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SYNTHESIS AND INVESTIGATION OF THE STABILITY OF ESTERS OF 6'-CARBAMOYLMETHYLTHIO-5'-CYANO-1',4'-DIHYDRO-3,4'- AND -4,4'-BIPYRIDINE-3'-CARBOXYLIC ACIDS 1. ESTERS OF 6'-CARBAMOYLMETHYLTHIO-5'-CYANO-1',4'-DIHYDRO-3,4'-BIPYRIDINE-3'-CARBOXYLIC ACIDS

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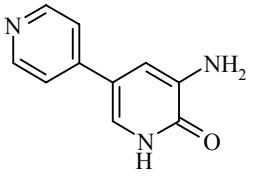
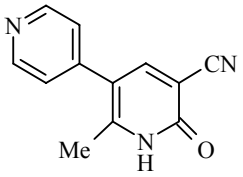
Esters of 6'-carbamoylmethylthio-5'-cyano-1',4'-dihydro-3,4'-bipyridine-3'-carboxylic acids are obtained by the alkylation of piperidinium 3'-alkoxycarbonyl-5'-cyano-1',4'-dihydro-3,4'-bipyridine-6'-thiolates with iodoacetamide. For an HPLC study of the stability of solutions of the abovementioned 1,4-dihydrobipyridines (solution pH 2.3-9.0) the appropriate esters of 6'-carbamoylmethylthio-5'-cyano-3,4'-bipyridine-3'-carboxylic acids and esters of 8-cyano-5-methyl(or phenyl)-3-oxo-7-pyridin-3-yl-2,3-dihydro-7H-thiazolo[3,2-a]pyridine-6-carboxylic acids were synthesized as reference compounds. Analysis by HPLC was carried out under conditions of reverse-phase chromatography. It was shown that solutions of the investigated compounds in a mixture of acetonitrile and phosphate buffer (pH 3.0-5.0) were stable for 1 month on storage protected from light. Under the action of light in all the solutions investigated irrespective of pH the formation occurs of the corresponding esters of 6'-carbamoylmethylthio-5'-cyano-3,4'-bipyridine-3'-carboxylic acids. The presence of esters of 8-cyano-5-methyl(or phenyl)-3-oxo-7-pyridin-3-yl-2,3-dihydro-7H-thiazolo[3,2-a]pyridine-6-carboxylic acids (no more than 4%) was detected only in 0.1% solutions of phosphoric acid (pH 2.3) under conditions of storage of the latter protected from light. A series of as yet unidentified products was detected in solutions of pH 7.0-9.0.

Keywords: 3,4'-bipyridines, 2,3-dihydro-7H-thiazolo[3,2-*a*]piperidines, HPLC.

3.4'-Bipyridines have aroused considerable interest for more than 20 years since among them are cardiotonic agents for the treatment of cardiac insufficiency. The preparations amrinone (5-amino-1H-3,4'-bipyridin-6-one) and milrinone (2-methyl-6-oxo-1,6-dihydro-3,4'-bipyridine-5-carboxylic acid nitrile) show a marked inotropic action on the heart, simultaneously displaying a vasodilating effect [1-6].



Amrinone



Milrinone

* Dedicated to E. Lukevics on his 70th birthday

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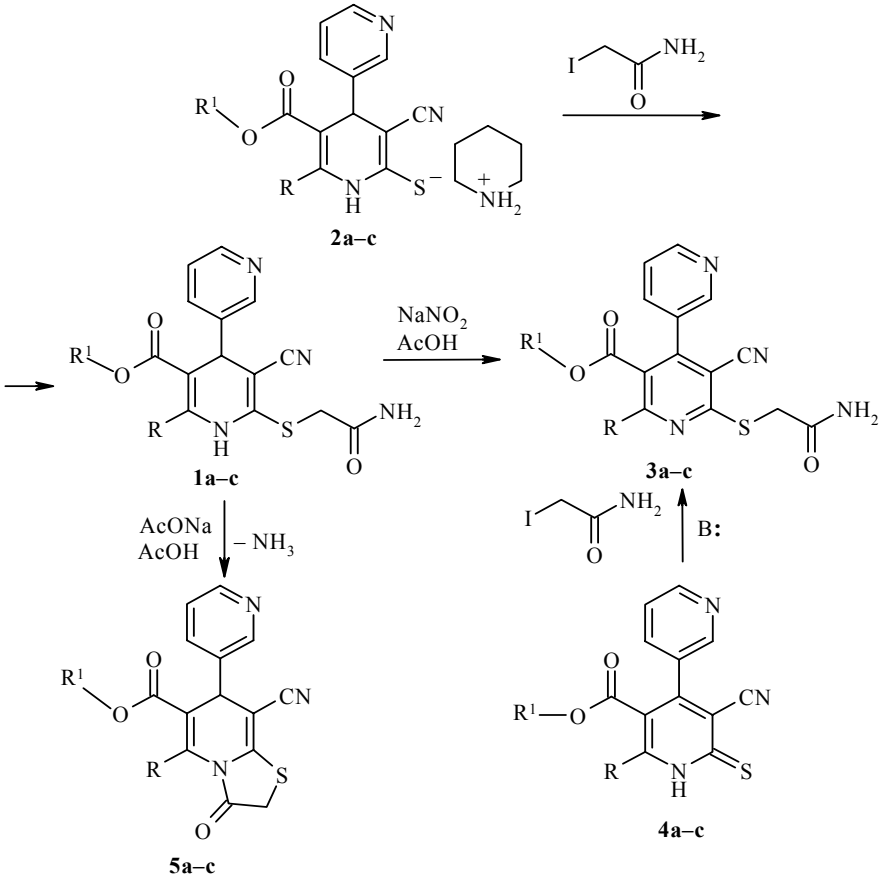
It was established that ethyl esters of 5'-cyano-1',4'-dihydro-3,4'-bipyridin-3'-carboxylic acids, depending on the degree of hydrogenation, show a double inotropic action [7], and the nitrile of 3'-acetyl-6'-carbamoylmethylthio-2'-hydroxy-2'-methyl-1',2',3',4'-tetrahydro-3,4'-bipyridine-5'-carboxylic acid (dose 0.1 mg/kg) increases blood flow in the femoral artery by 125%, but the effect is brief due to the instability of the compound [8]. In addition the antioxidant activity of 6'-alkylthio-1',4'-dihydro-3,4'-bipyridines was shown by us in [9], however it was established that some of them were unstable in dilute, especially acidic, solutions.

The 1,4-dihydropyridine ring of esters of 6'-carbamoylmethylthio-5'-cyano-1',4'-dihydro-3,4'-bipyridine-3'-carboxylic acids **1** in dilute solution may be oxidized by oxygen of the air to a pyridine ring, since compounds **1** are antioxidants. In view of the potential practical value of 1',4'-dihydro-3,4'-bipyridines **1**, in the present work we have investigated their stability in aqueous solution (pH 2.3-9.0).



a R = R^1 = Me; **b** R = Me, R^1 = Et; **c** R = Ph, R^1 = Et

Compounds **1** were obtained by the alkylation of 3'-alkoxycarbonyl-5'-cyano-1',4'-dihydro-3,4'-bipyridine-6'-thiolates **2** with iodoacetamide. The highest yields were achieved on carrying out these reactions under mild conditions with gradual addition of iodoacetamide, which enables an excess of thiolate to be maintained.

Standard compounds were used in the study of the stability of aqueous solutions of the 1',4'-dihydro-3,4'-bipyridines synthesized by us. These were esters of 6'-carbamoylmethylthio-5'-cyano-3,4'-bipyridine-3'-carboxylic acids **3** obtained by the action of sodium nitrite on the corresponding 1',4'-dihydro-3,4'-bipyridines **1** in boiling acetic acid. It should be mentioned that in the presence of a carbamoylmethylthio substituent in position 6 of the 1,4-dihydropyridine ring this simple method of oxidation [10] has a drawback, *viz.* elimination of a molecule of ammonia occurs with the formation of a 7H-thiazolo[3,2-*a*]pyridine ring, which is stable to oxidation. Bipyridines **3** were also obtained by the alkylation of pyridine-6(1H)-thiones **4** with iodoacetamide.

TABLE 1. Investigation of the Stability of Solutions of Compounds **1** by Reverse-Phase HPLC*

Com-pound	Content of studied compound in solution being analyzed, % **											
	Solution **I** (pH 2.3)				Solution **II** (pH 3.0)		Solution **III** (pH 5.0)		Solution **IV** (pH 7.0)		Solution **V** (pH 9.0)	
	Directly after solution	After 1 day *hν*	After 1 month		After 1 month		After 1 month		After 1 month		After 1 month	
			T	*hν*	T	*hν*	T	*hν*	T	*hν*	T	*hν*
1a	97.7	95.2	90	—	97	—	97.7	—	93	—	54	—
3a	1	3.5	1	83	0.8	70	0.5	75	1	40	1	25
5a	0.3	0.3	3.4	—	0.7	—	0.5	—	—	—	—	—
1aA	—	—	—	—	—	—	—	—	4.6	—	41	—
1aB	—	—	—	—	—	—	—	—	—	23	—	30
1aC	—	—	—	—	—	—	—	—	0.2	1.3	2.6	8
aX* **	1	1	5.6	15	1.5	30	1.3	25	1.2	35.7	1.4	36
n	4	4	5	18	4	18	4	18	4	20	4	20
1b	98.5	95.8	90.5	—	98.5	—	98.4	—	94	—	89	—
3b	0.2	3.3	0.2	82	0.2	62	0,2	70	0.6	40	—	24
5b	0.3	0.3	4.0	—	0.9	—	0.8	—	—	—	—	—
1bA	—	—	—	—	—	—	—	—	4.4	—	9	—
1bB	—	—	—	—	—	—	—	—	—	20	—	23
1bC	—	—	—	—	—	—	—	—	0.5	1.3	1.3	7.4
1bX* **	1	0.6	5.3	18	0.4	38	0.6	30	1	38.7	0.7	45.6
n	5	5	6	18	5	18	5	18	5	20	5	20
1c	97.7	97	95.4	78.2	97.1	38.5	94.2	62.4	93.4	72.6	85.6	47.2
3c	2.3	2.9	2.2	19.3	2.1	58.8	5.2	36	2.8	21.5	2	14
5c	—	—	2.1	2.3	0.7	—	0.2	0.1	—	—	—	—
1cA	—	—	—	—	—	—	—	—	3.4	—	9.1	6
1cB	—	—	—	—	—	—	—	—	—	—	—	—
1cC	—	—	—	—	—	—	—	—	0.3	2.4	3.2	17.6
1cX* **	—	0.1	0.1	0.4	0.1	2.7	0.4	1.5	0.1	3.5	0.1	15.2
n	—	1	1	4	1	11	4	12	1	10	1	12

* Conditions of chromatographic analysis and preparation of solutions for analysis are given in EXPERIMENTAL.

** Quantitative analysis was carried out by normalizing areas (λ = 254 nm) [14]. *hν* solutions (in bottles of normal glass) were stored unprotected from light. T solutions (in bottles of dark glass) were stored protected from light.

***Overall content of unidentified contaminants (*n* is number of unidentified contaminants on the chromatogram).

Boiling 6'-carbamoylmethylthio-5'-cyano-1'.4'-dihydro-3,4'-bipyridine-3'-carboxylic acids **1** in acetic acid with added sodium acetate gave esters of 8-cyano-5-methyl(or phenyl)-3-oxo-7-(3'-pyridyl)- 2,3-dihydro-7*H*-thiazolo[3,2-*a*]pyridine-8-carboxylic acids **5**, used later as standard compounds for studying the stability of 1',4'-dihydro-3,4'-bipyridines **1**.

In the last decade high performance liquid chromatography (HPLC) has occupied a significant place in the analytical chemistry of organic compounds. The reverse-phase variant has the widest application in chromatographic practice, including the analysis of 1,4-dihydropyridines [11-13].

Investigation of the stability of compounds **1** in aqueous solution (pH 2.3 solution **I**; pH 3.0 solution **II**; pH 5.0 solution **III**; pH 7.0 solution **IV**; pH 9.0 solution **V**; see EXPERIMENTAL) was carried out by reverse-phase chromatography in gradient mode. The results obtained are given in Table 1.

On introducing a test sample of solution **I** to be analyzed (directly after solution of compound **1** in the mobile phase) peaks corresponding to 1',4'-dihydro-3,4'-bipyridines **1** (97.7-98.5%) predominated, the peaks corresponding to bipyridines **3** amounted to 0.3-2.3%, and the content of thiazolopyridines **5** in the solutions did not exceed 0.3%.

After 24 h (solutions **I** being analyzed were stored unprotected from light) a reduction was observed in the area of peaks corresponding to 1',4'-dihydro-3,4'-bipyridine **1**, and an increase was seen in the areas of peaks corresponding to bipyridine **3**. While the content of compounds **3a** and **3b** increased more than three times, the area of the peak corresponding to compound **3c** grew only from 2.3 to 2.9% in 24 h. Evidently the presence of a phenyl substituent (R = Ph) in the compound **1c** molecule makes the solution more stable towards oxidation of the 1,4-dihydropyridine ring to a pyridine ring compared with compounds **1a** and **1b** containing a methyl group as R.

One month after introducing the sample, chromatograms of solutions **I-III** (solutions stored unprotected from light) were obtained in which peaks for 1',4'-dihydro-3,4'-bipyridines **1a** and **1b** were absent, but peaks corresponding to bipyridine **3** amounted to 62-83% (see Table 1). It should be mentioned that apart from bipyridines **3a** and **3b** there was also a whole series of unidentified contaminants on the chromatograms, however the individual content of the latter did not exceed 5%. In solutions **I** (pH 2.3) and **III** (pH 5.0) of compound **1c** (R = Ph) the formation of bipyridine **3c** occurs significantly more slowly (19.3% at pH 2.3, 36% at pH 5.0), while in solution **II** (pH 3.0) of compound **1c** the oxidation goes more rapidly and the area of the bipyridine **3c** peak amounted to 58.8%. The content of unidentified contaminants did not exceed 2.7%.

Solutions **I-III** stored in the dark for 1 month were in practice stable. On the corresponding chromatograms only an insignificant increase was observed in the areas of peaks corresponding to thiazolopyridine **5** (particularly in the value at pH 2.3 by 4%, see Table 1).

TABLE 2. Characteristics of Compounds **A-C**

Com-pound	Retention time, t_r, min*	UV spectrum, λ, nm**	Effect of light***
1aA	5.28	195; 218; 258; 295; 373	On storing solutions in the dark
1bA	5.89	194; 218; 258; 295; 374	
1cA	7.17	192; 258; 382	On storing a solution both in the dark and in the light (pH 9)
1aB	4.90	205	Under the action of light
1bB	5.55	205	
1aC	7.15	203; 289	Both under the action of light and in the dark
1bC	7.92	199; 288	
1cC	10.05	199; 298	

*Conditions of chromatographic analysis, see EXPERIMENTAL.

**Data of spectrophotometric detector on a ProStar 330 diode matrix.

***Preparation of solutions, see in EXPERIMENTAL; pH 7.0; 9.0.

Analysis of solutions **IV** (pH 7.0) and solutions **V** (pH 9.0) (1 month storage in the dark) showed the absence of the corresponding thiazolopyridines **5**. In solutions **IV** the content of 1',4'-dihydro-3,4'-bipyridines **1** was 93-94%, but in solutions **V** it varied from 54 (solution of compound **1a**) to 89% (solution of compound **1b**), the main contaminant was an unidentified compound, conditionally designated **A** (see Tables 1 and 2).

TABLE 3. Characteristics of the Synthesized Compounds

Compound	Empirical formula	Found, % / Calculated, %				mp, °C	Yield, % (method)
		C	H	N	S		
1a	$C_{16}H_{16}N_4O_3S$	55.52 / 55.80	4.59 / 4.68	16.09 / 16.27	9.19 / 9.31	192-194	72
1b	$C_{17}H_{18}N_4O_3S$	56.77 / 56.97	4.97 / 5.06	15.39 / 15.63	8.89 / 8.95	196-197	34
1b·HCl	$C_{17}H_{18}N_4O_3S$·HCl	51.77 / 51.71	4.97 / 4.85	14.39 / 14.19	8.12 / 8.12	181-182	53
1c	$C_{22}H_{20}N_4O_4S$	62.77 / 62.84	4.97 / 4.79	13.39 / 13.32	7.63 / 7.63	183-185 [7]	79
3a	$C_{16}H_{14}N_4O_3S$	56.13 / 56.13	4.12 / 4.12	16.36 / 16.36	9.37 / 9.37	185-186	67 (A) 23 (B)
3b	$C_{17}H_{16}N_4O_3S$	57.29 / 57.29	4.53 / 4.53	15.72 / 15.72	9.00 / 9.00	137-138	70 (A)
3c	$C_{22}H_{18}N_4O_3S$	62.97 / 63.14	4.20 / 4.34	13.19 / 13.39	7.49 / 7.66	167-169 [7]	65 (A)
5a	$C_{16}H_{13}N_3O_3S$	58.70 / 58.70	3.84 / 4.00	12.81 / 12.84	9.80 / 9.79	148-150	34
5b	$C_{17}H_{15}N_3O_3S$	59.77 / 59.81	4.43 / 4.43	12.31 / 12.31	9.39 / 9.39	125-126	30
5c	$C_{22}H_{17}N_3O_3S$	65.52 / 65.49	4.00 / 4.25	10.84 / 10.41	7.79 / 7.95	168-170	59

No 1',4'-dihydro-3,4'-bipyridines **1** were observed in solutions **IV** and **V**, stored unprotected from light. The exceptions were solutions of compound **1c** in which the content was 72.6% at pH 7.0 and 47.2% at pH 9.0 respectively. The formation of bipyridines **3** occurs under the action of light in all solutions. However the content of bipyridines **3** in solutions **V** of compounds **1a** and **1b** did not exceed 25% and the content of unidentified compounds **B** (see Tables 1 and 2) was more than 20%. In analogous solutions of compound **1c** the presence of compound **1cB** was not detected, but on the chromatogram peaks were present for the conditionally designated **1cA** and **1cC** (see Tables 1 and 2).

Under the action of light in all the investigated solutions irrespective of pH oxidation of the 1,4-dihydropyridine ring occurs with the formation of the corresponding bipyridines **3**. In solutions **IV** and **V** of compounds **1a** and **1b**, together with bipyridines **3**, the formation is also observed of as yet unidentified compounds **B** (no more than 30%). The presence of a phenyl substituent (R = Ph) in the molecule of **1c** gives solutions that are more stable towards oxidation. The maximum (58.8%) formation of bipyridine **3c** is observed in solution **II** (pH 3.0) and the minimum (14%) in solution **V** (pH 9.0).

On storing the solutions of 1',4'-dihydro-3,4'-bipyridines **1** being investigated for 1 month *protected* from light the most stable were solutions in **II** (pH 3.0) and **III** (pH 5.0). Solutions in **I** were less stable, after 1 month the formation was observed of thiazolopyridines **5** (but no more than 4%). Solutions in **IV** and **V** were characterized by the formation of as yet unidentified compounds, conditionally designated **A** (pH 7.0 no more than 4.6%, pH 9.0 up to 41% for a solution of compound **1a**) and **C** (pH 7.0 no more than 2.4%, pH 9.0 up to 17.6% for a solution of compound **1c**).

The problem of the constitution of compounds **A-C** remains open and will become a subject of study in our subsequent investigations.

TABLE 4. Spectral Characteristics of Compounds **1**, **3**, and **5**

Com-pound	IR spectrum, ν, cm^{-1}	^1H NMR spectrum, δ, ppm (*J*, Hz)*
1a	1628, 1668, 1702 (C=O); 2200 (C≡N); 3120, 3300 (NH, NH_2)	2.36 (3H, s, 6-CH_3); 3.55 (3H, s, OCH_3); 3.64 and 3.80 (2H, d and d, *J* = 16, SCH_2); 4.50 (1H, s, H-4); 7.32-8.55 (4H, m, C_5H_4N), 7.64 and 7.94 (2H, br. s and br. s, $CONH_2$); 10.54 (1H, s, NH)
1b	1646, 1680, 1702 (C=O); 2196 (C≡N); 3164, 3332 (NH, NH_2)	1.07 and 3.97 (5H, t and q, *J* = 7.0, OC_2H_5); 2.32 (3H, s, 6-CH_3); 3.67 and 3.78 (2H, d and d, *J* = 16, SCH_2); 4.58 (1H, s, H-4); 7.30-8.52 (4H, m, C_5H_4N); 7.67 and 7.93 (2H, br. s and br. s, $CONH_2$); 10.52 (1H, s, NH)
3a	1672, 1728 (C=O); 2225 (C≡N); 3130, 3340 (NH_2)	2.66 (3H, s, 6-CH_3); 3.57 (3H, s, OCH_3); 3.96 (2H, s, SCH_2); 5.90 and 6.53 (2H, br. s and br. s, NH_2); 7.40-7.84 and 8.58-8.84 (4H, m and m, C_5H_4N)
3b	1673, 1718 (C=O); 2222 (C≡N); 3330 (NH_2)	0.96 and 4.08 (5H, t and q, *J* = 7.0, OC_2H_5); 2.67 (3H, s, 6-CH_3); 3.97 (2H, s, SCH_2); 5.86 and 6.55 (2H, br. s and br. s, $CONH_2$); 7.42-8.80 (4H, m, C_5H_4N)
3c [7]	1674, 1728 (C=O); 2224 (C≡N); 3120, 3312 (NH_2)	0.70 and 3.84 (5H, t and q, *J* = 7.0, OC_2H_5); 4.06 (2H, s, SCH_2); 7.26 and 7.56 (2H, br. s and br. s, $CONH_2$); 7.40-8.78 (9H, m, C_6H_5 and C_5H_4N)
5a	1654, 1722, 1736 (C=O), 2198 (C≡N)	2.68 (3H, s, 6-CH_3); 3.64 (3H, s, OCH_3); 3.98 (2H, s, 2-CH_2); 4.80 (1H, s, H-7); 7.30-7.70 and 8.38-8.74 (4H, m and m, C_5H_4N)
5b	1673, 1720, 1743 (C=O), 2200, 2006 (C≡N)	1.12 and 4.06 (5H, t and q, *J* = 7.0, OEt); 2.66 (3H, s, 6-CH_3); 3.97 (2H, s, 2-CH_2); 4.76 (1H, s, H-7); 7.30-8.60 (4H, m, C_5H_4N)
5c	1672, 1757 (C=O), 2206 (C≡N)	0.69 and 3.68 (5H, t and q, *J* = 7.0, OC_2H_5); 3.89 (2H, s, 2-CH_2); 4.86, (s, H-7); 7.14-7.50, 7.68-7.77, 8.54-8.68 (9H, m, C_6H_5 and C_5H_4N)

*^1H NMR spectra were taken in DMSO-D_6 (compounds **1a,b, 3a,c**, and **5a,c**) and $CDCl_3$ (compounds **3b** and **5b**).

EXPERIMENTAL

The IR spectra were taken on a Perkin-Elmer 580B spectrometer in nujol. The ^1H NMR spectra were recorded on a WH 90/DC (90 MHz) spectrometer. Internal standard was HMDS (δ 0.05 ppm). A check on the progress of reactions and the purity of substances was effected by TLC on Silufol 254 plates, eluent was chloroform–hexane–acetone, 2 : 1 : 1. Compounds were recrystallized from ethanol. The synthesis of compound **1c** is described in [7].

Esters of 6'-Carbamoylmethylthio-5'-cyano-2'-methyl(or phenyl)-1',4'-dihydro-3,4'-bipyridine-3'-carboxylic Acids 1 (general procedure). A mixture of 3'-alkoxycarbonyl-5'-cyano-1',4'-dihydro-3,4'-bipyridine-6'-thiolate **2** [15] (10 mmol) and iodoacetamide (10 mmol) in ethanol (20-40 ml) was stirred at 40-50°C for 15-30 min. The resulting solid was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml).

Esters of 6'-carbamoylmethylthio-5'-cyano-2'-methyl(or phenyl)-3,4'-bipyridine-3'-carboxylic Acids 3. A. A mixture of 6'-carbamoylmethylthio-5'-cyano-2'-methyl(or phenyl)-1',4'-dihydro-3,4'-bipyridine-3'-carboxylic acid (**1**) (10 mmol) in acetic acid (20 ml) was heated to boiling and sodium nitrite (30 mmol) was added. After the end of NO_2 evolution the reaction mixture was poured into water (20 ml), and neutralized with ammonia. The solid was filtered off, washed with water (10 ml), and compounds **3a-c** were obtained.

B. A mixture of pyridine-6(1H)-thione **4a** (0.285 g, 1 mmol), piperidine (0.1 ml, 1 mmol), and iodoacetamide (0.19 g, 1 mmol) in ethanol (2 ml) was heated to boiling, cooled to 0°C, and the solid filtered off. The solid was washed with ethanol (2 ml) and with water (5 ml). Compound **3a** (0.08 g, 23%) was obtained.

Esters of 8-Cyano-5-methyl(or phenyl)-3-oxo-7-(3'-pyridyl)-2,3-dihydro-7H-thiazolo[3,2-*a*]pyridine-8-carboxylic Acids 5. A mixture of 6-carbamoylmethylthio-5-cyano-2-methyl(or phenyl)-1,4-dihydro-4,3'-bipyridine-3'-carboxylic acid ester **1** (10 mmol) and sodium acetate (5 mmol) in acetic acid (20 ml) was boiled for 1-4 h. After cooling, the reaction mixture was poured into water (20 ml), and neutralized with ammonia. The solid was filtered off, and washed with water (10 ml).

The characteristics of the synthesized compounds are given in Tables 3 and 4.

The chromatographic investigation was carried out on a Varian "Prostar" chromatograph consisting of a Prostar 240 gradient pump, a Prostar 330 spectro-photometric detector on a diode matrix (λ = 254 nm), and a Prostar 410 autosampler. The column (150 x 4.6 mm) (Agilent) was packed with Zorbax SB-C18 sorbent. Mobile phase was acetonitrile–0.1% phosphoric acid solution in water (pH 2.3). The linear gradient was 15 min from 5 to 95% acetonitrile. Consumption of mobile phase was 1.0 ml/min. The test sample (10 μl, concentration 0.5 μg/ml in mobile phase) was introduced by the autosampler.

Retention times, t_r, min: **1a**) 5.54; **3a**) 7.32; **5a**) 6.90; **1b**) 6.17; **3b**) 8.13; **5b**) 7.84; **1c**) 7.60; **3c**) 9.98; **5c**) 9.42 min. Retention time t_0 of an apparently unabsorbed substance (uracil) was 1.55 min.

Solutions of 0.01 M phosphate buffer (pH 3.0; 5.0; 7.0; 9.0) were obtained by titration of 0.01 M phosphoric acid with 1 M potassium hydroxide to the desired pH value [16].

Solutions for Analysis. Solution **I**. Compound **1a** (5 mg) was dissolved in a 5% solution (10 ml) of acetonitrile in 0.1% phosphoric acid solution in water. The obtained solution (about 1 ml) was placed in four autosampler bottles of volume 1.5 ml (three bottles were of ordinary glass and one was of dark glass) which were tightly closed with appropriate caps. One of the three bottles of ordinary glass was placed in the autosampler and was analyzed directly after preparation of the solution to be analyzed. The second and third bottles were stored unprotected from light, in the first case for 1day and in the second for 1 month, and only then was HPLC analysis of the solutions carried out. The solution in the fourth bottle of dark glass was also stored for 1 month and then analyzed by HPLC.

Solutions of compounds **1b** and **1c** were prepared for analysis analogously.

Solution **II**. Compound **1a** (5 mg) was dissolved in a solution (10 ml) of 25% acetonitrile in 0.01 M phosphate buffer (pH 3.0). The solution obtained (about 1 ml) was placed in one bottle of ordinary glass and in one of dark glass (the bottleswere tightly closed with appropriate caps). The solutions were stored unprotected from light for 1 month and then analyzed by HPLC.

Solutions of compounds **1b** and **1c** were prepared for analysis analogously.

Solution **III**. Compound **1a** (5 mg) was dissolved in a solution (10 ml) of 25% acetonitrile in 0.01 M phosphate buffer (pH 5.0). The solution obtained (about 1 ml) was placed in one bottle of ordinary glass and one of dark glass (the bottles were tightly closed with appropriate caps). The solutions were stored unprotected from light for 1 month and then analyzed by HPLC.

Solutions of compounds **1b** and **1c** were prepared for analysis analogously.

Solution **IV**. Compound **1a** (5 mg) was dissolved in a solution (10 ml) of 30% acetonitrile in 0.01 M phosphate buffer (pH 7.0). The solution obtained (about 1 ml) was placed in one bottle of ordinary glass and one of dark glass (the bottles were tightly closed with appropriate caps). The solutions were stored unprotected from light for 1 month and then analyzed by HPLC.

Solutions of compounds **1b** and **1c** were prepared for analysis analogously.

Solution **V**. Compound **1a** (5 mg) was dissolved in a solution (10 ml) of 30% acetonitrile in 0.01 M phosphate buffer (pH 9.0).The solution obtained (about 1 ml) was placed in one bottle of ordinary glass and one of dark glass (the bottles were tightly closed with appropriate caps). The solutions were stored unprotected from light for 1 month and then analyzed by HPLC.

Solutions of compounds **1b** and **1c** were prepared for analysis analogously.

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