synthesis of various nitrogen heterocycles.

Table 1. Spectral characteristics of synthetized acridine derivatives (**4**, **5a-g**, **6a-e** and **7a-g**).

Compd.	Absorbance		Fluorescence[a]			Compd.	Absorbance		Fluorescence[a]		
	λ_{max} [nm]	ε [$mmol^{-1}.cm^{-1}$]	λ_{max} [nm]	F [a.u]	F/F_0		λ_{max} [nm]	ε [$mmol^{-1}.cm^{-1}$]	λ_{max} [nm]	F [a.u]	F/F_0
4	366.8	11.67	502	64.5	10.45	**6c**	433.4	11.13	476	5.3	0.83
5a	434.0	12.00	491	3.52	0.57	**6d**	434.0	13.43	474	3.7	0.60
5b	434.0	11.72	475	4.91	0.80	**6e**	434.7	10.21	498	0.49	0.08
5c	409.6	11.79	452	2293.1	371.7	**7a**	414.0	12.88	476	0.63	0.10
5d	430.7	10.37	472	845.3	137.0	**7b**	414.0	13.22	440	1.45	0.24
5e	430.3	8.79	464	109.0	17.67	**7c**	413.4	10.67	450	6.54	1.06
5f	433.0	11.02	450	170.7	27.7	**7d**	358.0	3.07	470	18.66	3.02
5g	433.0	11.31	450	91.4	14.8	**7e**	413.7	12.56	481	0.40	0.06
6a	434.4	13.55	480	0.47	0.08	**7f**	413.0	12.76	435	84.24	13.63
6b	434.4	13.55	475	0.52	0.08	**7g**	413.2	13.79	439	35.17	5.70

[a]Fluorescence emission spectra were recorded in the region 420-600 nm at excitation wavelengths 410 nm. Relative fluorescence **F/F_0,** where $F_0 = 1$ corresponds the concentration 1.6 x 10^{-6} $mol.L^{-1}$ of 9-isothiocyanatoacridine **1a** at λ_{em}= 446 nm in acetonitrile.

EXPERIMENTAL

Melting points were determined on a Koffler hot-stage apparatus and are uncorrected. Infrared spectra (cm^{-1}) were measured on a Specord 75 IR spectrophotometer (Zeiss) in chloroform or KBr discs. ^{1}H NMR spectra were obtained on a Varian VXR (300.131 MHz) and Tesla BS 587 (80.118 MHz) spectrometers at room temperature in deuteriochloroform or a mixture deuteriochloroform-hexadeuteriodimethyl sulfoxide. Chemical shifts are given in ppm towards an internal standard tetramethylsilane (0.00 ppm). ^{13}C NMR spectra were taken on a Varian VXR (75.475 MHz) spectrometer in deuteriochloroform at room temperature. Chemical shifts were calibrated to deuteriochloroform signal (77. 00 ppm). MS spectra were measured on a mass spectrometer SSQ 710 Finnigan with a direct inlet (Ee = 70 eV, T = 150 ºC, Ie = 200μA). An elemental analysis was done on a Perkin-Elmer analyzer CHN 2400.

Absorption spectra of 9-isothiocyanatoacridine and its derivatives were obtained using UV-3000 Shimadzu spectrophotometer at concentrations 1.5-2.0x10^{-5} $mol.L^{-1}$ in acetonitrile. Extinction coefficients are expressed in $mmol^{-1}.cm^{-1}$ (Figure 1, Table 1). Fluorescence measurements were performed on a Shimadzu RF-5000 spectrofluorometer. Emission spectra were recorded in

the region 420-600 nm at the excitation wavelength 410 nm. Obtained fluorescence spectra are averages of 3-6 subsequent scans at the same excitation wavelength. Fluorescence intensities expressed in arbitrary units [a.u.] (Figure 2, Table 1) correspond to emission of compounds at concentration $1.0x10^{-6}$ mol.L^{-1} in acetonitrile. All the measurements were performed at 25 ^{o}C.

10-Methyl-9-isothiocyanatoacridinium trifluoromethanesulfonate (4). To a stirred solution of 9-isothiocyanatoacridine (**1a**) (378 mg, 1.6 mmol) in chloroform (20 mL) methyl trifluoromethanesulfonate (300 mg, 1.83 mmol) was added and the mixture was stirred for 24 h at laboratory temperature. The reaction course was followed by TLC monitoring (eluent benzene/acetone 5:2) whereby the starting compound did not move from the start. Triflate (**4**) precipitated by addition of ether (10 mL) was filtered off , washed with ether (5 mL) and crystallized from acetonitrile. mp 179-181 ^{o}C, yield 576 mg (90 %), orange-red crystals. IR ($CHCl_3$) cm^{-1}: 638, 1029, 1173, 1240, 1377, 1418, 1488, 1580, 1640, 2010 (broad, N=C=S); ^{1}H NMR (DMSO-d_6) δ: 4.87 (s, 3H, NCH_3), 7.90-8.85 (m, 8H, AcrH). MS m/z (rel. intensity): 251(15, M^{+}- CF_3SO_3), 236(74), 225(100). Anal. Calcd for $C_{16}H_{11}N_2O_3F_3S_2$: C, 48.00; H, 2.77; N, 7.00. Found C, 47.90; H, 2.79; N, 6.95.

General procedure for the preparation of *O*-alkyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethanesulfonates (5a-g). A suspension of 10-methyl-9-isothiocyanatoacridinium triflate (**4**) (200 mg, 0.5 mmol) in appropriate alcohol (5 mL) was heated until **4** was completely dissolved. Simultaneously, the color of reaction mixture turned into red which indicated the end of reaction. After cooling and addition of ether (10 mL) the product precipitated in the form of red needles. The crystals were filtered off, washed with ether (5 mL) and dried.

***O*-Methyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethanesulfonate (5a):** mp 147-153ºC (methanol); yield 85 %. IR (KBr) cm^{-1}: 515, 638, 752, 1030, 1068, 1149 (C-O-C), 1219, 1246, 1291, 1317, 1378, 1440, 1502 (NHCS), 1560, 1580, 1617 (C=N), 1631, 2960, 3150 (NH). ^{1}H NMR (DMSO-d_6) δ: 4.07 (s, 3H, OCH_3), 4.85 (s, 3H, NCH_3), 7.75-8.75 (m, 8H, AcrH). Anal. Calcd for $C_{17}H_{15}N_2O_4F_3S_2$: C, 47.22; H, 3.50; N, 6.48. Found C, 47.07; H, 3.58; N, 6.35.

***O*-Ethyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethanesulfonate (5b):** mp 130-138ºC (ethanol); yield 85 %. IR (KBr) cm^{-1}: 515, 633, 761, 802, 1028, 1159 (C-O-C), 1214, 1253, 1287, 1342, 1388, 1463, 1500 (NHCS), 1558, 1580, 1612 (C=N), 1623, 2960, 3166 (NH). ^{1}H NMR ($CDCl_3$) δ: 1.50 (t, J=7.0 Hz, 3H, CH_3), 4.65 (q, J=7.0 Hz, 2H, OCH_2), 4.77 (s, 3H, NCH_3), 7.63-8.87 (m, 8H, AcrH). Anal. Calcd for $C_{18}H_{17}N_2O_4F_3S_2$: C, 48.43; H, 3.84; N, 6.27. Found C, 48.30; H, 3.71; N, 6.10.

***O*-Isopropyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethanesulfonate (5c):** mp 190-197ºC (isopropanol), yield 80 %. IR (KBr) cm^{-1}: 625, 763, 1027, 1161 (C-O-C), 1243, 1291, 1373, 1458, 1500 (NHCS), 1552, 1578, 1608 (C=N), 2948, 3140 (NH). ^1H NMR ($CDCl_3$) δ: 1.40 (d, J=6.7 Hz, 6H, 2xCH_3), 4.72 (s, 3H, NCH_3), 5.60 (m, 1H, OCH), 7.60-8.75 (m, 8H, AcrH). Anal. Calcd for $C_{19}H_{19}N_2O_4F_3S_2$: C, 49.56; H, 4.16; N, 6.08. Found C, 49.27; H, 4.35; N, 5.94.

***O*-Cyclohexyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethanesulfonate (5d):** mp 218-222 ºC (acetonitrile), yield 80 %. IR (KBr) cm^{-1}: 635, 755, 1030, 1159 (C-O-C), 1250, 1380, 1462, 1485 (NHCS), 1568, 1617 (C=N), 1628, 2860, 2930. ^1H NMR ($CDCl_3$ - DMSO-D_6) δ: 1.05-2.60 (m, 10H, CH_2), 4.75 (s, 3H, NCH_3), 5.50 (m, 1H, OCH), 7.70-8.70 (m, 8H, AcrH). Anal. Calcd for $C_{22}H_{23}N_2O_4F_3S_2$: C, 52.79; H, 4.63; N, 5.60. Found C, 52.93; H, 4.82; N, 5.48.

***O*-n-Butyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethanesulfonate (5e):** mp 180-185 ºC (acetonitrile-ether), yield 82 %. IR (KBr) cm^{-1}: 633, 755, 1021, 1155 (C-O-C), 1183, 1263, 1285, 1300, 1363, 1458, 1486 (NHCS), 1595 (C=N), 1630, 2853, 2925. ^1H NMR ($CDCl_3$) δ: 0.95 (t, J=6.6 Hz, 3H, CH_3), 1.12-2.00 (m, 4H, CH_2CH_2), 4.52 (t, J=7.7 Hz, 2H, OCH_2), 4.70 (s, 3H, NCH_3), 7.52-8.70 (m, 8H, AcrH). Anal. Calcd for $C_{20}H_{21}N_2O_4F_3S_2$: C, 50.62; H, 4.46; N, 5.90. Found C, 50.41; H, 4.60; N, 5.73.

***O*-sec-Butyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethanesulfonate (5f):** mp 220ºC (decomp.) (acetonitrile-ether), yield 62 %. IR (KBr) cm^{-1}: 630, 767, 1029, 1168 (C-O-C), 1245, 1292, 1377, 1458, 1498 (NHCS), 1548, 1579, 1609 (C=N), 2940, 2965, 3140 (NH). ^1H NMR ($CDCl_3$-DMSO-d_6) δ: 0.83 (t, J=8.7 Hz, 3H, CH_3), 1.28 (d, J=7.3 Hz, 3H, CH_3), 1.60 (m, 2H, CH_2), 4.87 (s, 3H, NCH_3), 5.47 (m, 2H, OCH), 7.75-8.70 (m, 8H, AcrH). Anal. Calcd for $C_{20}H_{21}N_2O_4F_3S_2$: C, 50.62; H, 4.46; N, 5.90. Found C, 50.85; H, 4.44; N, 6.05.

***O*-Isobutyl [(10-methylacridinium-9-yl)amino]methanethioate trifluoromethanesulfonate (5g):** mp 193-195 ºC (acetonitrile-ether), yield 68 %. IR (KBr) cm^{-1}: 637, 751, 1027, 1164 (C-O-C), 1215, 1274, 1320, 1376, 1456, 1505 (NHCS), 1550, 1578, 1608 (C=N), 1622, 1633, 2964, 3140 (NH). ^1H NMR ($CDCl_3$) δ: 0.95 (d, J = 8.0 Hz, 6H, 2xCH_3), 2.09 (m, 1H, CH), 4.30 (d, J=8.5 Hz, 2H, OCH_2), 4.71 (s, 3H, NCH_3), 7.60-8.88 (m, 8H, AcrH). Anal. calcd for $C_{20}H_{21}N_2O_4F_3S_2$: C, 50.62; H, 4.46; N, 5.90. Found C, 50.71; H, 4.44; N, 5.81.

General procedure for the preparation of *O*-alkyl [(10-methyl-10*H*-acridin-9-ylidene)amino]methanethioates (6a-g). To a solution of triflates (**5a-g**) (0.6 mmol) in methanol (10 mL) a solution of sodium methoxide (54.005 mg, 1 mmol) in methanol (10 mL) was added dropwise untill the pH of the reaction mixture changed from acid to neutral. The mixture was

stirred for 2 h at slightly increased temperature and the course of reaction was monitored on TLC plates. Compounds (**5a-g**) remained on the start, the reaction products **6a-g** moved with eluent (benzene/acetone 5:2, R_f ~ 0.65). After the reaction was finished, ether (10 mL) was added and the separated precipitates of **6a-g** were filtered off, washed with ether (5 mL), dried and crystallized.

***O*-Methyl [(10-methyl-10*H*-acridin-9-ylidene)amino]methanethioate (6a):** mp 180-182ºC (acetonitrile-ether), yield 92 %. IR ($CHCl_3$) cm^{-1}: 642, 1118, 1136, 1176 (C-O-C), 1457, 1496, 1555, 1585, 1607 (C=N), 1623, 2950, 2992. ^1H NMR ($CDCl_3$) δ: 3.83 (s, 3H, NCH_3), 4.15 (s, 3H, OCH_3), 7.26, 7.50, 7.67, and 8.31 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 34.72 (NCH_3), 57.52 (OCH_3), 115.24 (CH), 118.09 (C-8a,9a), 122.31 (CH), 129.04 (CH), 134.21 (CH), 141.45 (C-4a,10a), 155.90 (C-9), 194.20 (C=S). Anal. Calcd for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92. Found C, 68.13; H, 4.98; N, 9.81.

***O*-Ethyl [(10-methyl-10*H*-acridin-9-ylidene)amino]methanethioate (6b):** mp 184-186ºC (acetonitrile-ether), yield 91 %. IR ($CHCl_3$) cm^{-1}: 640, 1118, 1166 (C-O-C), 1312, 1358, 1449, 1488, 1548, 1577, 1595 (C=N), 1615, 2985. ^1H NMR ($CDCl_3$) δ: 1.47 (t, J=7.1 Hz, 3H, CH_3), 3.92 (s, 3H, NCH_3), 4.63 (q, J=7.1 Hz, 2H, OCH_2), 7.30, 7.54, 7.72 and 8.36 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 14.40 (CH_3), 34.64 (NCH_3), 66.86 (OCH_2), 115.04 (CH), 118.24 (C-8a,9a), 122.07 (CH), 129.27 (CH), 133.97 (CH), 141.66 (C-4a,10a), 154.50 (C-9), 195.30 (C=S). MS m/z (rel. intensity): 296 (17, M^+), 251 (22), 219 (100), 194 (12). Anal. Calcd for $C_{17}H_{16}N_2OS$: C, 68.89; H, 5.44; N, 9.45. Found C, 68.71; H, 5.63; N, 9.37.

***O*-Isopropyl [(10-methyl-10*H*-acridin-9-ylidene)amino]methanethioate (6c):** mp 175-177ºC (acetonitrile-ether), yield 86 %. IR ($CHCl_3$) cm^{-1}: 1028, 1095, 1177 (C-O-C), 1242, 1458, 1490, 1577, 1602 (C=N), 1625, 2992. ^1H NMR ($CDCl_3$) δ: 1.46 (d, J=6.2 Hz, 6H, 2 x CH_3), 3.91 (s, 3H, NCH_3), 5.61 (septet, J=6.2 Hz, 1H, OCH), 7.28, 7.53, 7.71 and 8.34 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 21.89 (2 x CH_3), 34.64 (NCH_3), 74.45 (OCH), 115.00 (CH), 118.32 (C-8a,9a), 121.99 (CH), 129.29 (CH), 133.88 (CH), 141.68 (C-4a,10a), 153.64 (C-9), 195.06 (C=S). Anal. Calcd for $C_{18}H_{18}N_2OS$: C, 69.65; H, 5.84; N, 9.02. Found C, 69.83; H, 5.73; N, 8.90.

***O*-Cyclohexyl [(10-methyl-10*H*-acridin-9-ylidene)amino]methanethioate (6d):** mp 196-198 ºC (acetonitrile), yield 87 %. IR ($CHCl_3$) cm^{-1}: 1120, 1177 (C-O-C), 1460, 1495, 1588, 1605 (C=N), 1623, 2863, 2942. ^1H NMR ($CDCl_3$) δ: 1.16 – 1.70 (m, 6H, 3 x CH_2), 1.80 (m, 2H, CH_2), 2.17 (m, 2H, CH_2), 3.88 (s, 3H, NCH_3), 5.35 (m, 1H, OCH), 7.26, 7.50, 7.69 and 8.34 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$): 24.12 (2 x CH_2), 25.42 (CH_2), 31.73 (2 x CH_2), 34.56 (NCH_3), 79.53 (OCH), 114.93 (CH), 118.25 (C-8a,9a), 121.86 (CH), 129.30 (CH), 133.72 (CH), 141.66 (C-4a,10a), 153.33 (C-9), 195.37 (C=S). Anal. Calcd for $C_{21}H_{22}N_2OS$: C, 71.97; H, 6.33; N, 7.99. Found C, 71.90; H, 6.45; N, 8.01.

***O*-n-Butyl [(10-methyl-10*H*-acridin-9-ylidene)amino]methanethioate (6e):** mp 136-138 ºC (acetonitrile), yield 88 %. IR (KBr) cm^{-1}: 637, 745, 1047, 1124, 1172 (C-O-C), 1265, 1317, 1357, 1455, 1495, 1583, 1604 (C=N), 1621, 2870, 2953. ^1H NMR ($CDCl_3$) δ: 0.97 (t, J=7.4 Hz, 3H, CH_3), 1.47 (m, 2H, CH_2), 1.83 (m, 2H, CH_2), 3.93 (s, 3H, NCH_3), 4.57 (t, J=6.7 Hz, 2H, OCH_2), 7.30 m, 2H; 7.54 m, 2H; 7.72 m, 2H and 8.35 dd, J=8.2 and 1.4 Hz, 2H (AcrH). ^{13}C NMR ($CDCl_3$) δ: 13.81 (CH_3), 19.27 (CH_2), 30.83 (CH_2), 34.63 (NCH_3), 71.00 (OCH_2), 115.01 (CH), 118.30 (C-8a,9a), 122.06 (CH), 129.26 (CH), 133.91 (CH), 141.69 (C-4a,10a), 154.20 (C-9), 195.81 (C=S). Anal. Calcd for $C_{19}H_{20}N_2OS$: C, 70.34; H, 6.21; N, 8.63. Found C, 70.41; H, 6.10; N, 8.77.

***O*-sec-Butyl [(10-methyl-10*H*-acridin-9-ylidene)amino]methanethioate (6f):** mp 168-170 ºC (dichloromethane-ether), yield 72 %. IR (KBr) cm^{-1}: 638, 740, 1035, 1088, 1115, 1165, 1180 (C-O-C), 1250, 1362, 1452, 1497, 1587, 1600 (C=N), 1624, 2925, 2970. ^1H NMR ($CDCl_3$) δ: 1.04 (t, J=7.4 Hz, 3H, CH_3), 1.45 (d, J=6.2 Hz, 3H, CH_3), 1.72 and 1.91 (m, 2H, CH_2), 3.87 (s, 3H, NCH_3), 5.43 (m, 1H, OCH), 7.27-8.34 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 9.98 (CH_3), 19.35 (CH_3), 28.94 (CH_2), 34.56 (NCH_3), 79.23 (OCH), 114.98 (CH), 118.18 (C-8a,9a), 121.93 (CH), 129.21 (CH), 133.81 (CH), 141.60 (C-4a,10a), 153.54 (C-9), 195.17 (C=S). Anal. Calcd for $C_{19}H_{20}N_2OS$: C, 70.34; H, 6.21; N, 8.63. Found C, 70.22; H, 6.28; N, 8.50.

***O*-Isobutyl [(10-methyl-10*H*-acridin-9-ylidene)amino]methanethioate (6g):** m.p. 156-158 ºC (dichloromethane-ether), yield 75 %. IR (KBr) cm^{-1}: 644, 751, 1043, 1120, 1171 (C-O-C), 1260, 1278, 1315, 1366, 1465, 1496, 1595, 1608 (C=N), 1630, 2875, 2958. ^1H NMR ($CDCl_3$) δ: 1.02 (d, J=6.7 Hz, 6H, 2xCH_3), 2.19 (m, 1H, CH), 3.90 (s, 3H, NCH_3), 4.34 (d, J=6.8 Hz, 2H, OCH_2), 7.29-8.35 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 19.37 (2xCH_3), 27.91 (CH), 34.60 (NCH_3), 77.30 (OCH_2), 115.01 (CH), 118.25 (C-8a, 9a), 122.05 (CH), 129.18 (CH), 133.88 (CH), 141.63 (C-4a, 10a), 154.09 (C-9), 195.75 (C=S). Anal. Calcd for $C_{19}H_{20}N_2OS$: C, 70.34; H, 6.21; N, 8.63. Found C, 70.25; H, 6.08; N, 8.43.

General procedure for the preparation of *O*-alkyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylates (7a-g): To a solution of methanethioates (**6a-g**) (0.6 mmol) in acetonitrile (15 mL) mesityl nitriloxide (112.84 mg, 0.7 mmol) was added and the mixture was stirred for 1 h at rt. The end of reaction was followed by TLC on the silica plates, eluent benzene/acetone (5:2). A spot of product exhibited an intensive yellow fluorescence under the UV irradiation at 254 nm. The solvent was evaporated to dryness and the residue was washed with petrol ether (2x10 mL) to remove an excess of mesityl nitriloxide and mesityl isothiocyanate arising as a by-product during the reaction. The product was crystallized from a mixture dichloromethane-ether or acetonitrile.

***O*-Methyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylate (7a):** mp 149-151 ºC (dichloromethane-ether), yield 95 %.

IR ($CHCl_3$) cm^{-1}: 1130, 1172 (C-O-C), 1250, 1274, 1294, 1367, 1459, 1488, 1589 (C=N), 1618, 1673 (C=O), 2956, 3007. ^1H NMR ($CDCl_3$) δ: 3.82 (s, 3H, NCH_3), 3.89 (s, 3H, OCH_3), 7.22-8.20 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 34.36 (NCH_3), 52.98 (OCH_3), 114.63 (CH), 119.58 (C-4a,10a), 121.53 (CH), 127.51 (CH), 132.95 (CH), 141.55 (C-8a,9a), 156.14 (C-9), 164.42 (C=O). Anal. Calcd for $C_{16}H_{14}N_2O_2$: C, 72.17; H, 5.30; N, 10.52. Found C, 72.10; H, 5.33; N, 10.43.

***O*-Ethyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylate (7b):** mp 158-160 ºC (dichloromethane-ether), yield 93 %. IR ($CHCl_3$) cm^{-1}: 1127, 1167 (C-O-C), 1242, 1267, 1287, 1362, 1460, 1486, 1588 (C=N), 1618, 1668 (C=O), 3005. ^1H NMR ($CDCl_3$) δ: 1.36 (t, J=7.1 Hz, 3H, CH_3), 3.75 (s, 3H, NCH_3), 4.35 (k, J=7.1 Hz, 2H, OCH_2), 7.17-8.21 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 14.45 (CH_3), 34.25 (NCH_3), 61.80 (OCH_2), 114.56 (CH), 119.50 (C-4a,10a), 121.31 (CH), 127.50 (CH), 132.81 (CH), 141.45 (C-8a,9a), 155.72 (C-9); 163.94 (C=O). MS m/z (rel. intensity): 280 (20, M^+), 235 (100), 221(20), 208 (83), 192 (15). Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84, H, 5.75; N, 9.99. Found C, 72.70; H, 5.86; N, 10.18.

***O*-Isopropyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylate (7c):** mp 183-185 ºC (acetonitrile), yield 94 %. IR ($CHCl_3$) cm^{-1}: 1101, 1135 (C-O-C), 1170, 1247, 1272, 1291, 1364, 1462, 1490, 1592 (C=N), 1620, 1664 (C=O), 2942, 2988, 3027. ^1H NMR ($CDCl_3$) δ: 1.36 (d, J=6.3 Hz, 6H, 2xCH_3), 3.78 (s, 3H, NCH_3), 5.19 (septet, J=6.3 Hz, 1H, OCH), 7.18-8.23 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 21.99 (2xCH_3), 34.30 (NCH_3), 69.28 (OCH), 114.52 (CH), 119.64 (C-4a,10a), 121.26, 127.67, 132.81 (CH), 141.51 (C-8a,9a), 155.39 (C-9), 163.49 (C=O). Anal. Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found C, 73.29; H, 6.22; N, 9.41.

***O*-Cyclohexyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylate (7d):** mp 220-222 ºC (acetonitrile), yield 94 %. IR ($CHCl_3$) cm^{-1}: 1130, 1166 (C-O-C), 1240, 1269, 1287, 1362, 1460, 1486, 1590 (C=N), 1619, 1662 (C=O), 2865, 2947, 3008. ^1H NMR ($CDCl_3$) δ: 1.12-1.65 (m, 6H, 3xCH2), 1.77 (m, 2H, CH_2), 2.08 (m, 2H, CH_2), 3.77 (s, 3H, NCH_3), 4.93 (m, 1H, OCH), 7.18-8.25 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 24.14, 25.41 (3xCH_2); 31.93 (2xCH_2), 34.27 (NCH_3), 74.41 (OCH), 114.46 (CH), 119.68 (C-4a, 10a), 121.21 (CH), 127.72 (CH), 132.71(CH), 141.53 (C-8a,9a), 155.25 (C-9), 163.59 (C=O). Anal. Calcd for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38. Found C, 75.26; H, 6.74; N, 8.30.

***O*-n-Butyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylate (7e):** mp 105-107 ºC (acetonitrile), yield 96 %. IR ($CHCl_3$) cm^{-1}: 1130, 1168 (C-O-C), 1240, 1268, 1290, 1327, 1359, 1458, 1484, 1593 (C=N), 1619, 1666 (C=O), 2963, 3008. ^1H NMR ($CDCl_3$) δ: 0.93 (t, J=6.6 Hz, 3H, CH_3), 1.12 - 1.98 (m, 4H, CH_2CH_2), 3.81 (s, 3H, NCH_3), 4.29 (t, J=6.5 Hz, 2H, OCH_2), 7.00-8.22 (m, 8H, AcrH). ^{13}C NMR ($CDCl_3$) δ: 13.80 (CH_3), 19.39 (CH_2), 30.96 (CH_2), 34.58 (NCH_3), 71.16 (OCH_2),

115.17 (CH), 117.37 (C-4a,10a), 122.18 (CH), 129.51 (CH), 133.88 (CH), 141.71 (C-8a,9a), 156.30 (C-9), 163.81 (C=O). Anal. Calcd for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.08. Found C, 74.08; H, 6.68; N, 9.00.

***O*-sec-Butyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylate (7f):** mp 136-138 ºC (dichloromethane), yield 86 %. IR (KBr) cm^{-1}: 648, 747, 1106, 1135, 1170 (C-O-C), 1224, 1270, 1294, 1370, 1493, 1593 (C=N), 1624, 1680 (C=O), 2873, 2922, 2972. ^{1}H NMR ($CDCl_3$) δ: 0.95 (t, J=7.4 Hz, 3H, CH_3), 1.35 (d, J=6.3 Hz, 3H, CH_3), 1.63 and 1.72 (m, 1H, CH_2), 3.76 (s, 3H, NCH_3), 4.99 (m, 1H, OCH), 7.17-8.24 (m, 8H, AcrH). Anal. Calcd for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.08. Found C, 73.81; H, 6.70; N, 8.95.

***O*-Isobutyl [(10-methyl-10*H*-acridin-9-ylidene)amino]carboxylate (7g):** mp 143-145 ºC (dichloromethane-ether), yield 78 %. IR ($CHCl_3$) cm^{-1}: 1134, 1172 (C-O-C), 1247, 1273, 1292, 1367, 1467, 1490, 1596 (C=N), 1625, 1670 (C=O), 2950, 3005. ^{1}H NMR ($CDCl_3$) δ: 0.97 (d, J=6.8 Hz, 6H, 2xCH_3), 2.07 (m, 1H, CH), 3.80 (s, 3H, NCH_3), 4.09 (d, J=6.6 Hz, 2H, OCH_2), 7.00-8.23 (m, 8H, AcrH). Anal. Calcd for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.08. Found C, 73.85; H, 6.65; N, 9.02.

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