Synthesis and Physicochemical Characterization of Fluorescent (*E*)-2-Stilbenyloxyalkylthiouracils and Isomer Differentiation using EIMS

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Twelve new fluorescent (*E*)-2-stilbenyloxyalkylthiouracils and 6-methyluracils **5a**-**5l** were prepared. EI induced mass spectral fragmentation of these compounds was investigated. Fragmentation pathways are proposed on the basis of accurate mass and metastable transition measurements. Correlation between the intensities of the $M^{+\cdot}$ and the selected fragment ions of these compounds is discussed. The data obtained permit a distinction of the metamers. The ^1H and ^{13}C NMR spectra of these compounds were assigned unambiguously using a combination of heteronuclear (HETCOR) spectra and the chemical shifts. The data derived from these spectra can be used to differentiate the isomers.

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Introduction.

Thio derivatives of pyrimidine bases are of interest because of their biological and pharmacological activities [1-7]. Many of the modified nucleobases and nucleosides both with additional five- and six-membered rings are fluorescent [8-11] and offer a possibility of gaining information concerning DNA and RNA structure and dynamic [12-14]. Many have been shown to enter biochemical pathways and have given indications about their binding to different enzymes [15-16]. The fluorescent derivatives of cytosine and cytidine have been widely reported in the literature [11,17-20]. However, to the best of our knowledge, very little work has been published on the synthesis and physicochemical properties of fluorescent derivatives of 2-thiouracils. This fact has stimulated us to prepare a series of (*E*)-2-stilbenyloxyalkylthiouracils (**5a**,**5c**,**5e**,**5g**,**5i**,**5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5d**,**5f**,**5h**,**5j**,**5l**) with the idea that these compounds bearing on pyrimidine ring (*E*)-stilbenyloxyalkylthio substituents with strongly fluorescent (*E*)-stilbene moiety, would certainly be fluorescent [21,22].

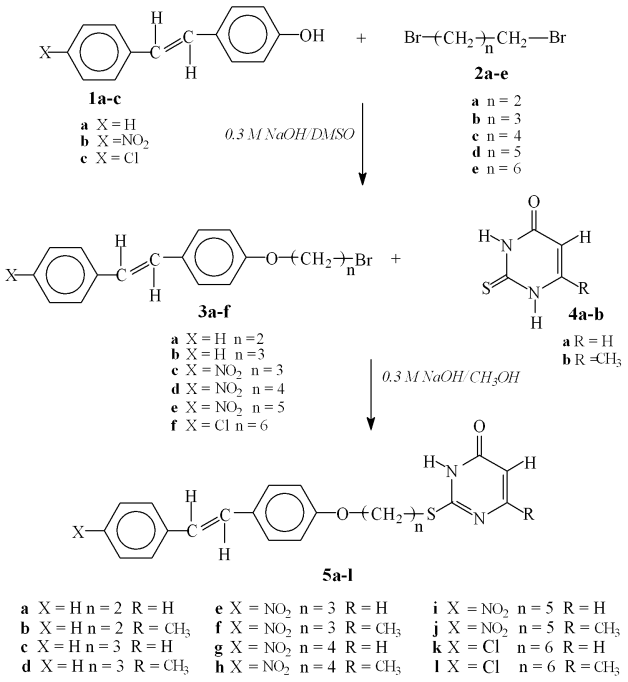
This paper deals with the synthesis and physicochemical properties of **5a**-**5l**. The UV/VIS, IR, ^1H NMR and ^{13}C NMR of (*E*)-bromoalkoxystilbenes (**3a**-**3f**) have also been investigated because these compounds are also unknown in the literature. The analyses of ^1H NMR, ^{13}C NMR and EI mass spectra of **5a-5l** have been performed to check the possibility of differentiation of (*E*)-2-stilbenyloxyalkylthiouracils (**5a**,**5c**,**5e**,**5g**,**5i**,**5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5d**,**5f**,**5h**,**5j**,**5l**). The investigation of electron-impact induced mass spectra was also undertaken to discover whether it is possible to distinguish the metamers with molecular formulas $C_{21}H_{20}N_2O_2S$ (**5b**,**5c**); $C_{22}H_{21}N_3O_4S$ (**5f**,**5g**) and $C_{23}H_{23}N_3O_4S$ (**5h**,**5i**) on the basis of differences in values of μ_1 and μ_2 *i.e.* the ratio of the intensity of selected fragment ion peaks to that of the parent ion peak, and to compare the data with those previously obtained in our laboratory [23-25].

Results and Discussion.

A few series of twelve new fluorescent (*E*)-2-stilbenyloxyalkylthiouracils (**5a**,**5c**,**5e**,**5g**,**5i**,**5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5d**,**5f**,**5h**,**5j**,**5l**) have been synthesized in the reaction of 2-thiouracil (or 2-thio-6-methyluracil) with corresponding (*E*)-bromoalkoxystilbenes **3a**-**3f**. Treatment of 2-thiouracil (or 2-thio-6-methyluracil) with **3a**-**3f** in 0.3 *M* solution of NaOH in methanol at room temperatures afforded **5a**-**5l** (Scheme 1). A series of new **3a**-**3f** have been synthesized in the reaction of (*E*)-stilbenol-4, [(*E*)-4'-chlorostilbenol-4, (*E*) 4'-nitrostilbenol-4)] with 1,2-dibromoethane (1,3-dibromopropane; 1,4-dibromobutane; 1,5-dibromopentane; 1,6-dibromohexane) in 0.3 *M* NaOH solution of DMSO at room temperature. The structures of all compounds obtained were determined by examining their UV/VIS, IR, ^1H NMR and ^{13}C NMR spectra as well as on the basis of elemental analyses (Tables 1-7).

The ^1H and ^{13}C NMR data of **3a-3f** and **5a-5l** are given in Tables 3-7. Assignments of the ^1H NMR and ^{13}C NMR resonances of these compounds were deduced on the basis of signal multiplicities, and by the concerted application of the two–dimensional NMR technique HETCOR. The HETCOR results allow unequivocal assignment of the ^{13}C NMR spectra proposed on the basis of chemical shifts theory, additivity rules and by comparing the measured and calculated chemical shifts [31]. (*E*) configuration in the stilbene part of the molecules of **3a-3f** and **5a-5l** was determined on the basis of their UV/VIS and IR spectra. It has been pointed out that in the UV/VIS spectra of **3a-3f** λ_{max} are in the range 320-378 nm (Table 3), as well as **5a-5l** 318.5-362.5 nm (Table 4) respectively. According to the literature [26-28] (*E*)-stilbenes exhibited the values of λ_{max} in range 290-360 nm and for (*Z*)-stilbenes values of λ_{max} fall in the range 260-280 nm. The infrared spectra of **3a-3f** and **5a-5l** show a strong band in the range 960-987 cm^{-1} which according to the literature [29-30] can be attributed to the out-of-plane deformation vibration of the C-H bond of the (*E*)-ethylene bridge of the stilbene skeletone (Tables 3,4).

Scheme 1



In the ^{1}H NMR spectra of (*E*)-2-stilbenyloxyalkylthiouracils (**5a**,**5c**,**5e**,**5g**,**5i**,**5k**) are seen two doublets of CV-H and CVI-H protons of uracil ring. The values of the chemical shifts of the signals of these protons were established in the range 6.52-6.60 δ and 7.45-8.00 δ, respectively (Table 4). In the ^{1}H NMR spectra of (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5d**,**5f**,**5h**,**5j**,**5l**) are seen two singlets of CV-H and CVI-CH_3 protons of 6-methyluracil ring. The values of the chemical shifts of the signals of these protons fall in the range 6.37-6.83 δ and 2.24-2.49 δ, respectively. The presence of these signals in the ^{1}H NMR spectra of 2-(*E*)-stilbenyloxyalkylthiouracils and 2-(*E*)-stilbenyloxyalkylthio-6-methyluracils allows for the determination of the presence of uracil or 6-methyluracil ring in the molecules of **5a**-**5l**. The determination of the presence of 6-methyl-uracil ring in the molecules of **5b**, **5d**, **5f**, **5h**, **5j** and **5l** is also possible on the basis of the presence in the ^{13}C NMR spectra of these compounds the signals of carbons of C VI -methyl group of uracil ring. The values of the chemical shifts of the signals of these carbons fall in the range 18.94-24.23 δ.

A comparison of the number and positions of the C II, C IV, C VI carbon atom signals in the region 160-170 ppm of ^{13}C NMR spectra of (*E*)-2-stilbenyloxyalkylthiouracils (**5a**,**5c**,**5e**,**5g**,**5i**,**5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5d**,**5f**,**5h**,**5j**,**5l**) allows a differentiation between metameric **5b** and **5c** [$C_{21}H_{20}N_2O_2S$], **5f** and **5g** [$C_{22}H_{21}N_3O_4S$] as well as **5h** and **5i** [$C_{23}H_{23}N_3O_4S$]. This data are given in tabular form below:

5b (E)-2-[stilbenyl-4-oxyethylthio-6-metyluracil];

C II 167.05 ppm
C IV 160.63 ppm
C VI 162.24 ppm

5c (E)-2-[stilbenyl-4-oxypropylthiouracil]

C II 166.40 ppm
C IV 161.56 ppm
C VI 149.76 ppm

5f (E)-2-[4'-nitrostilbenyl-4-oxypropylthio-], 6-methyluracil,

C II 165.70 ppm
C IV 164.56 ppm
C VI 160.01 ppm

5g (E)-2-[4'-nitrostilbenyl-4-oxybutylthio-]uracil

C II 161.70 ppm
C IV 160.89 ppm
C VI 146.18 ppm

5h (E)-2-[4'-nitrostilbenyl-4-oxybutylthio-], 6-methyluracil

C II 165.70 ppm
C IV 164.56 ppm
C VI 160.30 ppm

Table 1

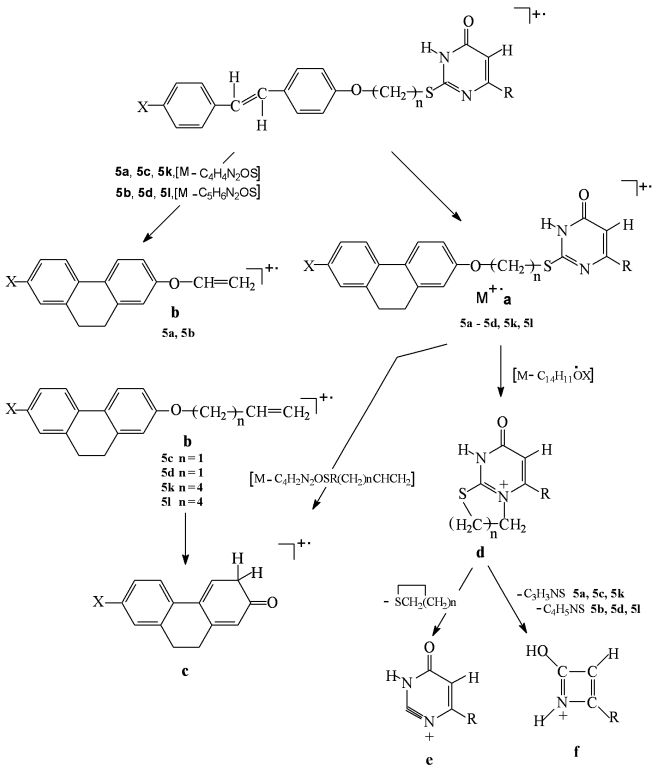
Chemical and Physical Data of Compounds **3a-f**

Comp.	Formula (mol. weight)	M.p. [°C]	Yield %	Rf TLC	Elemental analysis Calculated (Found) C	H	N
3a	$C_{16}H_{15}OBr$ 303.20	123-4	72	0.50	63.36 (63.13)	4.95 (5.15)	-
3b	$C_{17}H_{17}OBr$ 317.22	108-9	62	0.52	64.35 (63.74)	4.95 (5.23)	-
3c	$C_{17}H_{16}NO_3Br$ 362.23	72-4	30	0.74	56.35 (56.15)	4.42 (4.62)	3.84 (3.60)
3d	$C_{18}H_{18}NO_3Br$ 376.25	82-3	16	0.74	57.45 (57.85)	4.79 (4.80)	3.72 (3.78)
3e	$C_{19}H_{20}NO_3Br$ 408.25	77-8	17	0.72	55.88 (55.65)	5.39 (5.11)	3.43 (3.41)
3f	$C_{20}H_{22}ONBrCl$ 394.20	136-8	72	0.51	60.91 (60.60)	6.69 (6.73)	-

Table 2
Chemical and Physical Data of Compounds **5a-l**

Comp.	Formula (mol. weight)	M.p. [°C]	Yield %	Rf TLC	Elemental analysis Calculated (Found)			
					C	H	N	S
5a	$C_{20}H_{18}N_2O_2S$ 350.06 x $^1/_2$ H_2O	246-8	20	0.73	66.85 (66.77)	5.01 (4.94)	7.79 (7.60)	8.91 (8.82)
5b	$C_{21}H_{20}N_2O_2S$ 364.04	213-5	53	0.59	69.23 (66.92)	5.49 (5.56)	7.69 (7.40)	8.79 (8.70)
5c	$C_{21}H_{20}N_2O_2S$ 364.04	222-3	19	0.66	69.23 (69.94)	5.49 (5.59)	7.69 (7.41)	8.79 (8.62)
5d	$C_{22}H_{22}N_2O_2S$ 405,3 x $1^1/_2$ H_2O	187-9	24	0.57	65.18 (65.44)	5.43 (5.52)	6.91 (6.41)	7.90 (7.82)
5e	$C_{21}H_{19}N_3O_4S$ 409,41	93-4	73	0.48	61.61 (61.72)	4.65 (4.62)	10.26 (10.05)	7.82 (7.51)
5f	$C_{22}H_{21}N_3O_4S$ 423.44	175-7	40	0.51	62.41 (62.58)	4.96 (5.02)	9.93 (9.98)	7.56 (7.51)
5g	$C_{22}H_{21}N_3O_4S$ 432.44 x $^1/_2$ H_2O	154-6	77	0.54	61.11 (61.04)	5.09 (5.23)	9.72 (9.41)	7.56 (7.62)
5h	$C_{23}H_{23}N_3O_4S$ 437.46	132-4	65	0.47	63.16 (63.12)	5.26 (5.36)	9.60 (9.47)	7.32 (7.39)
5i	$C_{23}H_{23}N_3O_4S$ 437.46	148-50	57	0.54	63.10 (63.05)	5.26 (5.24)	9.60 (9.57)	7.32 (7.40)
5j	$C_{24}H_{25}N_3O_4S$ 451.49	139-41	81	0.57	63.86 (63.56)	5.54 (5.48)	9.31 (9.18)	7.09 (7.00)
5k	$C_{24}H_{25}N_2O_2SCl$ 449.45 x $^1/_2$ H_2O	189-91	16	0.67	62.88 (63.00)	5.89 (5.56)	6.11 (6.23)	7.12 (8.06)
5l	$C_{25}H_{27}N_2O_2SCl$ 463.45 x $^1/_2$ H_2O	169-71	19	0.65	64.79 (64.83)	5.83 (5.86)	6.04 (5.86)	6.91 (6.78)

Scheme 2



5i (E)-2-[4'-nitrostilbenyl-4-oxypentylothiouracil

C II 165.65 ppm
C IV 164.86 ppm
C VI 146.00 ppm

On the basis of the low and high resolution electron–impact mass spectra, as well as fragment ion spectra from the first field–free region, recorded using linked scans at constant B/E (Tables 8, 9), the principal mass spectral fragmentation routes of the molecular ions of compounds **5a**-**5l** are interpreted as shown in Scheme 2 and 3. The stilbenyloxyalkyl substituents of the molecular ions of **5a**-**5l** might have a tricyclic dihydrophenanthrenyloxyalkyl structure, as it was postulated by us earlier in the cases of the mass decompositions of the molecular ions of *N*- (*E*)-stilbenyloxyalkylcarbonyl substituted hydrazones of arylaldehydes [25,32,33]. The common features of the mass spectral fragmentation of the molecular ions of **5a**-**5l** are simple cleavages of Csp^3-O bonds in the thioalkyloxy chain *i.e.* the elimination of stilbenyloxy radicals. By these simple inductive cleavage the even-electron fragment ions **d** are derived. These ions might have a bicyclic structure with the positive charge probably situated on the annular nitrogen atom, as was proposed earlier for the mass fragmentation of alkoxycarbonylalkylthiouracils [34].

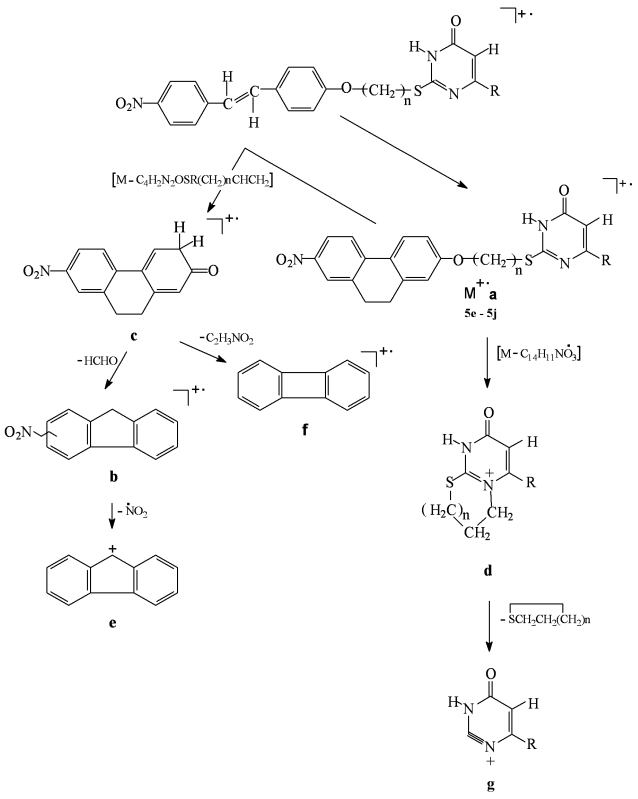
It ought to be pointed out that cleavages of Csp^2-S and Csp^3-N bonds in these even-electron fragment ions leading to ejection of neutral alkenethiols molecules to give even-electron fragment ions situated in the mass spectra of (*E*)-2-stilbenyloxyalkylthio-6-methylurails (**5a**,**5c**,**5e**,**5g**,**5i**,**5k**) at

Table 3

UV/VIS, IR and ^{1}H NMR Data of **3a**-**f**

Comp.	UV/VIS λ max	(lg ε)	IR (cm^{-1}) ν C=C	HC=CH trans	νC-O-	^{1}H NMRδ (ppm)
3a	240	(3.95)	1606			OCH_2 4.32 t J= 6Hz
	306	(4.43)	1595	961	1255	$BrCH_2$ 3.66 t J= 6Hz
	320	(4.42)	1511			Ar 6.88-7.51 m
3b	240	(3.97)	1603			OCH_2 4.13 t J= 6Hz
	308	(4.42)	1588	968	1246	$BrCH_2$ 3.61 t J= 6Hz
	320	(4.41)	1511			Ar 6.88-7.51 m
3c	259	(4.14)	1604			OCH_2 4.33 t J= 6Hz
	276	(4.12)	1588	969	1253	$BrCH_2$ 3.66 t J= 6Hz
	376	(4.30)	1511			Ar 6.88-7.51 m
3d	258	(4.08)	1604			OCH_2 4.13 t J= 6Hz
	275	(4.05)	1589	974	1254	$BrCH_2$ 3.61 t J= 6Hz
	376	(4.32)	1512			Ar 6.88-7.51 m
3e	258	(4.12)	1604			OCH_2 3.98 t J= 6Hz
	276	(4.09	1589	960	1249	$BrCH_2$ 3.48 t J= 6Hz
	378	(4.35)	1510			Ar 6.86-7.52 m
3f	240	(4.05)	1605			OCH_2 3.98 t J= 6Hz
	310	(4.44)	1573	968	1253	$BrCH_2$ 3.48 t J= 6Hz
	328	(4.47)	1511			Ar 6.86-7.45 m

Scheme 3



m/z 95 [$C_4H_3N_2O$] (ion **e**, Table 8, Scheme 2; ion **g** Table 9, Scheme 3) and in the mass spectra of (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5d**,**5f**,**5h**,**5j**,**5l**) at m/z 109 [$C_5H_5N_2O$] (ion **e**, Table 8, Scheme 2; ion **g** Table 9, Scheme 3). The presence in the mass spectra of **5b**, **5d**, **5f**, **5h**, **5j** and **5l** the even-electron fragment ions situated at m/z 109 [$C_5H_5N_2O$] allows one to identify the presence of the 6-methyl group on the uracil moiety of these compounds.

It was found that the cleavage of the Csp^3-O bond in the thioalkoxy chain of **5a**-**5l** follows also McLafferty rearrangement involving the migration of a hydrogen atom to a double bond of the phenyl ring of the (*E*)-stilbene skeleton. Odd-electron fragment ions **c** (Scheme 1) or **b** (Scheme 2) were obtained by this fragmentation pathways. The differences in the fragmentation of metameric pairs of (*E*)-2-stilbenyloxyalkylthiouracils (**5a**,**5c**,**5e**,**5g**,**5i**,**5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5d**,**5f**,**5h**,**5j**,**5l**) *i.e.* **5b** and **5c**; **5f** and **5g**; **5h** and **5i** have been quantified by comparing the calculated values of the coefficients μ_1 and μ_2 *i.e.* the abundances of the selected fragment ions relative to the abundances of the molecular ions.

$$\mu_1 = \frac{\%\ \text{rel. int. } \mathbf{d}^+}{\%\ \text{rel. int. } \mathbf{a}^{+\cdot}} \qquad \mu_2 = \frac{\%\ \text{rel. int. } \mathbf{c}^+}{\%\ \text{rel. int. } \mathbf{a}^{+\cdot}}$$

It has been established by us previously that the differences in the values of coefficients μ are useful for differentation of the positional isomers of benzylthiouracils [35-37].

As can be seen from the data in Table 10, the differences between the values of the coefficients μ_1 and μ_2 [*i.e.* the calculated quotients of the relative intensities of the selected fragment ions **d** (μ_1) and **c** (μ_2) as well as molecular ions M$^{+\cdot}$ **a**] are sufficient to differentiate isomers. The (*E*)-2-stilbenyloxyalkylthiouracils (**5b**, **5f**, **5h**) are distinguished from metameric (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5c**, **5g**, **5i**) on the basis of the lower values of μ_1 and the higher values of μ_2.

Table 4

UV/VIS, IR and ^{1}H NMR Data of **5a-l**

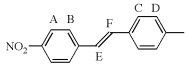
Comp.	UV/VIS	IR (cm^{-1})				^{1}H NMR δ (ppm)	
	λ max (lg ε)	νC=C	νC=O	HC=CH	νSCH_2		
			trans			**5e-j**	
5a	292.5 (4.43) 318.5 (4.47)	1605 1535 1511	1658	961	2443	OCH_2 4.45 t J= 6Hz SCH_2 3.74 t J= 6Hz CVI H 8.00 d J= 7Hz CV H 6. 52 d J= 7Hz Ar 6.90-7.49 m	
5b	292.5 (4.45) 318.5 (4.47)	1603 1577 1510	1648	962	2453	OCH_2 4.45 t J= 6Hz SCH_2 3.75 t J= 6Hz CV H 6.43 s CVI CH_3 2.49 s Ar 6.93-7.51 m	
5c	292.5 (4.45) 318.5 (4.44)	1602 1595 1511	1703	968	2449	OCH_2 4.50 t J= 6Hz SCH_2 3.50 t J= 6Hz CVI H 8.07 d J= 7Hz CV H 6. 54 d J= 7Hz Ar 6.93-7.51 m	
5d	292.5 (4.44) 318.5 (4.45)	1604 1592 1511	1644	967	2445	OCH_2 4.42 t J= 6Hz SCH_2 3.61 t J= 6Hz CV H 6.86 s CVI CH_3 2.39 s Ar 6.93-7.51 m	
5e	265.0 (4.32) 359.5 (4.17)	1603 1587 1511	1705	970	2445	OCH_2 4.16 t J= 6Hz SCH_2 3.53 t J= 6Hz CVI H 7.49 d J= 7Hz CV H 6.53 d J= 7Hz	d_A 8.20 J=9Hz d_B 7.61 J=9Hz d_C 6.95 J=9 Hz d_D 9.92 J=9Hz d_E 6.97 J=16Hz d_F 7.24 J=16Hz
5f	266.0 (3.88) 359.5 (3.72)	1605 1587 1511	1635	968	2446	OCH_2 4.19 t J= 6Hz SCH_2 3.43 t J= 6Hz CV H 6.84 s CVI CH_3 2.29 s	d_A 8.20 J=9Hz d_B 7.60 J=9Hz d_C 6.94 J=9 Hz d_D 9.91 J=9Hz d_E 6.99 J=16Hz d_F 7.23 J=16Hz
5g	271.0 (4.27) 361.0 (4.08)	1605 1589 1511	1657	964	2449	OCH_2 4.11 t J= 6Hz SCH_2 3.43 t J= 6Hz CVI H 7.49 d J= 7Hz CV H 6.60 d J= 7Hz	d_A 8.21 J=9Hz d_B 7.61 J=9Hz d_C 6.95 J=9Hz d_D 6.91 J=9Hz d_E 6.99 J=16Hz d_F 7.24 J=16Hz
5h	270.0 (4.29) 359.5 (4.09)	1605 1589 1512	1644	961	2453	OCH_2 4.06 t J= 6Hz SCH_2 3.45 t J= 6Hz CVI H 6.49 s CV CH_3 2.30 s	d_A 8.19 J=9Hz d_B 7.58 J=9Hz d_C 6.94 J=9Hz d_D 6.91 J=9Hz d_E 6.97 J=16Hz d_F 7.23 J=16Hz
5i	271.5 (4.30) 362.5 (4.12)	1607 1585 1511	1668	987	2452	OCH_2 4.03 t J= 6Hz SCH_2 3.63 t J= 6Hz CVI H 7.49 d J= 7Hz CV H 6.53 d J= 7Hz	d_A 8.21 J=9Hz d_B 7.59 J=9Hz d_C 6.96 J=9Hz d_D 6.91 J=9Hz d_E 6.94 J=16Hz d_F 7.23 J=16Hz
5j	271.0 (4.28) 360.5 (4.05)	1605 1590 1510	1655	958	2455	OCH_2 4.00 t J= 6Hz SCH_2 3.41 t J= 6Hz CVI H 6.49 s CV CH_3 2.24 s	d_A 8.21 J=9Hz d_B 7.59 J=9Hz d_C 6.94 J=9Hz d_D 6.91 J=9Hz d_E 6.94 J=16Hz d_F 7.22 J=16Hz

Table 4(continued)

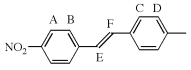
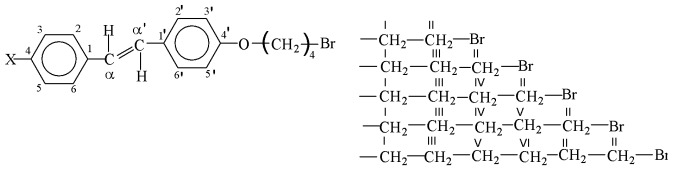
Comp.	UV/VIS λ max (lg ε)	IR (cm^{-1}) νC=C	νC=O	HC=CH	νSCH_2	^1H NMR δ (ppm) 
			trans			**5e-j**
5k	292.5 (4.40) 326.0 (4.46)	1604 1592 1511	1659	968	2401	OCH_2 4.11 t J= 6Hz SCH_2 3.38 t J= 6Hz CVI H 7.45 d J= 7Hz CV H 6.63 d J= 7Hz	d_A 8.07 J=9Hz d_B 7.43 J=9Hz d_C 7.16 J=9H d_D 6.76 J=9Hz d_E 6.92 J=16Hz d_F 7.29 J=16Hz
5l	293.0 (4.37) 323.0 (4.41)	1604 1575 1511	1648	967	2669	OCH_2 4.03 t J= 6Hz SCH_2 3.26 t J= 6Hz CV H 6.37 s CVI CH_3 2.46 s	d_A 7.43 J=9Hz d_B 6.96 J=9Hz d_C 6.90 J=9Hz d_D 6.63 J=9Hz d_E 7.00 J=16Hz d_F 7.64 J=16Hz

Table 5

^{13}C NMR Shifts of **3a**-**3f**



Carbon	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**
C-1	137.55	137.63	144.30	144.14	144.31	136.20
C-2,6	127.10	126.78	128.42	128.31	128.43	128.87
C-3,5	128.67	128.64	124.92	124.04	124.15	128.76
C-4	127.34	127.25	146.48	146.25	146.40	132.64
C-α	126.31	126.26	124.17	123.99	124.03	127.77
C-α'	128.02	126.16	132.90	132.76	132.92	125.14
C-1'	130.96	130.45	129.13	128.87	128.89	129.65
C-2',6'	127.81	127.75	128.42	126.38	126.48	127.36
C-3',5'	115.01	114.76	114.92	114.73	114.84	114.72
C-4'	157.79	158.43	159.29	159.35	159.68	158.98
C-I	67.94	65.39	68.87	66.94	67.66	67.76
C-II	28.97	29.91	29.83	29.47	28.93	29.02
C-III	-	32.34	32.48	33.46	33.56	32.62
C-IV	-	-	-	27.90	28.34	27.87
C-V	-	-	-	-	24.77	25.24
C-VI	-	-	-	-	-	33.71

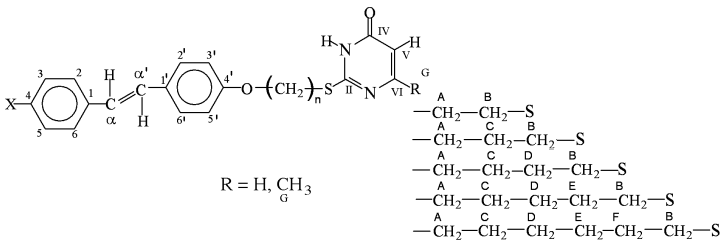
The fluorescence properties of compounds **5a**-**5l** have been investigated, the fluorescence emission maxima and quantum yields are summarized in Table 4. The (*E*)-2-stilbenyloxyalkylthiouracils (**5a**,**5c**,**5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5d**,**5l**) are moderately fluorescent, having emission bands at 370-380 nm with the main excitation peak at 320-360 nm at dioxane. The (*E*)-2-[4'-nitrostilbenyl-4-oxyalkylthio]-6-methyluracils (**5f**,**5h**,**5j**) are highly fluorescent, having emissions bands at 520 nm with the main excitation peak at 360 nm in dioxane. Clearly, the substitution of (*E*)-stilbenylaxyalkyl group at the angular sulfur atom of 2-thiouracil (or 2-thio-6-methyluracil) serves as a useful method of converting these compounds into fluorescent derivatives.

Conclusions.

- It follows from the obtained results that the fluorescence of **5a** - **5l** can be attributed to the introduced fluorophore *i.e.* (*E*)-stilbenyl moiety.

Table 6

^{13}C NMR Shifts of **5a**-**5f**



R = H, CH_3 (G)

Carbon	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**
C-1	136.06	137.50	137.44	137.31	146.18	146.49
C-2,6	126.32	126.41	127.35	126.29	128.34	126.51
C-3,5	128.14	128.69	127.81	129.72	124.46	124.16
C-4	127.61	127.37	127.35	128.34	146.12	146.30
C-α	126.23	126.34	126.20	126.64	136.50	132.97
C-α'	128.00	127.81	127.47	129.23	124.13	124.31
C-1'	128.65	128.74	128.71	129.83	129.53	128.57
C-2',6'	127.75	127.71	127.71	128.94	129.16	126.56
C-3',5'	114.16	114.75	114.88	115.75	114.81	115.04
C-4'	155.62	157.76	157.60	159.35	160.09	159.92
C-II	168.89	167.05	166.4	169.02	165.65	165.70
C-IV	161.18	160.63	161.56	161.70	160.65	164.56
C-V	108.67	108.86	108.85	109.30	109.11	108.42
C-VI	145.35	162.24	149.76	162.84	146.00	160.01
C_A	67.31	67.44	65.61	67.50	65.74	65.98
C_B	32.63	32.54	28.51	28.57	28.47	28.87
C_C	-	-	28.24	29.42	28.34	27.32
C_D	-	-	-	-	-	-
C_E	-	-	-	-	-	-
C_F	-		-	-	-	-
C_G	-	19.17	-	18.94	-	24.05

- (*E*)-4'-Nitrostilbenyl-4-oxyalkylthiouracils (**5e**,**5g**,**5i**) and (*E*)-4'-nitrostilbenyl-4-oxyalkylthio-6-methyluracils (**5f**,**5h**,**5j**) may well be the best fluorescent derivatives of uracil yet prepared.
- (*E*)-Configuration in the stilbene part of the molecules of **5a**-**5l** can be determined on the basis of the analysis of UV/VIS and IR spectra of these compounds.
- The presence of the 6-methyl substituent in the uracil ring of (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5d**,**5f**,**5h**,**5j**,**5l**) can be identified on the basis of analysis of ^{1}H NMR, (Table 4), ^{13}C NMR (Table 6) and EI mass spectra (Tables 8 and 9) of these compounds.
- The differences in the ^{13}C NMR spectra of **5a**-**5l** in the number and positions in the range 160-170 ppm of the C II, C IV and C VI carbon atoms of uracil ring, allow the differentiation of metamers **5b**, **5c** and **5f**, **5g** as well as **5h**, **5i**.
- The basic mass fragmentation of the molecular ion of **5a**-**5l** is due to cleavages of Csp^3–O bonds of oxyalkylthio chains of (*E*)-stilbenyloxy-alkylthiouracils.
- The inductive cleavage of these bonds produces even electron fragment ions **d**. The cleavage of these bonds according to McLafferty rearrangement produces odd-electron fragment ions **c** (Schemes 2,3; Tables 9, 10).
- The values of μ_1 and μ_2 (*i.e.* the ratio of intensities of **d** and **c** fragment ions peaks to those of the molecular ion peaks $M^{+\cdot}$) depend on the structure of (*E*)-2-stilbenyloxyalkylthiouracils (**5c**,**5g**,**5i**) and (*E*)-stilbenyloxyalkylthio-6-methyluracils (**5b**,**5f**,**5h**). The differences in the values of μ_1 and μ_2 are useful for differentiation between metamers of these compounds derivatives of uracil (**5b**,**5f**,**5h**) and 6-methyluracil (**5c**,**5g**,**5i**).

EXPERIMENTAL

The purity of all described compounds was checked by m.p.'s, TLC and elemental analysis. Melting points (uncorrected) were determined on a Böetius microscope hot stage. R_f values refer to TLC silica gel F_{254} TLC plates (Merck) developed with $CHCl_3$ – *n*-hexane 4:1 (**3a**-**3f**) and $CHCl_3$ - MeOH 5:1 (**5a**-**5l**) and observed under UV light (λ=254 and 366 nm). UV/VIS spectra were recorded with a Specord UV/VIS spectrophotometer in dioxane. IR spectra were recorded with a FT-

Table 7

^{13}C NMR Shifts of **5g**-**5l**

Carbon	**5g**	**5h**	**5i**	**5j**	**5k**	**5l**
C-1	144.31	146.49	146.27	144.18	135.89	136.20
C-2,6	123.83	126.51	126.38	126.39	127.88	127.87
C-3,5	124.04	124.16	123.98	123.98	128.78	130.03
C-4	146.22	146.30	146.27	144.18	132.92	132.79
C-α	132.83	132.97	132.77	132.86	126.06	125.63
C-α'	124.15	124.16	124.05	124.07	128.50	128.84
C-1'	129.14	129.14	128.85	128.83	131.17	130.35
C-2',6'	126.47	128.41	124.31	128.32	127.41	127.44
C-3',5'	116.00	114.89	114.77	114.80	115.49	115.02
C-4'	160.00	159.41	159.41	159.92	157.38	158.60
C-II	161.70	165.70	165.65	165.40	163.91	157.15
C-IV	160.89	164.56	164.86	163.98	161.88	160.87
C-V	109.68	108.42	108.22	108.33	109.45	109.03
C-VI	146.18	160.30	146.0	160.92	145.52	165.09
C_A	67.72	65.48	67.26	67.73	69.22	68.16
C_B	31.27	28.47	30.24	30.57	31.65	31.08
C_C	28.56	28.34	28.17	28.86	28.59	28.82
C_D	-	-	25.87	25.20	25.04	25.21
C_E	-	-	-	28.75	27.93	28.56
C_F	-	-	-	-	27.67	27.99
C_G	-	24.05	-	24.23	-	21.54

Table 8

Elemental Composition and Relative Intensities of the Ion Peaks in the spectra of **5a**-**5d** and **5k**-**l** According to High Resolution Data

Ion	m/z	Elemental compositon	% Relative intensity					
			5a	**5b**	**5c**	**5d**	**5k**	**5l**
M+· a	350	$C_{20}H_{18}N_2O_2S$	10	-	-	-	-	-
	364	$C_{21}H_{20}N_2O_2S$	-	7	18	-	-	-
	378	$C_{22}H_{22}N_2O_2S$	-	-	-	28	-	-
	440	$C_{24}H_{25}N_2O_2SCl$	-	-	-	-	72	-
	454	$C_{25}H_{27}N_2O_2SCl$	-	-	-	-	-	18
b	222	$C_{16}H_{14}O$	7	8	-	-	-	-
	236	$C_{17}H_{16}O$	-	-	3	6	-	-
	312	$C_{20}H_{21}OCl$	-	-	-	-	4	3
c	196	$C_{14}H_{12}O$	27	14	14	22	-	-
	230	$C_{14}H_{11}OCl$	-	-	-	-	77	100
d	155	$C_6H_7N_2OS$	100	-	-	-	-	-
	169	$C_7H_9N_2OS$	-	100	100	-	-	-
	183	$C_8H_{11}N_2OS$	-	-	-	100	-	-
	211	$C_{10}H_{15}N_2OS$	-	-	-	-	100	-
	225	$C_{11}H_{17}N_2OS$	-	-	-	-	-	27
e	95	$C_4H_3N_2O$	13	-	7	-	13	-
	109	$C_5H_5N_2O$	-	11	-	6	-	5
f	70	C_3H_4NO	8	-	7	-	20	-
	84	C_4H_6NO	-	9	-	10	-	25

IR Bruker IFS – 113 v spectrophotometer in KBr pellets. The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Mercury Spectrometer operating at 300.07 MHz (proton or 75.46 MHz (carbon). Data were obtained from $CDCl_3$ solutions. The chemical shifts were referenced to tetramethylsilane. Chemical shifts are given in the δ scale (ppm) and coupling constants in Hertz. ^{1}H NMR (300.07) spectra were recorded with spectral width 9 KHz, acquisition time 2.0 s, pulse width 6 μs and double precision acquisition. ^{13}C NMR (75.460 MHz) spectra were recorded with spectral width 18.76 KHz, acquisition time 1.0 s, recycle delay 1.0 s and pulse width 15 μs. Heteronuclear 2D ^{13}C NMR- ^{1}H NMR chemical shift correlation experiments were carried out using HETCOR spectra. The spectra were acquired with 2K data points, 256 increments and spectral width 19.63 KHz for ^{13}C and 4.97 KHz for ^{1}H.

Elemental analyses were performed with a Vector Euro EA 3000 analyzer. Low- and high-resolution mass spectra were recorded on an AMD - Intectra GmbH-Harpstedt D-27243 Model 402 two - sector mass spectrometer (ionizing voltage 70 eV, accelerating voltage 8 kV, resolution 10,000). Samples were introduced by a direct insertion probe at the source temperature

Table 9

Elemental Composition and Relative Intensities of the Ion Peaks in the Spectra of **5e**-**5j** According to High Resolution Data

Ion	m/z	Elemental compositon	% Relative intensity 5e	5f	5g	5h	5i	5j
M+· a	409	$C_{21}H_{19}N_3O_4S$	3	-	-	-	-	-
	423	$C_{22}H_{21}N_3O_4S$	-	6	16	-	-	-
	437	$C_{23}H_{23}N_3O_4S$	-	-	-	4	27	-
	451	$C_{24}H_{25}N_3O_4S$	-	-	-	-		20
b	211	$C_{13}H_9NO_2$	37	11	9	7	14	7
c	241	$C_{14}H_{11}NO_3$	30	12	14	9	45	26
d	169	$C_7H_9N_2OS$	100	-	-	-	-	-
	183	$C_8H_{11}N_2OS$	-	100	100	-	-	-
	197	$C_9H_{13}N_2OS$	-	-	-	100	100	-
	211	$C_{10}H_{15}N_2OS$	-	-	-	-	-	93
e	165	$C_{13}H_9$	59	25	22	19	30	24
f	152	$C_{12}H_8$	11	11	6	3	8	7
g	109	$C_5H_5N_2O$	-	12	-	16	-	40
	95	$C_4H_3N_2O$	13	-	35	-	25	-

Table 10

Values of μ Calculated from the EI Mass Spectra of Metameric **5b**, **5c**, **5f**, **5g**,**5h**, **5i**

Comp	**5b**	**5c**	**5f**	**5g**	**5h**	**5i**
μ_1	0.7	1.8	0.6	1.6	0.4	2.7
μ_2	2.0	0.7	2.0	0.8	2.25	1.66

Table 11

UV/VIS and Fluorescence Spectra of **5a**-**5l**

Comp.	UV/VIS λ max[nm], (log ε) (dioxane)	Fluorescence Excitation wave [nm] (dioxane)	Emission wave [nm] (dioxane)	Quantum yields [ϕ]
5a	303.0 (4.53)	320	370	0.01
	320.0 (4.49)			
5b	304.5 (4.51)	320	370	0.01
	320.0 (4.49)			
5c	303.5 (4.53)	320	380	0.01
	320.0 (4.49)			
5d	304.5 (4.50)	320	370	0.01
	320.5 (4.48)			
5e	265.0 (4.32)	360	520	0.002
	359.0 (4.17)			
5f	266.0 (4.32)	360	530	0.002
	359.0 (3.72)			
5g	271.0 (4.27)	360	520	0.002
	261.0 (4.08)			
5h	270.0 (4.29)	360	520	0.003
	259.5 (4.09)			
5i	271.9 (4.30)	360	520	0.002
	362.5 (4.12)			
5j	271.9 (4.28)	360	520	0.003
	360.5 (4.05)			
5k	308.0 (4.50)	326	380	0.01
	326.5 (4.50)			
5l	308.5 (4.53)	326	380	0.01
	326.0 (4.54)			

of ~150 °C. The elemental composition of the ions were determined by a peak matching method relative to perfluorokerosene and using the same instrument. All masses measured were in agreement with those of the composition given in column 3 of Tables 8 and 9 to within ± 2 ppm. The B/E linked scan spectra in the first field - free region were investigated using helium as the collision gas at a pressure of 1.73 x 10^{-5} with the ion source temperature of 180 °C, ionization energy of 70 eV and an accelerating voltage of 8 kV. The values of μ_1 and μ_2 were calculated as averages of three measurements. Fluorescence corrected spectra were taken in dioxane (isocratic grade for liquid chromatography-Merck) with a Perkin Elmer MPF-3 instrument. Fluorescence quantum yields (in the same solvent) were calculated on the basis of the value 0.55 for quinine sulfate. (*E*)-Stilbenol-4, (*E*)-4'-nitostilbenol-4 and *E*-4'-chlorostilbenol-4 were obtained according to the literature [38].

General Procedure for the Preparation of (*E*)-Bromoalkoxystilbenes **(3a-3f)**.

A 20 ml solution of (*E*)-stilbenol-4, (2 mmole) or (*E*)-4'-nitrostilbenol-4, (*E*)-4'-chloro-stilbenol-4) in of 0.3 *M* NaOH in DMSO was stired at room temperature while 4 mmole of dibromoalkanes (1,2-dibromoethane; 1,3-dibromopropane; 1,4-dibromobutane; 1,5-dibromo- pentane; 1,6-dibromohexane) was added dropwise. After stiring for 3 hours precipitated solid was filtered. The filtrate was then acidified (pH 3) with 6 *M* HCl and diluted with 80 ml of cold water. The precipitated solid was collected by filtration, washed with water and dried at room temperature under vacuum. Recrystallization from ethanol afforded compounds **3a**-**3f**.

General Procedure for the Preparation of (*E*)-2-Stilbenyloxyalkylthiouracils **(5a-5l)**.

2-Thiouracil (or 2-thio-6-methyluracil) (0.07 mole) in 10 ml of 0.3 *M* methanolic NaOH was stirred in room temperature while 0.07 mole of corresponding (*E*)-bromoalkoxystilbene (**3a-3f**) in 10 ml of acetone solution was added dropwise. The reaction mixture was stirred at room temperature for 8 hours. The obtained solid was filtered, the filtrate was acidified (pH 3) with 6 *M* HCl and diluted with 70 ml of cold water. The precipitated solid was collected by filtration, washed with water and dried at room temperature under vacuum. Recrystallization from ethanol afforded compounds **5a-5l**.

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