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SYNTHESIS, CARDIOVASCULAR ACTIVITY, AND ELECTROCHEMICAL OXIDATION OF NITRILES OF 5-ETHOXYCARBONYL-2-METHYL-THIO-1,4-DIHYDROPYRIDINE-3-CARBOXYLIC ACID

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Nitriles of 4-aryl-5-ethoxycarbonyl-2-methylthio-1,4-dihydropyridine-3-carboxylic acid have been obtained by the methylation of 1,4-dihydropyridine-2-thiolates; of 1,4-dihydropyridine-2(3H)-thiones in the presence of a stoichiometric amount of piperidine, and of a mixture of 1,4,5,6-tetrahydro- and 1,4-dihydropyridine-2-thiolates with methyl iodide. One-pot multicomponent synthesis has also been used in the condensation of ethyl 2-arylmethyleneacetoacetate, 2-cyanothioacetamide, piperidine, and methyl iodide; of ethyl acetoacetate, 3-aryl-2-cyanothioacrylamide, piperidine, and methyl iodide; and of ethyl acetoacetate, an aromatic aldehyde, 2-cyanothioacetamide, piperidine, and methyl iodide. The latter, a five-component method, takes place rapidly and under mild conditions, it is efficient (yields of 75-96%, economy of time, labour, and resources) and "green" (there is no need to synthesize lachrymators, such as 3-aryl-2-cyanothioacrylamides).The cardiovascular activity and the electrochemical oxidation of the synthesized 2-methylthio-1,4-dihydropyridines have been investigated. A comparative analysis has been carried out of the ability towards electrochemical oxidation as a function of the electronic properties of the substituent at position 4 of the heterocycle.

Keywords: 1,4-dihydropyridines, cardiovascular activity, one-pot multicomponent synthesis, electrochemical oxidation.

Many of the widely studied esters of 4-substituted 1,4-dihydropyridine-3,5-dicarboxylic acids possess marked cardiovascular activity and many preparations for the treatment of cardiovascular diseases have been created from them [1-4]. An important role, determining the activity of these compounds, is played by the presence of alkoxycarbonyl groups at positions 3 or 5, a methyl group at positions 2 or 6, and an aryl group at position 4. Nitrophenyl, alkoxyphenyl, and halogen-substituted phenyl groups are characterized as effective pharmacophores [1].

2-Alkylthio-1,4-dihydropyridines display cardiovascular [5], hepatoprotective [6], antioxidant (AOA) [7], and antiradical (ARA) [8] activity, however these compounds are still insufficiently studied pharmacologically.

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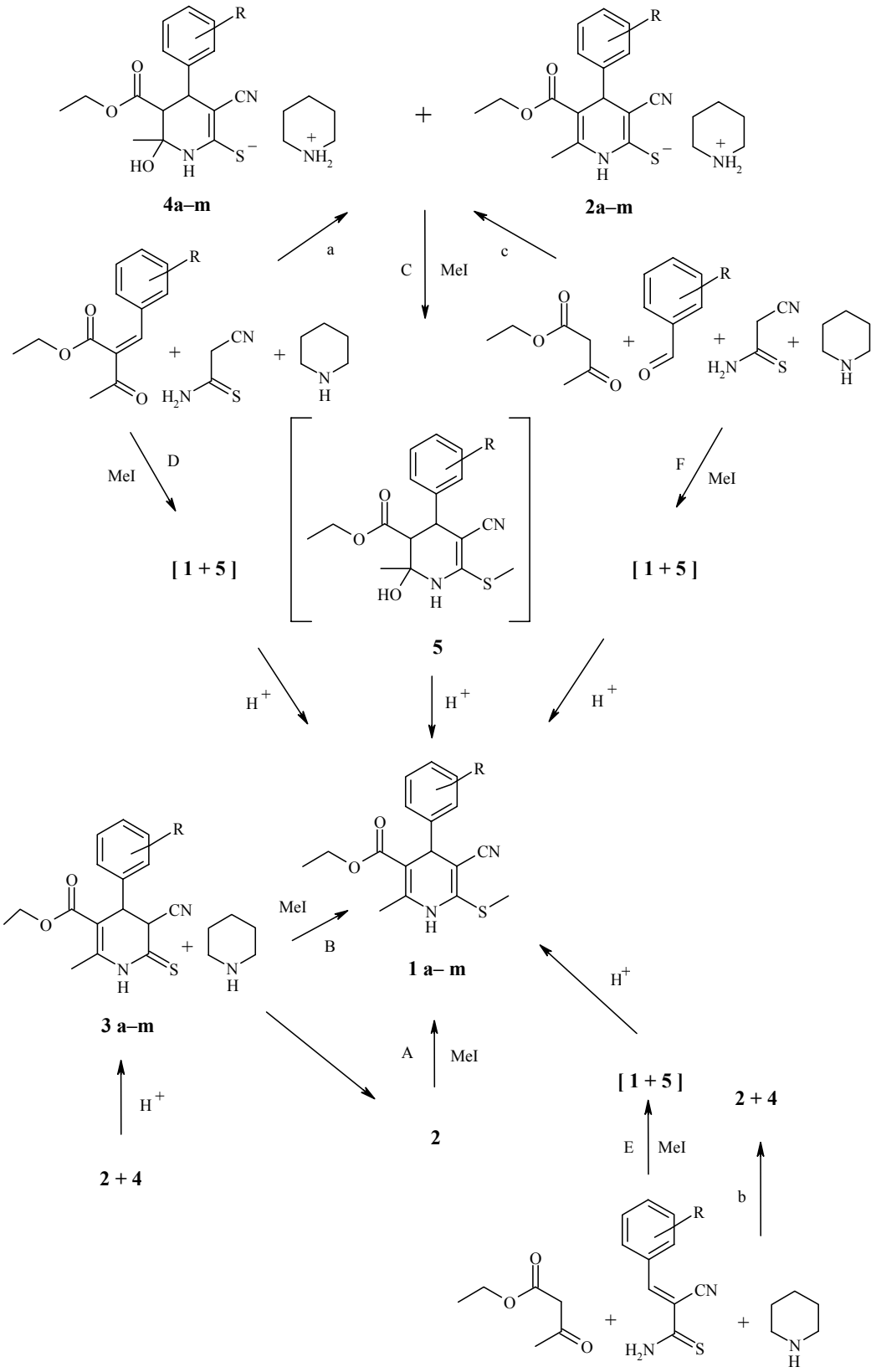

a R = H, **b** R = 4-OH; **c** R = 4-OMe; **d** R = 2-OMe; **e** R = 4-NMe_2; **f** R = 4-Cl; **g** R = 3-Cl; **h** R = 2-Cl; **i** R = 2,4-Cl_2; **j** R = 4-NO_2; **k** R = 3-NO_2; **l** R = 4-CN; **m** R = 3-CN

In a continuation of investigations on the synthesis and study of the properties of 2-alkylthio-1,4-dihydropyridines [9] and to improve the methods of obtaining them [10-11], we have synthesized a series of new nitriles of 4-aryl-5-ethoxycarbonyl-6-methyl-2-methylthio-1,4-dihydropyridine-3-carboxylic acid **1**, in which the substituents on the 4-phenyl residue are varied widely, and have investigated their cardiovascular activity. A comparative analysis has been carried out of the ability of 1,4-dihydropyridines **1** towards electrochemical oxidation as a function of the electronic properties of the substituents at position 4 of the heterocycle. Such data enable the search for biologically active substances to be carried out in a more purpose directed manner.

In principle the important problem, the construction of 1,4-dihydropyridine-2(3H)-thione ring, is extremely complex, since these compounds are subject to oxidation in dilute solutions. Three of the main methods were already described in 1983 [12] and have been widely used since [13-23]. For a comparative analysis of the methods 2-chlorophenyl and 2,4-dichlorophenyl-substituted 1,4-dihydropyridine-2(3H)-thiones **3h** and **3i** were obtained by the condensation of a) ethyl 2-arylmethyleneacetoacetate, 2-cyanothioacetamide, and piperidine, b) ethyl acetoacetate, 3-aryl-2-cyanothioacrylamide, and piperidine, and c) ethyl acetoacetate, aromatic aldehyde, 2-cyanothioacetamide, and piperidine. On acidification of the resulting mixture of salts **2** and **4** the 1,4-dihydropyridine-2(3H)-thiones **3h** and **3i** were obtained in overall yields of 36-60%. The overall yields, calculated on the initial aldehyde, were 31-50% (Table 1). It should also be noted that the ethyl esters of 2-arylmethyleneacetoacetic acid are formed as a mixture of *cis* and *trans* isomers [24] and their isolation is extended in time and laborious, since products of a further Michael reaction are formed on heating with the participation of ethyl acetoacetate as a methylene component. 3-Aryl-2-cyanothioacrylamides are lachrymators and in the presence of bases are inclined to dimerize [25].

The mentioned reactions using 2-cyanothioacetamide must be carried out under mild conditions. The use of stronger bases or boiling leads to a complex mixture (dimerization of 2-cyanothioacetamide, reaction of ethyl acetoacetate with 2-cyanothioacetamide [26]) and a reduction in yield. The use in these syntheses of a stoichiometric amount of piperidine [13-21] or N-methylmorpholine [22-23] was a fortunate finding. The intermediate piperidinium thiolates **4** or **2** are poorly soluble in ethanol, which provides their rapid separation from the reaction medium. Depending on the structure of the substituent in positions 5 and 6 and on the reaction temperature thiolates **4** or **2** are separated [15,16]. If under the reaction conditions a mixture of compounds **4** and **2** at a ratio of approximately 1:1 is formed then the products crystallize out with more difficulty. Too much dilution of the reaction mixture also hinders separation of the desired products and aids their oxidation to pyridine-2(3H)-thiones and bispyridyl disulfides [14].

TABLE 1. Characteristics of the Synthesized Compounds **2-4**

Compound	Empirical formula	Found, % / Calculated, %				mp, °C	Method	Yield, %*
		C	H	N	S			
4h + 2h							A	55 (40)
							B	63 (48)
							C	45 (45)
4i + 2i							A	73 (40)
							B	65 (60)
							C	57 (57)
3h	$C_{16}H_{15}ClN_2O_2S$	57.33 / 57.40	4.24 / 4.52	8.26 / 8.37	9.58 / 9.58	129-131	A	43(31)
							B	50 (38)
							C	36 (36)
3i	$C_{16}H_{14}Cl_2N_2O_2S \times$ $\times 0.5\ H_2O$	51.18 / 50.80	3.88 / 4.00	7.15 / 7.41	8.12 / 8.48	99-101	A	60 (33)
							B	53 (50)
							C	47 (47)

* The overall yield calculated on the aldehyde is given in parentheses.

In the case of thiones **3h** and **3i** the salts corresponding to them are also separated as mixtures **4h** + **2h** (~3:1) and **4i** + **2i** (~6:1), according to the ^{1}H NMR spectra.

6-Hydroxy-1,4,5,6-tetrahydropyridine-2-thiolates **4** on thermal treatment (at times a recrystallization is sufficient) may be converted into dihydropyridine-2-thiolates **2**, but to obtain the desired products **1** the mixture of salts **4** and **2** is practicable. A longer but productive method of obtaining pure thiolates **2** is the conversion of thiolates **4** into thiones **3**, and then back into 2-thiolates **2** by the action of piperidine. 1,4-Dihydropyridine-2(3H)-thiones **3** are also unstable in dilute solution and are readily oxidized [7,8], however pure thiolates **2** and thiones **3** are preserved for years under argon.

It should be noted that it is difficult to obtain thiones **3** containing electron-donating substituents in the 4-aryl group. Due to ready oxidation it was not possible to isolate thione **3d** containing a 4-(2-methoxyphenyl) substituent, and in the case of thiones **3a-c,e** reaction yields were low for the same reason.

Nitriles of 4-aryl-5-ethoxycarbonyl-2-methylthio-1,4-dihydropyridine-3-carboxylic acids **1** were obtained by six methods, A) by the methylation of 1,4-dihydropyridine-2-thiolates **2**, B) by the methylation of 1,4-dihydropyridine-2(3H)-thiones **3** in the presence of a stoichiometric amount of piperidine, C) by the methylation of mixtures of 1,4,5,6-tetrahydro- and 1,4-dihydropyridine-2-thiolates **4** and **2**, D) by the condensation of ethyl 2-arylmethyleneacetoacetate, 2-cyanothioacetamide, piperidine, and methyl iodide, E) by the condensation of ethyl acetoacetate, 3-aryl-2-cyanothioacrylamide, piperidine, and methyl iodide, and F) by the condensation of ethyl acetoacetate, an aromatic aldehyde, 2-cyanothioacetamide, piperidine, and methyl iodide. In the case of methods C-F it is necessary to acidify the reaction mixture to complete the reaction.

Allowing for the above, the target 2-methylthio-1,4-dihydropyridines **1** were obtained by the six methods, including the methylation of thiolates **2** and of thiones **3**. Methods of four-component synthesis (D, E) have insignificant advantages since the overall yields calculated on the initial aldehyde were higher. In the case of methods A-C yields were 24-50% and in the case of methods D and E 33-80%. Method F, the condensation of ethyl acetoacetate with aromatic aldehydes and 2-cyanothioacetamide in the presence of piperidine and subsequent action of methyl iodide, was the most productive, as indicated by the yields of reaction products (75-96%) and also by economy of labour and resources. The use of methods of multicomponent synthesis enables the preparation of 2-methylthio-1,4-dihydropyridine **1d**, which is not successful by the stepwise synthesis. It should be noted that in the case of methods C, D, E, and F 1,4-dihydropyridines **1** are formed with 6-hydroxy-1,4,5,6-tetrahydropyridines **5** as contaminant. On acidifying the reaction mixture of compounds **1** + **5** the final dehydration of **5** occurs with the formation of 1,4-dihydropyridines **1** exclusively.

It has therefore been shown that, on obtaining 1,4-dihydropyridines **1** by the stepwise methods A-E, significant reductions of product yields arise both in the synthesis of the initial ethyl esters of 2-arylmethyleneacetoacetic acids and 3-aryl-2-cyanothioacrylamides and also on isolating the unstable 1,4-dihydropyridine-2(3H)-thiones **3**. The five-component synthesis of 2-methylthio-1,4-dihydropyridines (method F), proceeding rapidly and under mild conditions, is efficient and "green", since the need to synthesize 3-aryl-2-cyanothioacrylamides, which are lachrymators, becomes superfluous.

The structures of compounds **1-5** were proved spectroscopically. In the IR spectra the most characteristic absorption bands were for the stretching vibrations of the cyano group, which are observed at 2156 cm^{-1} for mixtures of salts **2** and **4**, at 2190-2204 cm^{-1} for nitriles of 1,4-dihydropyridine-3-carboxylic acids **1**, and at 2248-2250 cm^{-1} for 1,4-dihydropyridine-2(3H)-thiones **3**. In the ^{1}H NMR spectra of compounds **4** the most characteristic signals were for the H-4 and H-5 protons at 2.68-2.72 and 4.64-4.72 ppm as doublets with coupling constants ~12 Hz, which indicates the *trans*-diaxial disposition of these protons [18]. In the case of salts **2** and 1,4-dihydropyridines **1** the signals of the H-4 protons were at 4.53-5.30 ppm. In the ^{1}H NMR spectra of thiones **3** the signals of the most characteristic protons H-3 and H-4 were strongly broadened and overlapped due to the thione–enethiol tautomeric equilibrium and *cis–trans* isomerism.

The characteristics of the synthesized compounds and the data of ^{1}H NMR and IR spectra are given in Tables 1-4.

TABLE 2. Characteristics of the Synthesized Compounds **1**

Compound	Empirical formula	Found, % / Calculated, %				mp, °C	Method	Yield, %*
		C	H	N	S			
1a	$C_{17}H_{18}N_2O_2S$	64.94 64.94	5.72 5.77	8.91 8.91	10.20 10.20	123-125 [3]	A D	90 (50) 53 (52)
1b	$C_{17}H_{18}N_2O_3S \times$ $\times H_2O$	58.64 58.61	5.63 5.78	8.08 8.04	8.89 9.12	146-148 [27]	E	81 (46)
1c	$C_{18}H_{20}N_2O_3S$	62.78 62.77	5.77 5.86	8.06 8.13	9.25 9.31	150-152	E	97 (33)
1d	$C_{18}H_{20}N_2O_3S$	62.53 62.77	5.84 5.86	8.04 8.13	9.06 9.31	163-165	E	77 (55)
1e	$C_{19}H_{23}N_3O_2S \times$ $\times 0.5\ H_2O$	61.93 62.28	6.48 6.60	11.31 11.46	8.57 8.75	140-142	B	67 (28)
1f	$C_{17}H_{17}ClN_2O_2S$	62.28 58.53	4.79 4.91	7.84 8.03	8.95 9.19	116-118 [3]	A D	82 (36) 74 (56)
1g	$C_{17}H_{17}ClN_2O_2S$	58.49 58.53	4.86 4.91	7.96 8.03	9.09 9.19	127-128	F	81 (81)
1h	$C_{17}H_{17}ClN_2O_2S$	58.50 58.53	4.80 4.91	7.93 8.10	9.12 9.19	174-176	B C D E F	76 (24) 96 (46) 80 (58) 91 (69) 96 (96)
1i	$C_{17}H_{16}Cl_2N_2O_2S$	53.31 53.27	4.10 4.21	7.21 7.31	8.32 8.37	186-188	C D E F	79 (47) 86 (47) 86 (80) 78 (78)
1j	$C_{17}H_{17}N_3O_4S$	56.76 56.81	4.77 4.77	11.52 11.69	8.90 8.92	161-162	B D	67 (37) 57 (38)
1k	$C_{17}H_{17}N_3O_4S$	56.54 56.81	4.77 4.77	11.69 11.69	8.69 8.92	102-104	B	83 (41)
1l	$C_{18}H_{17}N_3O_3S$	63.84 63.70	4.95 5.05	12.22 12.38	9.27 9.45	107-109	B	48 (24)
1m	$C_{18}H_{17}N_3O_2S$	63.22 63.70	4.84 5.05	12.33 12.38	9.40 9.45	135-137	F	75 (75)

* Yields calculated on the aldehyde are given in parentheses.

The electrochemical oxidation of compounds **1** was carried out in anhydrous acetonitrile at the stationary glass-graphite electrode. Compounds were adsorbed onto the surface of the electrode since the electrolysis was carried out in the presence of surface-active camphor at 0.1% concentration. For all the 1,4-dihydropyridine derivatives **1** (except for **1e**) one irreversible oxidation peak was recorded in the potential range E_p = 1.28-1.44 V relative to the saturated calomel electrode (Table 5). Polarograms were also recorded for compounds **1a** and **1f** at the rotating disk electrode with a ring, and their characteristics were compared with results obtained previously for compounds close in structure [11]. The electrochemical oxidation of compounds **1** proceeds along the previously established route. At the disk electrode one oxidation wave arises at a half-wave potential $E_{1/2}$ = 1.03 (for **1a**) or $E_{1/2}$ = 1.08 V (for **1f**), but at the ring electrode waves were recorded for the electroreduction products: $E_{1/2}$ = -1.08, –1.80 (for **1a**) and $E_{1/2}$ = -1.01, -1.73 V (for **1f**), indicating the formation of the corresponding pyridinium cation and pyridine as the oxidation products [11, 28-33].

The molecules of compounds **1a-m** have unsymmetrical substituents on the heterocycle, consequently it seemed of interest to clarify how the electronic properties of the cyano group in position 3 and the thiomethyl group in position 2 of the heterocycle influence electrooxidizability in comparison with the symmetrical 3,5-diethoxycarbonyl-2,6-dimethyl-4-phenyl derivative of 1,4-dihydropyridine. Cyclic voltammeric curves were recorded under the same experimental conditions for the model compounds 3,5-diethoxycarbonyl-2,6-dimethyl-

4-phenyl-1,4-dihydropyridine (**6**) and 3-cyano-5-ethoxycarbonyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine (**7**). It was shown that replacement of the ethoxycarbonyl group by cyano hinders the removal of the first electron by 90 mV (E_p = 1.13 for **6** and E_p = 1.22 V for **7**). Further replacement of a methyl group by thiomethyl (compound **1a**) in position 2 of the heterocycle hinders electrooxidation still more by 90 mV (E_p = 1.22 for **7** and E_p = 1.32 V for **1a**).

When considering the influence of substituents in the phenyl ring at position 4 of a 1,4-dihydropyridine, it was established that *meta* and *para* nitro groups hinder the electrooxidation by 120 and 140 mV respectively (compounds **1k** and **1j**). Compounds substituted in the *ortho* position (**1d** and **1h**) were oxidized more readily than the corresponding *para*- and *meta*-substituted compounds, due to intramolecular interactions. 4-(*p*-Dimethylaminophenyl)-1,4-dihydropyridine **1e** is oxidized stepwise with the formation of several oxidation

TABLE 3. Spectral Characteristics of Compounds **1**

Compound	IR spectrum, ν, cm^{-1}	^1H NMR spectrum ($CDCl_3$), δ, ppm
1a	1702 (C=O); 2198 (C≡N); 3278 (NH)	1.13 and 4.03 (5H, t and q, OC_2H_5); 2.36 (3H, s, 6-CH_3); 2.44 (3H, s, SCH_3); 4.68 (1H, s, H-4); 6.34 (1H, br. s, NH); 7.22 (4H, m, C_6H_5)
1b	1654, 1680 sh (C=O); 2202 (C≡N); 3190 (NH)	1.16* and 4.03 (5H, t and q, OC_2H_5); 2.32 (3H, s, 6-CH_3); 2.42 (3H, s, SCH_3); 4.53 (1H, s, 4-H); 6.74 and 7.00 (4H, d and d, C_6H_4); 8.14 (1H, br. s, NH)
1c	1676, 1698 (C=O); 2192, 2200 (C≡N); 3224, 3266 (NH)	1.16 and 4.03 (5H, t and q, OC_2H_5); 2.37 (3H, s, 6-CH_3); 2.47 (3H, s, SCH_3); 3.77 (3H, s, OCH_3); 4.63 (1H, s, H-4); 6.13 (1H, br. s, NH); 6.83 and 7.18 (4H, d and d, C_6H_4)
1d	1700 (C=O); 2190(C≡N); 3280 (NH)	1.00 and 3.98 (5H, t and q, OC_2H_5); 2.37 (3H, s, 6-CH_3); 2.42 (3H, s, SCH_3); 3.86 (3H, s, OCH_3); 5.15 (1H, s, H-4); 6.18 (1H, s, NH); 6.8-7.3 (4H, m, C_6H_4)
1e	1680 (C=O); 2194 (C≡N); 3226, 3278 (NH)	1.17 and 4.04 (5H, t and q, OC_2H_5); 2.35 (3H, s, 6-CH_3); 2.44 (3H, s, SCH_3); 2.90 [4H, s, $N(CH_3)_2$]; 4.57 (1H, s, H-4); 6.22 (1H, br. s, NH); 6.17 and 7.08 (4H, d and d, C_6H_4)
1f	1688 (C=O); 2202 (C≡N); 3280 (NH)	1.13 and 4.04 (5H, t and q, OC_2H_5); 2.35 (3H, s, 6-CH_3); 2.42 (3H, s, SCH_3); 4.66 (1H, s, H-4); 6.52 (1H, br. s, NH); 7.1-7.4 (4H, m, C_6H_4)
1g	1686 (C=O); 2196(C≡N); 3284 (NH)	1.13 and 4.04 (5H, t and q, OC_2H_5); 2.35 (3H, s, 6-CH_3); 2.43 (3H, s, SCH_3); 4.65 (1H, s, H-4); 6.30 (1H, br. s, NH); 7.0-7.3 (4H, m, C_6H_4)
1h	1688 (C=O); 2194 (C≡N); 3290 (NH)	1.08 and 3.98 (5H, t and q, OC_2H_5); 2.37 (3H, s, 6-CH_3); 2.42 (3H, s, SCH_3); 5.30 (1H, s, H-4); 6.40 (1H, br. s, NH); 7.0-7.4 (4H, m, C_6H_4)
1i	1708 (C=O); 2194 (C≡N); 3304 (NH)	1.12 and 3.98 (5H, t and q, OC_2H_5); 2.38 (3H, s, 6-CH_3); 2.45 (3H, s, SCH_3); 5.27 (1H, s, H-4); 6.18 (1H, br. s, NH); 7.22 and 7.38 (4H, m, C_6H_4)
1j	1640 (C=O); 2204(C≡N); 3195, 3260 (NH)	1.15 and 4.08 (5H, t and q, OC_2H_5); 2.43 (3H, s, 6-CH_3); 2.51 (3H, s, SCH_3); 4.83 (1H, s, H-4); 6.14 (1H, br. s, NH); 7.44 and 8.20 (4H, d and d, C_6H_4)
1k	1646, 1670 (C=O); 2202(C≡N); 3316 (NH)	1.14 and 4.06 (5H, t and q, OC_2H_5); 2.37 (3H, s, 6-CH_3); 2.47 (3H, s, SCH_3); 4.82 (1H, s, H-4); 6.58 (1H, br. s, NH); 7.4-8.2 (4H, m, C_6H_4)
1l	1634 (C=O); 2198, 2222 (C≡N); 3192, 3250 (NH)	1.12 and 4.06 (5H, t and q, OC_2H_5); 2.38 (3H, s, 6-CH_3); 2.48 (3H, s, SCH_3); 4.77 (1H, s, H-4); 6.18 (1H, s, NH); 7.37 and 7.64 (4H, d and d, C_6H_4)
1m	1700 (C=O); 2198, 2218 (C≡N); 3320 (NH)	1.14 and 4.03 (5H, t and q, OC_2H_5); 2.38 (3H, s, 6-CH_3); 2.47 (3H, s, SCH_3); 4.72 (1H, s, H-4); 6.43 (1H, br. s, NH); 7.40-7.65 (4H, m, C_6H_4)

* $CDCl_3$ + DMSO.

TABLE 4. Spectral Characteristics of Compounds **2-4**

Compound	IR spectrum, ν, cm^{-1}	^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz)
2h + 4h	1720 (C=O); 2156 (C≡N); 3170 (NH); 3400 (OH)	0.91 and 3.96 (5H, t and q, OC_2H_5); 1.48 (3H, s, 6-CH_3); 1.6-.8 [6H, m, $(CH_2)_3$]; 2.68 and 4.72 (2H, d and d, *J* = 12, H-5 and H-4); 2.9-.1 [4H, m, $N(CH_2)_2$]; 5.82 (1H, br. s, OH); 6.58 (1H, br. s, NH); 7.0-.5 (4H, m, C_6H_4); 1.07 and 3.96 (5H, t and q, OC_2H_5); 1.6-.8 [6H, m, $(CH_2)_3$]; 2.35 (3H, s, 6-CH_3); 2.9-.1 [4H, m, $N(CH_2)_2$]; 5.18 (1H, s, H-4); 7.0-.5 (4H, m, C_6H_4)
2i + 4i	1720 (C=O); 2156 (C≡N); 3206 (NH); 3406 (OH)	0.97 and 3.98 (5H, t and q, OC_2H_5); 1.48 (3H, s, 6-CH_3); 1.6-.8 [6H, m, $(CH_2)_3$]; 2.72 and 4.64 (2H, d and d, *J* = 12.2, H-5 and H-4); 2.9-3.1 [4H, m, $N(CH_2)_2$]; 5.84 (1H, br. s, OH); ~ 6.6 (1H, br. s, NH); 7.1-7.5 (4H, m, C_6H_4); 1.08 and 3.98 (5H, t and q, OC_2H_5); 1.6-1.8 [6H, m, $(CH_2)_3$]; 2.34 (3H, s, 6-CH_3); 2.9-3.1 [4H, m, $N(CH_2)_2$]; 5.12 (1H, s, H-4); 7.1-7.5 (4H, m, C_6H_4)
3h	1702 (C=O); 2248 (C≡N); 3280 (NH)	1.16, 4.10 and 4.20 (5H, t and q, OC_2H_5); 2.50 and 2.62 (3H, s and s, 6-CH_3); 4.9-5.3 (2H, m, H-3 and H-4); 6.9-7.6 (4H, m, C_6H_4); 8.42 (1H, br. s, NH)
3i	1698 (C=O); 2250 (C≡N); 3230 (NH)	1.20, 4.14 and 4.20 (5H, t and q, OC_2H_5); 2.54 and 2.63 (3H, s and s, 6-CH_3); 4.9-5.3 (2H, m, H-3 and H-4); 6.98, 7.22, 7.52 (3H, d, *J* = 10, dd, *J* = 2 and 10, d, *J* = 2, C_6H_3); 8.98 (1H, br. s, NH).

peaks at extremely positive potentials. A similar phenomenon was observed previously when oxidizing the corresponding symmetrical 1,4-dihydropyridines [34]. Facilitation of the oxidation of compound **1e** is explained by the prior electrooxidation of the dimethylamino group. To check this hypothesis oxidation of compound **1e** was carried out at the rotating disk electrode and at the ring electrode were recorded waves for the reduction of the oxidized products. It turned out that in the process of the electrooxidation of **1e** four waves were formed with half-wave potentials $E_{1/2}$ = 0.42, 0.80, 1.12, and 2.00 V relative to the $Ag/AgNO_3$ reference electrode (it is probable that at the stationary electrode the fourth wave is hidden by separation of the background). At the ring electrode, depending on the degree of oxidation of **1e,** two oxidized products are detected capable of being reduced at potentials $E_{1/2}$ = -1.29 V (oxidation was carried out at the potential of the limiting current of the third wave) and $E_{1/2}$ = -0.80 V (oxidation proceeds at the potential of the stationary current of the fourth wave), which corresponds to the pyridinium cation with various substituents at position 4 of the heterocycle. From the values of the reduction potentials it may be proposed that in the first stage the substituent partially retained electron-donating properties, and in the second the product contains a markedly electron-withdrawing substituent.

TABLE 5. Values of the Potentials of Oxidation Peaks on Cyclic Scanning of the Potential of the Stationary Electrode (E_p) for Compounds **1a-m** in Acetonitrile on a Base of 0.1 M $(C_4H_9)_4PF_6$ with Added 0.1% Camphor

No	Compound	E_p, V	No	Compound	E_p, V
1	**1a**	1.32	8	**1h**	1.36
2	**1b**	1.32	9	**1i**	1.40
3	**1c**	1.33	10	**1j**	1.44
4	**1d**	1.28	11	**1k**	1.42
5	**1e**	0.79; 0.99; 1.50	12	**1l**	1.42
6	**1f**	1.42	13	**1m**	1.39
7	**1g**	1.42			

The characteristics of the influence of substituents in the phenyl ring are mainly in agreement with the data given previously for symmetrical 3,5-dicarbethoxy-2,6-dimethyl-4-phenyl-substituted 1,4-dihydropyridines in [34,35]. However the introduction of a chlorine atom into the *para* or *meta* position of the phenyl ring (compounds **1f** and **1g**), in difference to the symmetrical 1,4-dihydropyridines, hinders oxidation like a nitro group in the *meta* position. Attempts were undertaken by the method of correlation-statistical analysis to trace quantitatively the influence of substituents in the phenyl ring on the electrooxidation potentials E_p. A linear correlation is observed with the Taft σ* constants. Nine representatives of the series (except for **1d-g**) fit onto a correlation straight line with parameters ρ* = 0.120 V (r = 0.977, ΔS = 0.011), and if **1c** and **1m** are also excluded from the equation then the remaining representatives of the series practically lie on the straight line with ρ* = 0.133 (r = 0.992, ΔS = 0.006). It is possible that the noted deviations from the straight line are caused by the specific properties of certain substituents, since the electro-oxidation potentials of 1,4-dihydropyridines at solid electrodes depends not only on electronic and steric effects but also on adsorbability, coplanarity, and the orientation of electroactive molecules on the electrode surface [36-38]. The sensitivity constant ρ* for the influence of substituents in the phenyl ring for compounds of the type of **1** differs little in size from that for symmetrical 4-phenyl-substituted 3,5-diethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridines (ρ* = 0.15 [34]). Consequently replacement of a methyl group by a thiomethyl in position 2 and an ethoxycarbonyl group by cyano in position 3 of the heterocycle has no significant influence on the effect of the substituents in the phenyl ring.

The cardiovascular activity of nitriles of 2-methylthio-1,4-dihydropyridine-3-carboxylic acid **4** was studied in experimental animals, in cats, dogs, and spontaneously hypertensive rats (SHR), and the acute toxicity was studied in mice. As is seen from Table 6 compounds **1f** and **1k** in high doses cause an increase in coronary bloodflow by 25-40% for 3-10 min, simultaneously reducing systemic arterial pressure by 29-53%. In experiments in dogs 1,4-dihydropyridine **1a** at low doses displays a more marked coronary dilatating action (30-58%) with a duration of 10-20 min, while not changing the systemic arterial pressure and pulse rate. Compound **1k**, containing the pharmacophoric 3-nitrophenyl group, gives an insignificant hypotensive effect in experiments in SHR.

The investigated compounds had low toxicity. The lethal dose was greater than 1000 mg/kg.

TABLE 6. Effect of 2-Methylthio-1,4-dihydropyridines **1** on Parameters of the Cardiovascular System and Their Acute Toxicity

Compound	Dose, mg/kg	Increase in coronary blood flow, %	Duration of effect, min	Change in pulse rate, %	Hypotensive activity, %	Hypotensive activity in SHR (10mg/kg), mm Hg	LD_{50}, mg/kg ip
1a	0.01	30*	10	n. i.	n. i.		>1000
	0.1	58*	20	n. i.	n. i.		
1f	0.1	40	3	8↓	41↓		>5000
	1.0	25	6	25↓	36↓		
	5.0	25	10	34↓	41↓		
1k	0.1	n. i.		14↓	14↓	13↓ 6 h	>2000
	1.0	30	5	31↓	29↓		
	5.0	36	10	27↓	53↓		

*Coronary blood flow was measured fluorometrically in dogs (intravenously).

EXPERIMENTAL

Chemical Section. The IR spectra of the initial compounds were taken on a Perkin-Elmer 580B spectrometer in nujol. The ^{1}H NMR spectra were recorded on a Bruker WH 90/DC (90 MHz) spectrometer in $CDCl_3$, internal standard was HMDS (δ 0.05 ppm). A check on the progress of reactions and the homogeneity of substances was effected by TLC on Silufol UV 254 plates, eluent was chloroform–hexane–acetone, 2:1:1. Compounds were recrystallized from ethanol. The initial ethyl esters of 2-arylmethyleneacetoacetic acid were synthesized according to [39], and the 3-aryl-2-cyanothioacrylamides according to [25]. Yields of products were: ethyl 2-phenylmethyleneacetoacetate 80%, ethyl 2-(4-chlorophenyl)methyleneacetoacetate 75%, ethyl 2-(2-chlorophenyl)methyleneacetoacetate 72%, ethyl 2-(2,4-dichlorophenyl)methyleneacetoacetate 55%, ethyl 2-(4-nitrophenyl)methyleneacetoacetate 66%, ethyl 2-(3-nitrophenyl)methyleneacetoacetate 80%, 2-cyano-3-(4-hydroxyphenyl)thioacrylamide 57%, 2-cyano-3-(4-methoxyphenyl)thioacrylamide 81%, 2-cyano-3-(2-methoxyphenyl)thioacrylamide 72%, 2-cyano-3-(4-dimethylaminophenyl)thioacrylamide 88%, 3-(2-chlorophenyl)-2-cyanothioacrylamide 76%, and 3-(2,4-dichlorophenyl)-2-cyanothioacrylamide 93%. Yields of thiolates were **2a** 69% [14], **2f** 59% [14], and thiones **3e** 48% [14], **3k** 61% [15], and **3l** 24% [21].

Cyclic voltammetric curves were recorded on a PAR 170 (USA) electrochemical system using a three-electrode cell with a stationary glass-graphite electrode. The reference electrode was an aqueous saturated calomel electrode, fitted with a transfer bridge for working in nonaqueous solvents. Electrochemical investigations by the method of a rotating disk electrode with a ring were carried out in equipment consisting of a PAR (USA) Ring-Disk-Electrode System Model 636 and a Bruker E 350 twin potentiostat. The disk and ring electrodes were prepared from glass-graphite. The calculated efficiency coefficient of the electrodes [40] was 0.39 and the rate of rotation of electrodes was 2000 rpm. All potentials were measured relative to a 0.1 N silver reference electrode ($Ag/AgNO_3$). All investigations were carried out in nonaqueous acetonitrile, purified by the procedure of [41], with the addition of 0.1% camphor. Depolarizer concentration was 5.10^{-4} M. Base electrolyte was $1 \cdot 10^{-1}$ M tetrabutylammonium hexafluorophosphate.

General Methods for the Synthesis of Nitriles of 4-Aryl-3-cyano-5-ethoxycarbonyl-6-methyl-2-thioxo-1,4-dihydropyridine-3-carboxylic Acids (3). a) A mixture of ethyl 2-arylmethyleneacetoacetate (5 mmol) and 2-cyanothioacetamide (5 mmol) in ethanol (15-20 ml) was heated briefly to dissolve the starting materials, piperidine (6 mmol) was added, and the mixture stirred for 10-30 min. The solid formed was filtered off and washed with ethanol (5-10 ml) cooled to 0°C. A mixture of compounds **4h** and **2h**, and **4i** and **2i** was obtained by this method (see Table 2 for ^{1}H NMR spectra). The mixture of salts **2** and **4** (5 mmol) and 1 M HCl in ethanol (10 ml) was heated briefly and stirred for 10-20 min at room temperature. The resulting solid was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml). Compounds **3h** and **3i** were obtained by this method.

b) A mixture of ethyl acetoacetate (5 mmol) and 3-aryl-2-cyanothioacrylamide (5 mmol) in ethanol (15-20 ml) was heated briefly to dissolve the starting materials. Piperidine (6 mmol) was added, and the mixture stirred for 10-30 min. The resulting solid was filtered off, and washed with ethanol (5-10 ml) cooled to 0°C. Mixtures of compounds **2d** and **4d**, **2h** and **4h**, and **2i** and **4i** were obtained by this method (see Table 4 for ^{1}H NMR spectra). The mixture of salts **2** and **4** (5 mmol) and 1M HCl in ethanol (10 ml) was heated briefly and stirred for 10-20 min at room temperature. The resulting solid was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml). Compounds **3h** and **3i** were obtained by this method.

c) A mixture of aromatic aldehyde (5 mmol), 2-cyanothioacetamide (5 mmol), and piperidine (1 mmol) in ethanol (15-20 ml) was heated briefly to effect solution. Then ethyl acetoacetate (5 mmol) and piperidine (5 mmol) were added with stirring at room temperature. The resulting solid was filtered off, and washed with ethanol (5-10 ml) cooled to 0°C. Mixtures of compounds **2d** and **4d**, **2h** and **4h**, and **2i** and **4i** were obtained by this method (see Table 4 for ^{1}H NMR spectra). Mixtures of salts **4** and **2** (5 mmol) and 1 M HCl in ethanol (10 ml) were heated briefly and stirred for 10-20 min at room temperature. The resulting solid was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml). Compounds **3h** and **3i** were obtained by this method.

General Method for the Synthesis of Nitriles of 4-Aryl-3-cyano-5-ethoxycarbonyl-6-methyl-2-methylthio-1,4-dihydropyridine-3-carboxylic Acids (1). A. A mixture of 1,4-dihydropyridine-2(3H)-thiolate (10 mmol) and methyl iodide (15 mmol) or dimethyl sulfate (12 mmol) in ethanol (20-40 ml) was boiled for 2-5 min on a water bath, then stirred for 1 h at room temperature. The solid which had formed was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml). Compounds **1a,f** were obtained by this method.

B. A mixture of 1,4-dihydropyridine-2(3H)-thione **3** (10 mmol), piperidine (11 mmol), and methyl iodide (15 mmol) or dimethyl sulfate (12 mmol) in ethanol (20-40 ml) was boiled on a water bath, then stirred for 1 h at room temperature. The resulting solid was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml). Compounds **1e,h,j,k,l** were obtained by this method.

C. A mixture of 1,4,5,6-tetrahydropyridin-2(3H)-thiolate **4** (with admixture of thiolate **2**) (10 mmol) and methyl iodide (15 mmol) in ethanol (20-40 ml) was boiled on a water bath, 3 M HCl in ethanol (2 ml) was added, and the mixture was then stirred for 1 h at room temperature. The precipitate which formed was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml). Compounds **1h,i** were obtained by this method.

D. A mixture of ethyl 2-arylmethyleneacetoacetate (5 mmol), 2-cyanothioacetamide (5 mmol), and piperidine (6 mmol) in ethanol (15-20 ml) was boiled briefly to effect solution, and after 10 min methyl iodide (10 mmol) was added. The mixture was boiled for 5 min on a water bath and 3 M HCl (2 ml) was added. The resulting solid was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml). Compounds **1a,f,h,i,j** were obtained by this method.

E. A mixture of ethyl acetoacetate (5 mmol), 3-aryl-2-cyanothioacrylamide, and piperidine (6 mmol) in ethanol (15-20 ml) was boiled briefly to effect solution and after 10 min methyl iodide (10 mmol) was added. The mixture was boiled for 5 min on a water bath and 3 M HCl in ethanol (2 ml) was added. The solid formed was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml). Compounds **1b-d,h,i** were obtained by this method.

F. A mixture of aromatic aldehyde (5 mmol), 2-cyanothioacetamide (5 mmol), and piperidine (1 mmol) in ethanol (15-20 ml) was boiled briefly to effect solution. While stirring at room temperature, ethyl acetoacetate (5 mmol) and piperidine (5 mmol) were added and then after 10 min methyl iodide (30 mmol) was added. The mixture was boiled for 5 min on a water bath, and 3 M HCl in ethanol (2 ml) was added. The solid formed was filtered off, washed with ethanol (5-10 ml) cooled to 0°C, and with water (10 ml). Compounds **1g-i,m** were obtained by this method.

Pharmacological Section. Experiments were carried out in cats of both sexes of weight 2.3-3.6 kg, anesthetized with chloralose (90 mg/kg, ip). Systemic arterial pressure was recorded by an electromanometric method from the common carotid artery. The *dp/dt* was calculated with the aid of a pressure processor (Nihon Kohden). ECG were drawn in standard I mode. The volume rate of coronary blood flow was determined by the method of N. V. Kaverina [42], and blood flow in the femoral artery with the aid of a MFV 1200 flowmeter (Nihon Kohden). All records were produced on a RM 6000 (Nihon Kohden) polygraph.

In experiments on mongrel dogs of both sexes of weight 13-24 kg, anesthetized with pentobarbital sodium (50 mg/kg, ip), systemic arterial pressure and ECG were recorded as in the experiments on cats.

Substances were dissolved in 50% dimethylacetamide and injected intra-venously through a cannula introduced into the femoral vein.

Each substance was investigated in 3-4 animals and mean data calculated.

In the experiments on awake, spontaneously hypertensive rats of the Okamoto-Aoki line [43] the systemic arterial pressure was determined by plethysmography [44] before administering the substance and 0.5, 1, 3, 6, and 24 h afterwards. Each dose of substance was investigated in 3-6 rats using aqueous suspensions prepared in Tween 80, which were introduced into the stomach through a probe.

Acute toxicity was investigated in white random-bred mice of weight 18-24 g. A suspension of the substance in water with added Tween 80 (0.05 ml 6% Tween per 5 mg substance) was administered intraperitoneally. Each dose was investigated in 3-6 mice, animals were observed for 10 d. The mean lethal dose (LD_{50}) was determined by the method of Litchfield and Wilcoxon.

REFERENCES

1. V. V. Kastron, R. O. Vitolin', and G. Ya. Dubur, *Khim.-farm. Zh.*, **24**, No. 6, 14 (1990).
2. D. J. Triggle, *Can. J. Physiol. Pharmacol.*, **68**, 1474 (1990).
3. N. Rojstaczer and D. J. Triggle, *Cardiovascular Drug Rev.*, **12**, 70 (1994).
4. V. Klusa, *Drugs of the Future*, **20**, 135 (1995).
5. A. A. Krauze, R. O. Vitolinya, M. R. Romanova, and G. Ya. Dubur, *Khim.-farm. Zh.*, **22**, 955 (1988).
6. A. A. Krauze, A. G. Odynets, A. A. Verreva, S. K. Germane, A. N. Kozhukhov, and G. Ya. Dubur, *Khim.-farm. Zh.*, **25**, No. 7, 40 (1991).
7. I. E. Kirule, A. A. Krauze, A. Kh. Velena, D. Yu. Antipova, G. Ya. Arnitsane, I. A. Vutsina, and G. Ya. Dubur, *Khim.-farm. Zh.*, **26**, No. 11/12, 59 (1992).
8. D. Tirzite, A. Krauze, A. Zubareva, G. Tirzitis, and G. Duburs, *Khim. Geterotsikl. Soedin.*, 902 (2002).
9. A. Krauze, J. Popelis, and G. Duburs, *Tetrahedron*, **54**, 9161 (1998).
10. A. Krauze, L. Sile, and G. Duburs, *Heterocycl. Commun.*, **7**, 375 (2001).
11. L. Baumane, A. Krauze, L. Chernova, L. Sile, G. Duburs, and J. Stradins, *Khim. Geterotsikl. Soedin.*, 1808 (2003).
12. A. A. Krauze, Yu. E. Pelcher, and Z. A. Kalme, *Abstracts of the All-Union Conference on Progress in the Chemistry of Nitrogen Heterocycles*, Rostov-on-Don, S70, 105, (1983).
13. A. A. Krauze, Yu. E. Pelcher, Z. A. Kalme, and G. Ya. Dubur, *Khim. Geterotsikl. Soedin.*, 1694 (1984).
14. A. A. Krauze, E. E. Liepin'sh, Yu. E. Pelcher, Z. A. Kalme, and G. Ya. Dubur, *Khim. Geterotsikl. Soedin.*, 95 (1985).
15. A. A. Krauze, R. O. Vitolinya, G. V. Zarin'sh, Yu. E. Pelcher, Z. A. Kalme, A. A. Kimenis, and G. Ya. Dubur, *Khim.-farm. Zh.*, **19**, 540 (1985).
16. A. A. Krauze, E. E. Liepin'sh, Yu. E. Pelcher, Z. A. Kalme, and G. Ya. Dubur, *Khim. Geterotsikl. Soedin.*, 630 (1986).
17. Yu. A. Sharanin, A. M. Shestopalov, L. A. Rodinovskaya, V. N. Nesterov, V. E. Shklover, Yu. T. Struchkov, V. K. Promonenkov, and V. P. Litvinov, *Zh. Org. Khim.*, **22**, 2600 (1986).
18. A. A. Krauze, E. E. Liepin'sh, Yu. E. Pelcher, Z. A. Kalme, and G. Ya. Dubur, *Khim. Geterotsikl. Soedin.*, 75 (1987).
19. A. A. Krauze, R. O. Vitolinya, M. R. Romanova, and G. Ya. Dubur, *Khim.-farm. Zh.*, **22**, 548 (1988).
20. A. A. Krauze, Kh.-I. En'sh, and G. Ya. Dubur, *Khim. Geterotsikl. Soedin.*, 1674 (1991).
21. A. Krauze and G. Duburs, *Khim. Geterotsikl. Soedin.*, 794 (2000).
22. S. G. Krivokolysko, V. D. Dyachenko, and V. P. Litvinov, *Izv. Akad. Nauk, Ser. Khim.*, 166 (1999).
23. S. G. Krivokolysko, V. D. Dyachenko, and V. P. Litvinov, *Izv. Akad. Nauk, Ser. Khim.*, 173 (2000).
24. P. Albough-Robertson and J. A. Katzenellenbogen, *J. Org. Chem.*, **48**, 5288 (1983).
25. J. S. A. Brunskill, A. De, and D. F. Ewing, *J. Chem. Soc., Perkin Trans. 1*, 629 (1978).
26. B. Y. Riad, A. M. Negm, S. E. Abdou, and H. A. Doboun, *Heterocycles*, **26**, 205 (1987).
27. A. Krauze, R. Verhe, and G. Duburs, *Latv. J. Chem.*, No. 1-2, 94 (1996).
28. Ya. P. Stradyn', G. Ya. Dubur, Yu. I. Beilis, Ya. R. Uldrikis, and A. F. Korotkova, *Khim. Geterotsikl. Soedin.*, 84, (1972).
29. V. Skala, J. Volke, V. Ohanka, and J. Kuthan, *Coll. Czech. Chem. Commun.*, **40**, 2632 (1975).
30. J. Ludvik, J. Klima, J. Volke, A. Kurfürst, and J. Kuthan, *J. Electroanal. Chem.*, **138**, 131 (1982).

31. Ya. V. Ogle, Ya. P. Stradyn', G. Ya. Dubur, V. K. Lusis, and V. P. Kadysh, *Khim. Geterotsikl. Soedin.*, 1263 (1980).
32. J. Stradins, L. Baumane, A. Kalnyn'sh, Ya. Uldrikis, E. Bisenieks, Ya. Poikans, and G. Duburs, *Khim. Geterotsikl. Soedin.*, 1360 (2000).
33. C. Lopez-Alarcon, L. J. Nunez-Vergara, and J. A. Squella, *Electrochim. Acta*, **48**, 2505 (2003).
34. Ya. P. Stradyn', Yu. I. Beilis, Ya. R. Uldrikis, G. Ya. Dubur, A. E. Sausin', and B. S. Chekavichus, *Khim. Geterotsikl. Soedin.*, 1525 (1975).
35. Ya. P. Stradyn', G. Ya. Dubur, Yu. I. Beilis, Ya. R. Uldrikis, and A. F. Korotkova, *Khim. Geterotsikl. Soedin.*, 84 (1972).
36. Ya. P. Stradyn', G. Ya. Dubur, Yu. I. Beilis, Ya. R. Uldrikis, A. E. Sausin', and B. S. Chekavichus, *Khim. Geterotsikl. Soedin.*, 1530 (1975).
37. N. R. Konopleva, Ya. P. Stradyn', N. P. Skvortsov, G. Ya. Dubur, and Yu. V. Vodzinskii, *Izv. Akad. Nauk Latv. SSR, Ser. Khim.*, 678 (1983).
38. V. P. Kadysh, Ya. P. Stradyn', Ya. R. Uldrikis, and G. Ya. Dubur, *Khim. Geterotsikl. Soedin.*, 1223 (1992).
39. *Practical Handbook of Organic Chemistry, Part II* [Russian translation], Mir, Moscow (1979), p. 147.
40. M. R. Tarasevich, E. I. Khrushcheva, and V. Yu. Filinovskii, *The Rotating Disk Electrode with a Ring* [in Russian], Nauka, Moscow (1987), p. 247.
41. D. Clark, M. Fleishmann, and D. Pletcher, *J. Electroanal. Chem.*, **36**, 137 (1972).
42. N. V. Kaverina, *Farmakol. Toksikol.*, **21**, 39 (1958).
43. K. Okamoto and K. Ajki, *Jpn. Circulat. J.*, **27**, 282 (1963).
44. H. Brauniger, *Arzneim.-Forsch.*, **6**, 222 (1956).