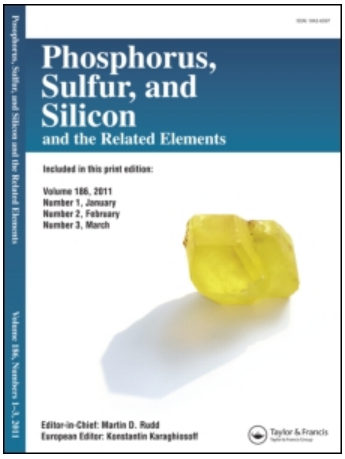

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New Fluorescent 2- Ortho -(Meta - and Para -) Chloro-(and Bromo-) Benzylthio- N -Phenylcytosines and 6-Methylcytosines

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NEW FLUORESCENT 2-*ORTHO*-(*META*- AND *PARA*-) CHLORO-(AND BROMO-) BENZYLTHIO-*N*-PHENYLCYTOSINES AND 6-METHYLCYTOSINES

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Twelve new fluorescent ortho-, meta-, *and* para-, *chloro-(bromo-) substituted derivatives of 2-benzylthio*-N-*phenylcytosines and 6-methylcytosines have been prepared. EI induced mass spectral fragmentation of these compounds was investigated. Fragmentation pathways are proposed on the basis of accurate mass and metastable transitions 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 isomers. The* ^{1}H *and* ^{13}C *NMR spectra of these compounds were assigned unambiguously using a combination of heternuclear (HETCOR) spectra and the chemical shifts. The data derived from these spectra can be used to differentiate the isomers.*

Keywords: ^{13}C NMR; fluorescence; ^{1}H NMR; 2-*ortho*-(*meta*- and *para*-) chloro-(bromo-) benzylthio-*N*-phenylcytosines; structural isomers

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 dynamics.[12–14] Many have been shown to enter biochemical pathways and have given the indications about their binding to different enzymes.[15–16] The fluorescent derivatives of cytosine and cytidine have been reported widely in literature.[11,17–20] However, to the best of our knowledge, no work has been published on the synthesis and physicochemical properties of fluorescent derivatives of 2-thiocytosine. This fact has stimulated us to prepare a series of substituted derivatives of 2-*ortho*-(*meta*- and

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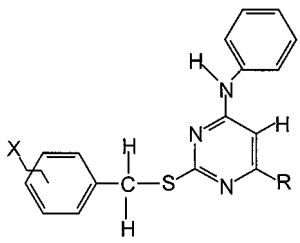
para-) chloro-(bromo-) benzylthio-*N*-phenylcytosines [**1–3**, **7–9**] and 2-*ortho*-(*meta*- and *para*-) chloro-(bromo-) benzylthio-*N*-phenyl-6-methylcytosines [**4–6**, **10–12**] with the idea that these compounds bearing two phenyl and one pyrimidine aromatic ring in the molecules would certainly be fluorescent.

This article deals with the synthesis and physicochemical properties of **1–12**. The UV/VIS, IR, ^{1}H NMR, and ^{13}C NMR as well as EI mass spectra of 2-thio-*N*-phenylcytosine **I** and 2-thio-*N*-phenyl-6-methylcytosine **II** also have been investigated, because, to the best of our knowledge, no work has been published on their spectral analysis. The analyses of ^{13}C NMR and EIMS mass spectra of **1–12** have been performed to check the possibility of differentation of positional isomers. We establish whether it is possible to determine the position of chloro-(or bromo-) substituents in the phenyl ring on the basis of differences in the values of μ, i.e., 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.[21–23]



1 - 12

1 R = H o - Cl
2 R = H m - Cl
3 R = H p - Cl

4 R = CH_3 o - Cl
5 R = CH_3 m - Cl
6 R = CH_3 p - Cl

7 R = H o - Br
8 R = H m - Br
9 R = H p - Br

10 R = CH_3 o - Br
11 R = CH_3 m - Br
12 R = CH_3 p - Br

FIGURE 1 The list of structures of compounds **1–12**.

RESULTS AND DISCUSSION

A few series of twelve new fluorescent *ortho*-(*meta*- and *para*-) chloro-(and bromo-) substituted 2-benzylthio-*N*-phenylcytosines (**1–3**, **7–9**) and 2-benzylthio-*N*-phenyl-6-methyl-cytosines (**4–6**, **10–12**) have been synthesized in the reaction of 2-thio-*N*-phenylcytosine (or 2-thio-*N*-phenyl-6-methylcytosine) with corresponding benzyl halides. Treatment of 2-thio-*N*-phenylocytosine (or 2-thio-*N*-phenyl-6-methylcytosine) with *o*-(*m*- and *p*-) chloro-(and bromo-) benzyl bromides (or chlorides) in 0.1 M solution of NaOH in methanol at room temperature afforded **1–12** (Figure 1).

The structures of all compounds obtained were determined by examining their UV/VIS, IR, ^{1}H NMR, and ^{13}C NMR spectra as well as on the basis of elemental analyses (Tables I–V). The obtained *N*-phenyl-2-thiocytosine (**I**) and *N*-phenyl-2-thio-6-methyl-cytosine (**II**) as well as 2-benzylthio-*N*-phenylcytosines (**1–3**, **7–9**) and 2-benzylthio-*N*-phenyl-6-methylcytosines (**4–6**, **10–12**) may appear theoretically in

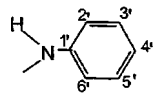
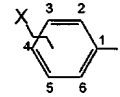
TABLE I Chemical and Physical Data of Compounds **1–12**

Comp.	R	X	Formula (mol. wt.)	m. p. [°C]	Yield (%)	R_f TLC	Reaction time [h]
1	H	o—Cl	$C_{17}H_{14}N_3SCl$ 327.83	155–157	80	0.80	6
2	H	m—Cl	$C_{17}H_{14}N_3SCl$ 327.83	146–148	82	0.80	6
3	H	p—Cl	$C_{17}H_{14}N_3SCl$ 327.83	153–155	82	0.84	6
4	CH_3	o—Cl	$C_{18}H_{16}N_3ClS$ 341.86	160–162	76	0.70	6
5	CH_3	m—Cl	$C_{18}H_{16}N_3ClS$ 341.86	119–121	71	0.90	6
6	CH_3	p—Cl	$C_{18}H_{16}N_3ClS$ 341.86	139–141	80	0.89	6
7	H	o—Br	$C_{17}H_{14}N_3SBr$ 372.28	143–146	79	0.83	8
8	H	m—Br	$C_{17}H_{14}N_3SBr$ 372.28	147–149	70	0.81	8
9	H	p—Br	$C_{17}H_{14}N_3SBr$ 372.28	163–165	74	0.87	8
10	CH_3	o—Br	$C_{18}H_{16}N_3SBr$ 386.31	205–207	82	0.86	8
11	CH_3	m—Br	$C_{18}H_{16}N_3SBr$ 386.31	159–162	69	0.83	8
12	CH_3	p—Br	$C_{18}H_{16}N_3SBr$ 386.31	147–150	71	0.90	8

TABLE II Elemental Analyses and UV/VIS, and IR Data of Compounds **1–12**

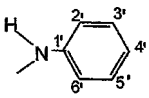
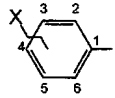
Comp.	UV/VIS λ_{max} [nm] (log ε) DMSO	IR ν [cm^{-1}] ν S-CH_2	ν C5-C6	δ NH	Elemental analysis (%) Calcul. C	H	N	Found C	H	N
1	302.5 (4.1)	2978	1631.0	1560.8	62.38	4.28	12.84	62.02	4.21	12.69
2	303.0 (4.1)	2970	1619.4	1562.2	62.38	4.28	12.84	62.49	4.50	13.10
3	302.5 (4.2)	2973	1604.7	1569.9	62.38	4.28	12.84	62.41	4.39	12.60
4	302.0 (4.1)	2885	1622.4	1571.2	63.34	4.69	12.31	63.15	4.50	12.10
5	302.0 (4.2)	2857	1621.6	1570.5	63.34	4.69	12.31	63.30	4.65	12.28
6	301.0 (4.2)	2973	1620.2	1571.2	63.34	4.69	12.31	63.25	4.72	12.60
7	301.5 (4.2)	2950	1619.9	1570.9	54.80	3.76	11.29	54.60	3.88	11.50
8	302.0 (3.72)	2936	1632.4	1560.5	54.80	3.76	11.29	54.86	3.80	11.36
9	303.0 (4.2)	2977	1631.2	1560.5	54.80	3.76	11.29	54.58	3.62	11.20
10	301.5 (4.0)	2962	1621.2	1570.1	55.95	4.14	10.88	55.58	4.36	10.48
11	301.5 (4.1)	2943	1612.7	1571.8	55.95	4.14	10.88	55.82	4.26	10.69
12	301.5 (4.2)	2978	1612.3	1567.8	55.95	4.14	10.88	55.74	3.92	10.54

TABLE III ^{1}H-NMR Shifts of **1–6**[a]

Comp.	S-CH_2 (s)	CV-H (d)	CV-H (s)	CVI-H (d) CVI-CH_3 (s)		
1	4.46	6.56	—	8.14	C-2′, 6′ H d 7.60	C-3 H d 7.71 C-4 H t 7.47
				—	C-3′, 5′ H t 7.40	C-5 H t 7.29 C-6 H d 7.19
					C-4′ H t 6.60	
2	4.44	6.76	—	8.16	C-2′, 6′ H d 7.60	C-2 H s 7.71 C-4 H t 7.47
				—	C-3′, 5′ H t 7.40	C-5 H t 7.29 C-6 H d 7.19
					C-4′ H t 6.60	
3	4.36	6.54	—	8.12	C-2′, 6′ H d 7.60	C-2, 6 H d 7.46
				—	C-3′, 5′ H t 7.40	C-3, 5 H d 7.36
					C-4′′ H t 6.60	
4	4.44	—	6.53	—	C-2′, 6′ H d 7.60	C-3 H d 7.70 C-4 H t 7.46
				2.27	C-3′, 5′ H t 7.40	C-5 H t 7.30 C-6 H d 7.20
					C-4′ H t 6.60	
5	4.35	—	6.38	—	C-2′, 6′ H d 7.60	C-2 H s 7.70 C-4 H t 7.45
				2.26	C-3′, 5′ H t 7.40	C-5 H t 7.20 C-6 H d 7.30
					C-4′ H t 6.60	
6	4.35	—	6.37	—	C-2′, 6′ H d 7.60	C-2, 6 H d 7.76
				2.26	C-3′, 5′ H t 7.40	C-3, 5 H d 7.36
					C-4′ H t 6.60	

[a]Spectra determined in dimethyl –d_6 sulfoxide at 25°C and shifts are reported in ppm (δ) downfield from tetramethylsilane.

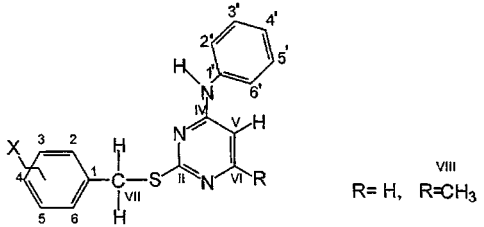
TABLE IV ^{1}H-NMR Shifts of **7–12**[a]

Comp.	S-CH_2 (s)	CV-H (d)	CV-H (s)	CVI-H (d) CVI-CH_3 (s)			
7	4.45	6.54	—	8.13	C-2′, 6′ H d 7.60	C-3 H d 7.71	C-4 H t 7.31
				—	C-3′, 5′ H t 7.40	C-5 H t 7.29	C-6 H d 7.48
					C-4′ H t 6.60		
8	4.43	6.22	—	7.55	C-2′, 6′ H d 7.60	C-2 H s 7.70	C-4 H t 7.42
				—	C-3′, 5′ H t 7.40	C-5 H t 7.19	C-6 H d 7.34
					C-4′ H t 6.60		
9	4.33	6.50	—	8.11	C-2′, 6′ H d 7.60	C-2, 6 H d 7.49	
				—	C-3′, 5′ H t 7.40	C-3, 5 H d 7.36	
					C-4′ H t 6.60		
10	4.53	—	6.64	—	C-2′, 6′ H d 7.60	C-3 H d 7.70	C-4 H t 7.31
				2.38	C-3′, 5′ H t 7.40	C-5 H t 7.29	C-6 H d 7.48
					C-4′ H t 6.60		
11	4.35	—	6.36	—	C-2′, 6′ H d 7.60	C-2 H s 7.50	C-4 H t 7.43
				2.26	C-3′, 5′ H t 7.40	C-5 H t 7.20	C-6 H d 7.34
					C-4′ H t 6.60		
12	4.33	—	6.36	—	C-2′, 6′ H d 7.60	C-2, 6 H d 7.50	
				2.25	C-3′, 5′ H t 7.40	C-3, 5 H d 7.36	
					C-4′ H t 6.60		

[a]Spectra determined in dimethyl –d_6 sulfoxide at 25°C and shifts are reported in ppm (δ) downfield from tetramethylsilane.

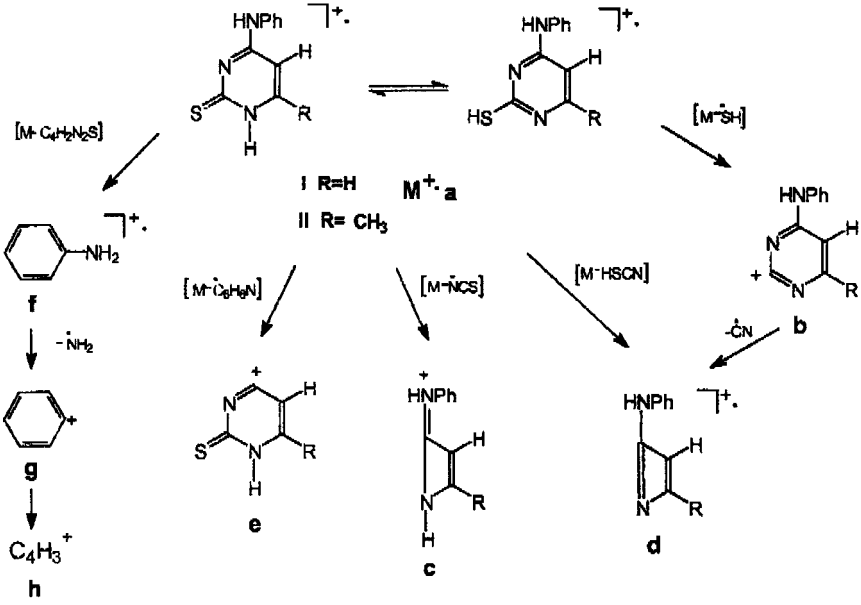
various tautomeric forms differing in the position of protons.[24] Literature provides only a few theoretical[28–34] or experimental[25–27] works on a systematic study of tautomerism of 2-thiocytosine and its derivatives. The reported results indicate that 2-thiocytosine exists predominantly in the thiol-amino tautomeric forms in the gas phase and the thione-amino tautomeric forms in solution.[34] The EI mass spectra of *N*-phenyl-2-thiocytosine **I** and *N*-phenyl-2-thio-6-methylcytosine **II** reveal the signals assigned to the fragmentaric ions [M–$\sqrt{^{\circ}SH}$], [M–$\sqrt{^{\circ}N=C=S}$] as well as [M–H– S–C≡N] (Table VIII, Scheme 1). The presence of these ions in the mass spectra of **I** and **II** suggests that the molecular ions exist in the form of the two thiol-amino and thione-amino tautomers. The UV/VIS spectra of *N*-phenyl-2-thiocytosine **I** and *N*-phenyl-2-thio-6-methylcytosine **II** as well as 2-benzylthio-*N*-phenylcytosine (**1–3**, **7–9**) and 2-benzylthio-*N*-phenyl-6-methylcytosine (**4–6**, **10–12**) show the absorption maxima at 294–303 nm (Tables II and VI). The IR spectra of **I**, **II** and **1–12** show absorption band in the region 2885–2978, which have been assigned to ν C–S. Moreover, the signals assigned to ν C5–C6 and δNH appear in the regions 1604–1632 and 1560–1571 cm^{-1} (Table II).

TABLE V ^{13}C NMR Shifts of **1–12**[a]



Carbon atom	Compounds											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
C II	169.1	168.1	168.3	168.6	168.6	168.7	169.1	169.8	169.2	168.2	168.8	168.6
C IV	159.1	159.6	159.7	164.9	164.7	164.8	159.7	159.7	159.7	164.8	164.9	164.6
C V	103.2	103.2	103.1	101.1	101.1	101.0	103.1	103.2	103	101.3	101.2	101.1
C VI	155.3	155.7	155.4	160.2	160.2	160.2	156.6	155.3	155.4	157.3	160.4	160.3
C VII	31.9	33.5	33.0	31.9	33.0	33.1	34.6	33.1	33.1	34.9	33.1	33.1
C VIII	—	—	—	23.3	23.4	23.4	—	—	—	19.3	23.3	23.2
C-1′	139.2	139.6	139.2	139.4	141.2	139.4	139.2	139.1	139.2	138.8	139.5	139.2
C-2′, 6′	120.7	121.9	120.3	120.3	120.3	120.3	120.2	120.3	120.3	120.5	120.5	120.5
C-3′, 5′	122.8	122.8	122.9	122.7	122.6	122.7	122.9	122.9	122.9	122.9	122.9	122.9
C-4′	128.7	122.8	128.7	128.7	128.6	128.7	128.8	128.9	128.7	129.0	128.9	128.8
C-1	135.3	137.4	137.4	135.5	139.4	137.6	136.9	141.3	137.8	136.1	141.7	138.1
C-2	133.3	128.6	130.5	133.3	128.7	130.6	124.1	131.3	130.9	124.0	131.7	131.2
C-3	129.4	132.9	128.2	129.3	132.7	128.2	132.6	121.4	131.2	132.6	121.5	131.0
C-4	128.9	127.2	131.6	128.9	127.4	131.4	127.7	129.6	119.9	127.8	129.8	119.9
C-5	127.6	130.2	128.2	127.2	130.1	128.2	129.3	129.7	131.2	129.7	130.5	131.0
C-6	130.9	127.5	130.5	131.0	130.1	130.6	132.6	127.7	130.9	131.8	127.9	131.2

[a]Spectra determined in dimethyl $-d_6$ sulfoxide at 25°C and shifts are reported in ppm (δ) downfield from tetramethylsilane.



SCHEME 1

The ^{1}H and ^{13}C NMR data of **1–12** are given in Tables III, V, and VI. Assignments of the ^{1}H 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.[23,35] The ^{1}H NMR spectra of **I** show dublets of CV-H, and CVI-H

TABLE VI UV/VIS, IR and ^{1}H NMR Data of Compounds N-Phenyl-2-Thiocytosine (**I**) and N-Phenyl-2-Thio-6-Methylcytosine (**II**)

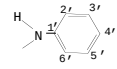
Comp.	UV/VIS λ_{max} [nm] (log ε) (CH_3CN)		IR ν [cm^{-1}] ν C=S ν C5=C6 δ NH	^{1}H NMR δ (ppm) CV-H (d) CV-H (s)	CVI-H (d) CVI-CH_3 (s)	
I	294.5	4.15	1273	6.22	7.74	C-2′, 6′ H d 7.55
	277.0	4.12	1633			C-3′, 5′ H t 7.37
			1577			C-4′ H t 7.13
II	294.5	4.17	1274	6.07	2.20	C-2′, 6′ H d 7.06
	277.5	4.14	1633			C-3′, 5′ H t 6.72
			1577			C-4′ H t 7.10

TABLE VII ^{13}C NMR Data of **I** and **II**

Carbon atom	**I**	**II**
C II	180.3	180.9
C IV	158.7	159.3
C V	98.1	96.8
C VI	142.8	154.0
CH_3	—	17.9
C-1′	138.9	138.9
C-2′, 6′	121.2	121.2
C-3′, 5′	123.7	123.7
C-4′	128.9	128.9

at 6.22 and 7.74 ppm respectively. The singlet of CV-H, and the singlet of CVI-CH3 in the ^{1}H NMR spectrum of **II** appear at 6.07 and 2.20 ppm. Moreover, the signals of protons of *N*-phenyl groups of **I** and **II** in these spectra are in the range 7.13–7.55 (**I**) and 6.72–7.10 ppm (Table VI). The ^{1}H NMR spectra of **1–12** (Tables III and IV) show singlets of CV-H, S-CH_2 as well as CVI-CH_3 protons at 6.36–6.64; 4.33–4.53 and 2.26–2.38 ppm respectively. The doublets of CVI-H appear at 7.55–8.16 ppm. The signals of protons of *ortho*-(*meta*- and *para*-) substituted benzyl groups of **1–12** appear in the range 7.19–7.76 ppm (Tables III and IV). Table V gives the ^{13}C NMR data for **1–12**, while Table VII provides data for **I** and **II**. In order to exemplify the attributions made for each compound on the basis of the analysis of HETCOR spectra, the case of **2** is discussed. For this compound the ^{1}H NMR spectrum exhibits two triplets at 7.47 and 7.29 ppm, ascribed to C–4H and C–5H respectively. The correlation between the pair of signals at 127.21 ppm and 130.22 ppm with ^{1}H NMR signals at 7.47 and 7.29 ppm allows the assignment of these signals to C-4 and C-5 respectively. Moreover, the doublet at 7.19 due to C–6H in the ^{1}H NMR spectrum correlates with the signal at 127.46 ppm in ^{13}C NMR spectrum, as well as the singlet at 7.71 ppm due to C—2H in ^{1}H NMR spectrum correlates with the signal at 128.57 ppm in ^{13}C NMR spectrum. The correlations allow the assignments of these signals to C-6 and C-2 respectively. The doublets in the ^{1}H NMR spectrum of **2** appearing at 8.16, 7.60, and 6.76 ppm ascribed to CVI-H, C—2′6′H, and CV-H correlate with the signals in ^{13}C NMR spectrum of **2** situated at 155.7, 121.9, and 103.2 ppm, respectively. These correlations allow the assignments of these signals to CVI, C—2′,6′ and CV respectively. The remaining three carbons at 33.5, 122.8, and 122.8 correspond to the singlet of S—CH_2 and triplets of C—3′, 5′H and C—4′H respectively. A comparison of the number and positions of the carbon atom signals in the range of 125–130 and 135–140 ppm in ^{13}C NMR spectra of **1–6**, as well as 115–125

and 140–145 in ^{13}C NMR spectra of **7–12** allows a differentiation between *ortho*-, *meta*-, and *para*- halo substituted in benzylthio groups isomers.

These data are given in tabular form below:

1–6 (Cl substituted isomers) 125–130 ppm and 135–140 ppm

ortho	*meta*	*para*
C–1′ 139.2 ppm	C–1′ 139.5 ppm	C–1′ 139.2 ppm
C–1 135.3 ppm	C–1 137.4 ppm	C–1 137.4 ppm
C–3 129.9 ppm	C–3′, 5′ 128.9 ppm	C–3′, 5′ 128.7 ppm
C–4′ 128.9 ppm	C–2 128.5 ppm	C–3 128.2 ppm
C–3′, 5′ 128.7 ppm	C–6 127.4 ppm	
C–5 127.6 ppm	C–4 127.2 ppm	

7–12 (Br substituted isomers) 115–125 ppm and 140–145 ppm

ortho	*meta*	*para*
C–2 124.1 ppm	C–1 141.3 ppm	C–4′ 122.9 ppm
C–4′ 122.9 ppm	C–4′ 122.9 ppm	C–2′,6′ 120.3 ppm
C–2′, 6′ 120.2 ppm	C–3′ 121.4 ppm	C–4 119.9 ppm
	C–2′, 6′ 120.3 ppm	

On the basis of the low and high resolution electron–impact as well as B/E linked scan mass spectra (Tables VIII–X), the principal mass spectral fragmentation routes of compounds **I**, **II** and **1–12** are interpreted as shown in Scheme 1 and Scheme 2. As can be seen from Scheme 1 and Table VIII the common features of the mass spectral fragmentation of the molecular ions of **I** and **II** are the eliminations of ˙SH and ˙N=C=S radicals, as well as H–S–C≡N neutral molecules. The even-electron fragment ions **b** and **c**, as well as the odd-electron fragment ion **d** are obtained on these ways of mass fragmentation. The presence in the mass spectra of **I** and **II** the fragmentaric ions **b**, **c**, and **d** suggests that, as it was mentioned above, the molecular ions of these compounds exist in the forms of thiol-amino and thione-amino tautomeric forms. The processes of mass decomposition of the molecular ions of **I** and **II** involve the inductive cleavages of the Csp2-N single bond with the subsequent eliminations of the $C_6H_6N^{\cdot}$ radicals, as well as dissociations of the same bonds accompanied by the neighbouring H-rearrangements. These cleavages lead to the even-electron fragment ion **e** and the odd-electron fragment ion **f** respectively. As can be seen from Scheme 2 and Tables IX and X, the principal mass fragmentation pathways of 2-*o*-(*m*- and *p*-) chlorobenzylthio-*N*-phenylcytosines **1–6** and 2-*o*-(*m*- and *p*-) bromobenzylthio-*N*-phenylcytosines **7–12** are similar to those of 2-benzyl-thiouracils investigated by us earlier.[21–23] The common features of the mass spectral fragmentation of the molecular ions of **1–12** are simple cleavages of Csp2-X and Csp3-S in the benzylthio substituent i.e. elimination of C_7H_6X (ions **f**) and X radicals (ions **c**). In **1–12**, during the processes of cleavages of Csp3-S bonds of the benzylthio substituent,

TABLE VIII Elemental Composition and Relative Intensities of the Ion Peaks in the Spectra of **I** i **II** According to High Resolution Data

Ion	M/z	Elemental composition	Relative intensities (%) **I**	**II**
$M^{+\cdot}$**a**	203	$C_{10}H_9N_3S$	100	—
	217	$C_{11}H_{11}N_3S$	—	100
b	170	$C_{10}H_8N_3$	13	—
	184	$C_{11}H_{10}N_3$	—	9
c	145	$C_9H_9N_2$	96	—
	159	$C_{10}H_{11}N_2$	—	78
d	144	$C_9H_8N_2$	6	—
	158	$C_{10}H_{10}N_2$	—	6
e	111	$C_4H_3N_2S$	7	—
	115	$C_5H_5N_2S$	—	2
f	93	C_6H_7N	13	13
g	77	C_6H_5	37	28
h	5	C_4H_3	19	12

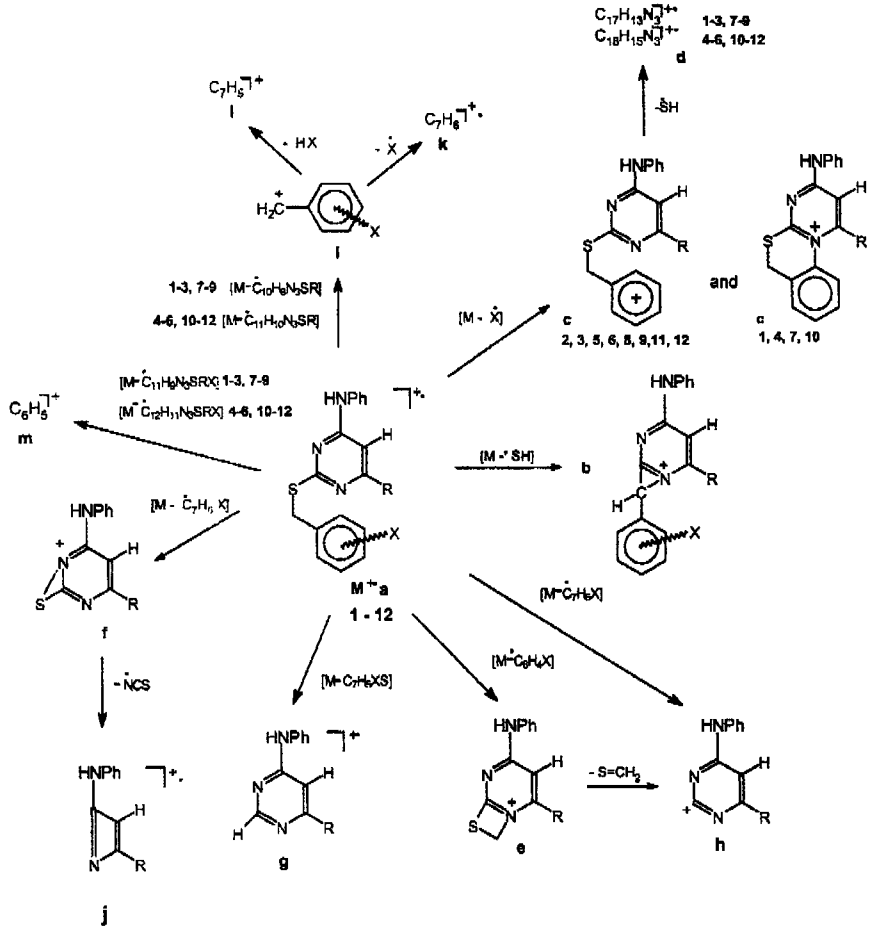
TABLE IX Elemental Composition and Relative Intensities of the Ion Peaks in the Spectra of **1–6** According to High Resolution Data

Ion	M/z	Elemental composition	Relative intensities (%) **1**	**2**	**3**	**4**	**5**	**6**
$M^{+\cdot}$**a**	327	$C_{17}H_{14}N_3SCl$	62	100	100	—	—	—
	341	$C_{18}H_{16}N_3SCl$	—	—	—	100	93	100
b	294	$C_{17}H_{13}N_3Cl$	15	44	29	—	—	—
	308	$C_{18}H_{15}N_3Cl$	—	—	—	11	14	14
c	292	$C_{17}H_{14}N_3S$	100	2	3	—	—	—
	306	$C_{18}H_{16}N_3S$	—	—	—	53	1	1
d	259	$C_{17}H_{13}N_3$	31	10	15	—	—	—
	273	$C_{18}H_{15}N_3$	—	—	—	34	4	10
e	216	$C_{11}H_{10}N_3S$	13	9	8	—	—	—
	230	$C_{12}H_{12}N_3S$	—	—	—	21	15	11
f	202	$C_{10}H_8N_3S$	7	7	6	—	—	—
	216	$C_{11}H_{10}N_3S$	—	—	—	9	6	7
g	171	$C_{10}H_9N_3$	39	38	61	—	—	—
	185	$C_{11}H_{11}N_3$	—	—	—	52	74	74
h	170	$C_{10}H_8N_3$	61	46	65	—	—	—
	184	$C_{11}H_{10}N_3$	—	—	—	93	100	95
i	125	C_7H_6Cl	31	25	37	55	31	34
j	144	$C_9H_8N_2$	5	4	5	—	—	—
	158	$C_{10}H_{10}N_2$	—	—	—	13	3	3
k	90	C_7H_6	8	8	7	11	8	7
l	89	C_7H_5	19	19	15	27	17	14
m	77	C_6H5	24	23	17	28	22	17

TABLE X Elemental Composition and Relative Intensities of the Ion Peaks in the Spectra of **7–12** According to High Resolution Data

Ion	M/z	Elemental composition	Relative intensities (%)					
			7	**8**	**9**	**10**	**11**	**12**
$M^{+\cdot}$ **a**	371/373	$C_{17}H_{14}N_3SBr$	38/39	99/100	92/93	—	—	—
	385/387	$C_{18}H_{16}N_3SBr$	—	—	—	66/68	81.82	99/100
b	338/340	$C_{17}H_{13}N_3Br$	3/3	34/35	25/24	—	—	—
	352/354	$C_{18}H_{15}N_3Br$	—	—	—	3/4	9/8	13/12
c	292	$C_{17}H_{14}N_3S$	100	3	2	—	—	—
	306	$C_{18}H_{16}N_3S$	—	—	—	100	2	1
d	259	$C_{17}H_{13}N_3$	43	23	30	—	—	—
	273	$C_{18}H_{15}N_3$	—	—	—	75	10	—
e	216	$C_{11}H_{10}N_3S$	8	13	10	—	—	—
	230	$C_{12}H_{12}N_3S$	—	—	—	19	20	12
f	202	$C_{10}H_8N_3S$	4	6	8	—	—	—
	216	$C_{11}H_{10}N_3S$	—	—	—	8	7	7
g	171	$C_{10}H_9N_3$	31	73	100	—	—	—
	185	$C_{11}H_{11}N_3$	—	—	—	37	78	81
h	170	$C_{10}H_8N_3$	42	72	80	—	—	—
	184	$C_{11}H_{10}N_3$	—	—	—	74	100	95
i	169/171	C_7H_6Br	12/11	13/12	24/23	20/19	15/14	20/18
j	144	$C_9H_8N_2$	3	5	6	—	—	—
	158	$C_{10}H_{10}N_2$	—	—	—	4	3	3
k	90	C_7H_6	19	24	31	27	25	26
l	89	C_7H_5	12	15	19	18	15	16
m	77	C_6H5	21	25	24	31	23	124

the positive charge is stabilized more effectively on the benzyl fragment (ions **i**). It was also found that the even-electron fragment ions [M-˙X] **c** in the mass spectra of **1**, **4**, **7**, and **10** have 53 and 100% relative intensity. In the mass spectra of **2**, **3**, **5**, **6**, **8**, **9**, **11**, and **12** the relative intensity of these ions is in the range 1–3%. Hence, it is obvious that the elimination of X in the cases of **1**, **4**, **7**, and **9** is strongly connected with the ortho effect. The loss of substituent radical from the ortho position of the phenyl ring is favored because it involves a formation of a very stable even-electron tricyclic ion characterized by the quaternization of N (1) of the pyrimidynyl moiety. The base peaks in the mass spectra of **2–4**, **6**, **8**, and **12** are the molecular ions **a**, as well as the even-electron fragment ions **h** ($C_{11}H_{10}N_3$-m/z 184) in the mass spectra **5**, **11** and the odd-electron fragment **g** ($C_{10}H_9N_3$-m/z 171) in the mass spectrum of **9**. It was found that even-electron fragment ions **h** are formed from the molecular ions by simple cleavage of Csp2-S bonds and eliminations XC_7H_6S. radicals, as well as the odd-electron fragment ions **g** are formed by cleavages of Csp2-S bonds accompanied by H-rearrangement and ejections



SCHEME 2

of XC_7H_5S neutral molecules. The cleavages of the Csp3-S and Csp2-S bonds of benzylthio substituent of **1–12** accompanied by eliminations of the C_7H_6X and C_6H_4X radicals produce even-electron fragment ions **f** and **e**. Both these ions may have bicyclic structure characterized by the quaternization of N-3 (or N-1) of the pyrimidynyl moiety. The differences in the fragmentation of isomeric *ortho*-(*meta*- and *para*-) substituted *N*-phenyl-2-benzylthiocytosines (**1–12**) have been quantified by comparing the calculated values of the coefficients μ_1–μ_5, i.e. the abundances of the selected fragment ions relative to the abundances of the molecular ions. It has been established by us previously that the differences in the values of coefficients μ are useful for differentation of the

TABLE XI The Values of μ_1–μ_5 Calculated from the EI Mass Spectra of **1–12**

Zwi_zek	μ_1	μ_2	μ_3	μ_4	μ_5
1	1.60	0.50	0.24	0.50	0.98
2	0.02	0.25	0.44	0.10	0.46
3	0.03	0.37	0.29	0.15	0.65
4	0.53	0.55	0.11	0.34	0.93
5	0.01	0.33	0.15	0.04	1.07
6	0.01	0.34	0.14	0.10	0.95
7	2.63	0.31	0.07	1.13	1.10
8	0.03	0.13	0.34	0.23	0.72
9	0.02	0.25	0.27	0.32	0.86
10	1.50	0.30	0.04	1.13	1.12
11	0.02	0.18	0.11	0.12	1.23
12	0.01	0.20	0.13	0.17	0.95

$$\boldsymbol{\mu_1} = \frac{\%\ \text{rel. int. c}^{]+}}{\%\ \text{rel. int. a}^{]+\cdot}};\ \boldsymbol{\mu_2} = \frac{\%\ \text{rel. int. j}^{]+\cdot}}{\%\ \text{rel. int. a}^{]+\cdot}};$$
$$\boldsymbol{\mu_3} = \frac{\%\ \text{rel. int. b}^{]+}}{\%\ \text{rel. int. a}^{]+\cdot}};\ \boldsymbol{\mu_4} = \frac{\%\ \text{rel. int. d}^{]+\cdot}}{\%\ \text{rel. int. a}^{]+\cdot}};$$
$$\boldsymbol{\mu_5} = \frac{\%\ \text{rel. int. i}^{]+}}{\%\ \text{rel. int. a}^{]+\cdot}}.$$

positional isomers of 2- and 4-substituted alkylthiouracils [36] as well as benzylthiouracils.[21–23,37]

As can be seen from the data in Table XI, the differences between the relative intensities of the peaks of selected fragment ions and $M^{+\cdot}$ ions i.e., the values of μ_1–μ_5 for 2-*ortho*-(*meta*- and *para*-) chloro- (and bromo-) substituted 2-benzylthio-*N*-phenylcytosines **1–12** are sufficient to differentiate isomers. The *ortho*-chloro-(and bromo-)substituted 2-benzylthio-*N*-phenylcytosines (**1**, **4**, **7**, **10**) are distinguished from isomeric *meta* (**2**, **5**, **8**, **11**) and *para* (**3**, **6**, **9**, **12**) substituted 2-benzylthio-*N*-phenylcytosines on the basis of the highest values of μ_1. The *ortho*-(*meta*- and *para*-) chloro-(bromo-) substituted isomers of 2-benzylthio-*N*-phenylcytosines **1–12** may be differentiated on the basis of the following sequences of the values of μ:

μ_4 *ortho* > μ_4 *para* > μ_4 *meta* **1–12**
μ_2, μ_5 *ortho* > μ_2, μ_5 *para* > μ_2, μ_5 *meta* **1–3**; **7–9**
μ_3 *meta* > μ_3 *para* > μ_3 *ortho* **1–3**, **7–9**
μ_5 *meta* > μ_5 *ortho* > μ_5 *para* **10–12**

The fluorescence properties of compounds **1–12** have been investigated, the fluorescence emission maxima and quantum yields are summarized in Table XII. The *ortho*-(*meta*- and *para*-) chloro- (bromo-)

TABLE XII UV/VIS and Fluorescence Spectra of **1–12**

Compound	UV/VIS λ_{max} [nm] (lg ε) CH_3CN	Fluorescence		Quantum yield Φ
		Excitation wave [nm] CH_3CN	Emission wave [nm] CH_3CN	
1	298 (4.0)	315	490	0.01
2	295 (4.1)	315	495	0.01
3	300 (4.1)	315	493	0.02
4	298 (4.1)	315	490	0.01
5	297 (4.1)	315	470	0.01
6	295 (4.2)	315	494	0.01
7	288 (4.3)	315	476	0.02
8	296 (4.2)	315	475	0.01
9	299 (4.1)	315	490	0.03
10	296 (4.2)	315	470	0.01
11	296 (4.0)	315	475	0.02
12	293 (4.2)	315	467	0.01

substituted isomers of 2-benzylthio-*N*-phenylcytosines **1–12** are highly fluorescent, having emission bands at 467–495 nm with the main excitation peak at 315 nm in CH_3CN. Clearly, the substitution of *ortho*-(*meta*- and *para*) chloro-(bromo-) benzyl group at the angular sulfur atom and phenyl group at the angular nitrogen atom of the molecule of 2-thiocytosine serves as a useful method of converting this compound into highly fluorescent derivatives. 2-*ortho*-(*meta*- and *para*-) chloro-(and bromo-) benzylthio-*N*-phenylcytosines (**1–3**, **7–9**) and *N*-phenyl-6-methylcytosines (**4–6**, **10–12**) may well be the best fluorescent derivatives of cytosine yet prepared.

The conclusions following from our investigation are:

- The basic mass fragmentation of *N*-phenyl-2-thiocytosine (**I**) and *N*-phenyl-2-thio-6-methylcytosine (**II**) suggests that the molecular ions of these compounds exist in the thiol-amino and thione-amino tautomeric forms.
- The basic mass fragmentation of **1–12** is due to cleavages of the Csp3-S and Csp2-S bonds of the S-benzyl group, as well as the Csp2-X bonds of X-substituted phenyl group.
- The values of μ_1–μ_5 (i.e., the ratio of intensities of the selected fragment ions peaks to those of the molecular ion peaks M^+) depend on the structures of *ortho*-(*meta*- and *para*-) substituted chloro-(bromo-)benzylthio-*N*-phenylcytosines (**1–12**). The differences in the values μ_1-μ_5 in the series of **1–12** are useful for differentation between *ortho*-(*meta*- and *para*-) substituted isomers of 2-benzylthio-*N*-phenylcytosines **1–12**.

- The differences in the ^{13}C NMR spectra of **1–12** in the number and positions of the signals of the carbon atoms in the range of 125–130 and 135–140 ppm (**1–6**) as well as 115–125 and 140–145 ppm (**7–12**) allow a differentiation between *ortho-*, *meta-*, and *para-* substituted in benzylthio groups isomers.

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 $CHCl_3$—MeOH 5:1 and observed under UV light ($\lambda = 254$ and 366 nm). UV/VIS spectra were recorded with a Specord UV/VIS spectrophotometer in DMSO-d_6 and acetonitrile. IR spectra were recorded with a FT-IR Bruker IFS–113 v spectrophotometer in KBr pellets. The ^1H NMR and ^{13}C NMR spectra were determined with a Varian Gemini 300 (300 MHz) spectrometer in DMSO-d_6 solution with TMS as internal standard. The ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Gemini 300 spectrometer in DMSO-d_6 solution at a concentration between 0.25 and 0.40 M in 5 mm sample tubes at ambient temperature. Chemical shifts are given in the δ scale (ppm) and coupling constants in hertz. ^1H NMR (300. 070) 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. Homonuclear ^1H-^1H shift-correlated two-dimensional diagrams were obtained on a Varian Gemini 300 spectrometer using the COSY pulse sequence. The spectral width was 4.97 KHz, acquisition time 0.206 s, number of increments in t_1 512 and number of scans 16. Heteronuclear 2D ^{13}C NMR - ^1H 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 ^1H.

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 of $\sim$150°C. The elemental compositions 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 VIII, IX, and X 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 1.73×10^{-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–μ_5, were calculated as averages of three measurements. Fluorescence corrected spectra were taken in acetonitrile (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. *N*-phenyl-2-thiocytosine (**I**) and *N*-phenyl-6-methyl-2-thiocytosine (**II**) were obtained according to literature.[38]

The Synthesis of 2-*Ortho*-(*meta*- and *para*-) chloro-(bromo-) benzylthio-*N*-phenylcytosines 1–12

A methanol solution consisting of 1 mmol of *N*-phenyl-2-tiocytosine (or N-phenyl-6-methyl-2-thiocytosine) in 50 ml of 0.1 M NaOH was stirred at room temperature while 1.1 mmol of corresponding *ortho*-(*meta*- and *para*-) chloro-(bromo-) benzyl halide were added dropwise. After stirring for 6–8 h (Table I) half of the volume of the solvent was removed in vacuo using a rotatory evaporator. The reaction mixture was acidified (pH 3) with 1 M HCl and was cooled in the refrigerator for 2 hs. Than the precipitated solids of **1–12** were filtered, dried, and recrystallized from ethanol.

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