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SYNTHESIS AND ANTIRADICAL ACTIVITY OF 5-ACETYL-2-ALKYLTHIO-4-ARYL-6-METHYL-1,4-DIHYDROPYRIDINE-3-CARBOXYLIC ACID NITRILES

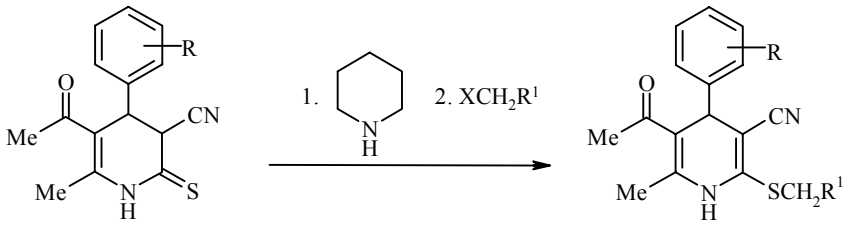
D. Tirzite, A. Krauze, A. Zubareva, G. Tirzitis, and G. Duburs

The alkylation of 3-cyano-1,4-dihydropyridine-2(3H)-thiones or the condensation of an aromatic aldehyde, cyanothioacetamide, acetylacetone, and methyl iodide in the presence of piperidine has given a series of novel 5-acetyl-2-alkylthio-4-aryl-6-methyl-1,4-dihydropyridine-3-carboxylic acid nitriles. A compound was obtained from 3,5-di(tert-butyl)-4-hydroxybenzaldehyde in the molecule of which were combined the active part of the antioxidant ionol and a 1,4-dihydropyridine ring. It was found that, among the compounds synthesized, the highest antiradical activity occurred in a compound having two hydroxyl groups in the 4-phenyl substituent.

Keywords: 1,4-dihydropyridines, 1,1-diphenyl-2-picrylhydrazyl, radical cation, trolox, antiradical activity.

There are many compounds amongst 4-substituted 1,4-dihydropyridine-3,5-dicarboxylic esters which possess cardiovascular activity [1]. Their antioxidant activity (AOA) and antiradical activity (ARA) have been widely investigated [2] as well as the relationship of AOA and ARA with cardiovascular properties [3, 4]. Unsymmetrical 2-alkylthio-1,4-dihydropyridines have been less studied but they also show marked cardiovascular [5], hepatoprotective [6], and antioxidant [7] activities.

Continuing our work on the synthesis and properties of 2-alkylthio-1,4-dihydropyridines [8], we have prepared a series of novel 5-acetyl-2-alkylthio-4-aryl-6-methyl-1,4-dihydropyridine-3-carboxylic acid nitriles and examined their ARA. The ARA and membranotropic activities are of value in a more purposeful search for biologically active substances, since the processes of peroxide oxidation of lipids and the regulation of these processes by membranotropic compounds have an important significance in a series of illnesses [9].



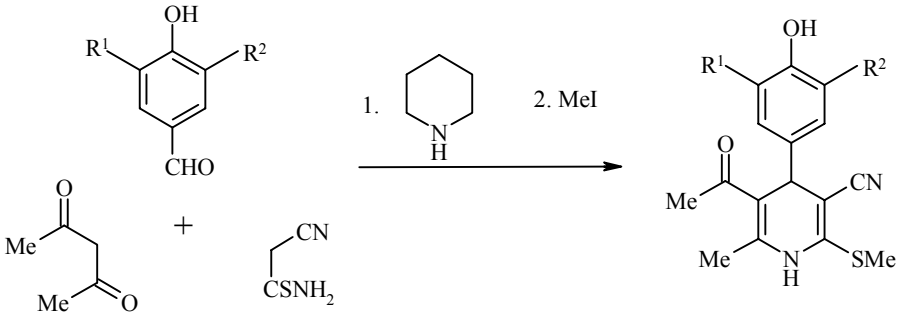
1 **2a–l**

a R = R^1 = H; **b** R = H, R^1 = Me; **c** R = H, R^1 = COOEt; **d** R = 4-OH, R^1 = H; **e** R = 4-Cl, R^1 = H; **f** R = 4-NO_2, R^1 = H; **g** R = 4-NO_2, R^1 = Me; **h** R = 3-NO_2, R^1 = H; **i** R = 3-NO_2, R^1 = Me; **j** R = 3-NO_2, R^1 = Et; **k** R = 3-NO_2, R^1 = CH=CH_2; **l** R = 3-NO_2, R^1 = $(CH_2)_{14}$Me. X = Br, I

Latvian Institute of Organic Synthesis, Riga LV-1006; e-mail: dace@osi.lv, krauze@osi.lv. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 7, pp. 902-907, July, 20002. Original article submitted March 25, 2002.

The 5-acetyl-2-alkylthio-4-aryl-6-methyl-1,4-dihydropyridine-3-carboxylic acid nitriles **2a-l** were prepared in high yields (compounds **2h-l** for the first time) by the alkylation of the corresponding 1,4-dihydropyridine-2(3H)-thiones **1** with an excess of the alkyl halide (the excess was increased in the case of the readily volatile methyl iodide and ethyl bromide) in the presence of piperidine. The length of the alkyl chain in the 2-alkylthio group was varied in order to increase the lipophilicity.

Up to now, the 4-(2,3-dihydroxy)phenyl- and 4-[3,5-di(*tert*-butyl)-4-hydroxy]phenyl-substituted 1,4-dihydropyridine-2(3H)-thiones have not been prepared. We obtained the 1,4-dihydropyridines **2m,n** in moderate yields by a four component condensation of the aromatic aldehyde, cyanothioacetamide, and acetylacetone with subsequent reaction with methyl iodide in the presence of an equimolar amount of piperidine. The 3,5-di(*tert*-butyl)-4-hydroxybenzaldehyde gave compound **2n** in which a fragment of ionol [3,5-di(*tert*-butyl)-4-methylphenol] and a 1,4-dihydropyridine ring are combined in a single molecule.

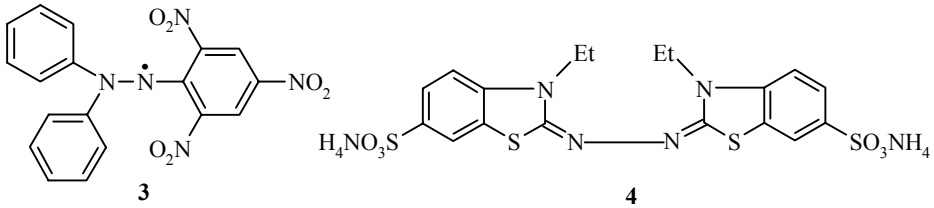


2m,**n**

m $R^1 = H$, $R^2 = OH$; **n** $R^1 = R^2 = CMe_3$

The structure of compound **2** was proved spectroscopically. In the IR spectra the most characteristic absorption bands are those of the stretching of the cyano group at 2198-2204 cm^{-1} and the C=O (which is significantly lowered to 1607-1612 cm^{-1} in the case of **2h-l** due to β-aminovinyl ketone type conjugation) and this is in agreement with published data [5, 6]. The ^1H NMR spectra of compound **2** show characteristic signals for the 4-H proton as a singlet at 4.82-4.86 ppm (dihydropyridines **2h-l**) and at 4.44-4.54 ppm (compounds **2m,n**). The parameters for the compounds synthesized for the first time are given in Tables 1 and 2.

The ARA was measured by the ability of the compound to react with the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (**3**) and with the radical cation 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (**4**).



3 **4**

In recent times, ABTS has been widely used to identify the antioxidant properties of blood plasma, plant extracts, and chemical compounds [10]. The radical cation $ABTS^{+\cdot}$ is formed through the reaction of ABTS with ferryl methemoglobin radical molecules which are generated by the reaction of methemoglobin ($HX–Fe^{3+}$) with H_2O_2.

$$HX - Fe^{3+} + H_2O_2 \rightarrow X^{\cdot} - [Fe^{4+}=O]$$
$$X^{\cdot} - [Fe^{4+}=O] + ABTS \rightarrow ABTS^{+\cdot} + HX - Fe^{3+}$$

TABLE 1. Characteristics for the Compounds Synthesized **2h-n**

Compound	Empirical formula	Found, % / Calculated, %				mp, °C	Yield, %
		C	H	N	S		
2h	$C_{17}H_{17}N_3O_3S$	59.43 / 59.46	4.88 / 4.99	12.20 / 12.24	9.42 / 9.34	164-166	79
2i	$C_{17}H_{17}N_3O_3S$	59.38 / 59.46	4.94 / 4.99	12.20 / 12.24	9.29 / 9.34	148-150	87
2j	$C_{18}H_{19}N_3O_3S$	60.42 / 60.49	5.22 / 5.36	11.80 / 11.76	9.01 / 8.97	129-131	61
2k	$C_{18}H_{17}N_3O_3S$	60.84 / 60.83	4.78 / 4.82	11.83 / 11.82	9.00 / 9.02	143-145	86
2l	$C_{31}H_{45}N_3O_3S$	68.82 / 68.99	8.54 / 8.40	8.01 / 7.78	5.71 / 5.94	76-78	80
2m	$C_{16}H_{16}N_2O_3S$	60.56 / 60.74	5.19 / 5.10	9.05 / 8.85	10.21 / 10.13	223-226	49
2n	$C_{24}H_{32}N_2O_2S$	69.91 / 69.87	8.03 / 7.82	6.76 / 6.79	7.72 / 7.77	193-194	62

TABLE 2. Spectroscopic Characteristics for Compounds **2h-n**

Com-pound	IR spectrum, ν, cm^{-1}	^1H NMR spectrum, δ, ppm*
2h	1608 (C=O); 2200 (C≡N); 3266 (NH)	1.20 (3H, t, $SCH_2\underline{CH_3}$); 2.14 (3H, s, $COCH_3$); 2.40 (3H, s, 6-CH_3); 2.90 (2H, m, $S\underline{CH_2}CH_3$); 4.82 (1H, s, 4-H); 6.44 (1H, br. s, NH); 7.42 and 8.18 (4H, dd and dd, 4-C_6H_4)
2i	1607 (C=O); 2198 (C≡N); 3162 , 3270 (NH)	1.22 (3H, t, $SCH_2\underline{CH_3}$); 2.17 (3H, s, $COCH_3$); 2.32 (3H, s, 6-CH_3); 2.92 (2H, m, $S\underline{CH_2}CH_3$); 4.84 (1H, s, 4-H); 6.62 (1H, br. s, NH); 7.4-8.2 (4H, system, 4-C_6H_4)
2j	1608 (C=O); 2198 (C≡N); 3160, 3278 (NH)	0.93 (3H, t, $SCH_2CH_2\underline{CH_3}$); 1.58 (2H, m, $SCH_2\underline{CH_2}CH_3$); 2.14 (3H, s, $COCH_3$); 2.40 (3H, s, 6-CH_3); 2.87 (2H, m, $S\underline{CH_2}CH_2CH_3$); 4.82 (1H, s, 4-H); 6.28 (1H, br. s, NH); 7.4–8.2 (4H, system, 4-C_6H_4)
2k	1612 (C=O); 2199 (C≡N); 3160 , 3272 (NH)	2.14 (3H, s, $COCH_3$); 2.37 (3H, s, 6-CH_3); 3.62 (2H, m, $S\underline{CH_2}CH=CH_2$); 4.86 (1H, s, 4-H); 4.90 and 5.08 (2H, dd and dd, $SCH_2CH=\underline{CH_2}$); 5.70 (1H, m, $SCH_2\underline{CH}=CH_2$); 7.6-8.2 (4H, system, 4-C_6H_4); 9.93 (1H, s, NH)
2l	1609 (C=O); 2198 (C≡N); 3272 (NH)	0.84 (3H, t, $S(CH_2)_{15}\underline{CH_3}$); 1.1-1.5 (28H, t, $SCH_2(\underline{CH_2})_{14}CH_3$); 2.17 (3H, s, $COCH_3$); 2.42 (3H, s, 6-CH_3); 2.86 (2H, m, $S\underline{CH_2}(CH_2)_{14}CH_3$); 4.82 (1H, s, 4-H); 6.28 (1H, s, NH); 7.4-8.2 (4H, system, 4-C_6H_4)
2m	1614, 1668 (C=O); 2202 (C≡N); 3250, 3314 (NH, OH)	2.02 (3H, s, $COCH_3$); 2.30 (3H, s, 6-CH_3); 2.57 (3H, s, SCH_3); 4.44 (1H, s, 4-H); 6.4-7.1 (3H, system, 4-C_6H_3); 8.7-9.0 (2H, br. s, 2 OH); 9.27 (1H, s, NH)
2n	1620,1652 (C=O); 2204 (C≡N); 3250 (NH), 3624 (OH)	1.38 (18H, s, 2 $C(CH_3)_3$); 2.12 (3H, s, $COCH_3$); 2.35 (3H, s, 6-CH_3); 2.47 (2H, s, S-CH_3); 4.54 (1H, s, 4-H); 6.66 (1H, s, OH); 7.06 (H, s, 4-C_6H_2); 9.36 (1H, s, NH)

* Spectra of compounds **2h-k** recorded in $CDCl_3$; **2l-n** in DMSO-d_6.

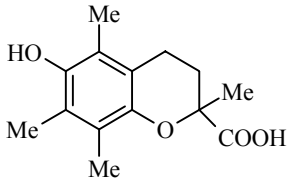
TABLE 3. Antiradical Activity of 5-Acetyl-2-alkylthio-4-aryl-6-methyl-1,4-dihydropyridine-3-carboxylic Acid Nitriles

Compound	K_{II}, l·mol^{-1}·sec^{-1}	TE, mM	Compound	K_{II}, l·mol^{-1}·sec^{-1}	TE, mM
2a	4.29±0.10	4.5	**2i**	0.85±0.10	3.0
2b	1.53±0.02	4.1	**2j**	1.12±0.21	2.5
2c	4.77±0.25	4.4	**2k**	0.39±0.02	2.9
2d	3.80±0.08	4.6	**2l**	0.40±0.03	0.3
2e	2.45±0.04	4.0	**2m**	346.1±17.4	2.8
2f	2.97±0.45	3.6	**2n**	4.26±0.05	4.1
2g	1.01±0.13	3.4	Trolox	562.4 ±33.9	5.0
2h	1.09±0.10	3.2	Ionol	*	3.7

* Does not react under the experimental conditions.

The long wavelength region of the UV spectrum of the ABTS cation radical shows characteristic absorption maxima at 660, 734, and 820 nm. The ARA is calculated from the decrease in intensity of the absorption of the ABTS cation radical at 734 nm in the presence of the test compound. The degree of inhibition is compared with the effect of the Vitamin E analog, trolox, and is expressed in trolox equivalents. One of our goals was the comparison of this ARA as determined by two different methods. The results obtained are given in Table 3.

According to the data in the Table, the most active compound in the reaction with DPPH is compound **2m** which has a 4-(3,4-dihydroxyphenyl) substituent. The ARA of compound **2m** is comparable with the activity of the standard antioxidant trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (**5**).



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The high ARA for the compound containing a dihydroxyphenyl substituent had been noted before in a study of the AOA activity of the symmetrical esters and amides of 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acids [11]. The presence of the ionol antioxidant group (the two *tert*-butyl and the hydroxyl group) in compound **2n** leads to a significant lowering of the value of the reaction rate constant. Compounds **2a-l** also react with the DPPH radical but the reaction rate is also significantly less than with **2m**. Introduction into the 2-alkylthio substituent of a long alkyl chain (**2l**) or the allyl radical (**2k**) significantly decreases the reaction rate constant.

By analysis of the results obtained by the ABTS method it can be seen that all of the investigated compounds how ARA activity which does not exceed that of trolox **5**. The most active in this system are the compounds **2a-e** and **2n**. The dihydropyridine **2l** is less active, as in the reaction with DPPH, and this has a long alkyl chain in the 2-alkylthio substituent. It should be noted that a correlation between the ARA determined by both methods is not observed. This is particularly marked when examining the ARA of compound **2m** which has the highest reaction rate constant with DPPH but shows only modest activity in the ABTS system. One should keep in mind that, in the ABTS test system, the investigated compounds can also act as a radical "trap" (reaction with the ABTS cation radical) and inhibit their formation [12], whereas in the DPPH system only the

bimolecular reaction with the radicals is possible. Addition of the investigated compounds during the course of the reaction in the ABTS system shows that, in fact, compound **2** also inhibits the formation of the ABTS cation radical and this fact is a possible reason for the difference in the results obtained in both systems.

Comparison of the effect of substituents on the ARA shows a relatively small change in ARA with the electronic nature of the substituents R and R'. The rather high ARA of the 1,4-dihydropyridine **2m**, containing the 4-(3,4-dihydroxyphenyl) substituent, is due to the *ortho*-dihydroxyphenyl group in addition to the remainder of the molecule. The absence of the ARA in the ionol analog **2n** is explained by the comparatively low ARA of the ionol molecule, particularly with rather low activity radicals. Hence with $ABTS^{+\cdot}$ (a more active radical) the 1,4-dihydropyridine **2n** is relatively more reactive than with DPPH.

EXPERIMENTAL

IR spectra were taken on a Perkin-Elmer 580B spectrometer using vaseline oil. ^{1}H NMR spectra were recorded on a WH 90/DS (90 MHz) spectrometer (90 MHz) using $CDCl_3$ or DMSO-d_6 solvent and TMS internal standard. Monitoring of the reaction course and the purity of the substances was carried out using TLC in Silfol UV-254 plates with chloroform–hexane–acetone (2:1:1) eluent. The compounds were recrystallized from ethanol. The synthesis of compounds **2a-c** has been reported in [6], **2d** in [13], and **2e-g** in [5].

General Method for the Synthesis of 5-Acetyl-2-alkylthio-4-aryl-6-methyl-1,4-dihydropyridine-3-carboxylic Acid Nitriles (2). A. A mixture of 1,4-dihydropyridine-2(3H)-thione [14] (10 mmol), piperidine (11 mmol), and the alkyl halide (30 mmol of methyl iodide, 30 mmol of ethyl bromide, 20 mmol of *n*-propyl bromide, 15 mmol of allyl bromide, or 12 mmol of hexadecyl bromide) in ethanol (30-50 ml) was refluxed for 10-15 min on a water bath and then stirred for 1 h at room temperature. The precipitate formed was filtered off and washed with ethanol cooled to 0°C (10-15 ml) and water (20 ml) was added to give the compounds **2h-l**.

B. The aromatic aldehyde (10 mmol) and cyanothioacetamide (10 mmol) in ethanol (30-50 ml) with piperidine (1 mmol) was heated until solution occurred. With stirring there were added acetylacetone (10 mmol) and piperidine (10 mmol) followed by methyl iodide (30 mmol) after 10 min. After a further 1 h, water (5 ml) was added (in the case of **2m** an additional 1 ml of conc. HCl). The precipitate formed was filtered off and washed with 50% ethanol (20 ml) and water (20 ml) to give compounds **2m,n**.

ARA Determination. Test System with DPPH. A mixture of an ethanol solution of DPPH (2.5 ml, 10^{-4} M) and an ethanol solution of the 1,4-DHP (50μl, 5×10^{-3} M) was incubated at 30°C (final cuvet concentration 10^{-4} M). The rate of reduction of the DPPH was calculated from the decrease in the absorption at 517 nm (Hitachi 557 UV-vis spectrophotometer). The ARA was expressed as a second order reaction rate constant and calculated using the equation [15]:

$$K_{II} = [DPPH] / [DPPH]_0 [DPPH]_t t \quad (l\cdot mol^{-1}\cdot sec^{-1})$$

Test System with ABTS. A solution of the diammonium salt of ABTS (300 μl, 5×10^{-4} M), methemoglobin (36 μl, 7×10^{-5} M), an ethanol solution of the investigated compound (blank = ethanol) (16 μl, 5×10^{-3} M), and physiological phosphate buffer (pH 7.4, 489 μl, 5×10^{-3} M) were placed in a cuvet. The ABTS and methemoglobin solutions were prepared in physiological phosphate buffer. A solution of H_2O_2 (167 μl, 0.01 M) was added with stirring and incubated at 37°C. The change in absorption at 734 nm was measured periodically on the Hitachi 557 UV-vis spectrophotometer. An ethanol solution (5×10^{-3} M) of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard. The ARA was calculated as trolox equivalents (TE) using the equation [16]:

$$TE = c_{trolox} \cdot (A_{control} - A_{sample}) / (A_{control} - A_{trolox})$$

where A is the absorption at 734 nm after 2 min.

The mean values for three experiments are given in Table 3. The relative error does not exceed ±10% of the basic value.

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