607. The test results are shown in Table 2, from which it will be seen that (VII) and (IV) show high tuberculostatic activity in vitro, while (VIII-XI) are inactive.

Also examined were the tolerance of white mice for (VII), and its therapeutic activity in experimental tuberculosis in white mice. The maximum tolerated dose following a single intragastric daily dose for five days was found to be greater than 2000 mg/kg. Treatment of mice with experimental tuberculosis for 39 days with a range of doses of the compound (from the MTD downwards) failed to influence the course of the disease.

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SYNTHESIS AND HEPATOPROTECTANT ACTIVITY OF 5-CARBAMOYL- AND 5-ACETYL-2-ALKYLTHIO-6-METHYL-4-ARYL-3-CYANO-1,4-DIHYDROPYRIDINES

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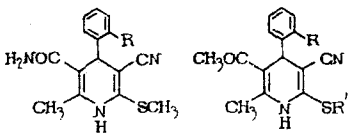
Continuing our work on the synthesis and biological activity of 2-alkylthio-1,4-dihydropyridines [1, 2], we have now obtained some 5-carbamoyl- and 5-acetyl-2-alkylthio-6-methyl-4-aryl-3-cyano-1,4-dihydropyridines (Ia-c) and (IIa-g), and examined their hepatoprotectant activity.



Ia-c IIa-g

Although hepatoprotectant activity in 1,4-dihydropyridines is well known and has been widely reported [3-5, 8-10, 13], we have found hepatoprotectant and antioxidant activity in related compounds, namely 1,4-dihydropyridine-2(3H)-thiones [6].

Alkylated derivatives of the latter (2-methylthio-6-methyl-4-aryl-5-carbamoyl-3-cyano-1,4-dihydropyridines (I)) have now been obtained for the first time. The 4-phenyl compound (Ia) was obtained in 81% yield by condensation of acetoacetamide, benzaldehyde, cyanothioacetamide and piperidine, followed by treatment with methyl iodide. The 4-(o-chlorophenyl)- and 4-(o-difluoromethoxyphenyl)-1,4-dihydropyridines (Ib) and (Ic) were prepared in 73 and 74% yields respectively by condensation of acetoacetamide with the appropriate 3-[o-chloro-(or o-difluoromethoxy)phenyl]-2-cyanoacrylthioamides in the presence of piperidine, followed by treatment with methyl iodide.

The 2-alkylthio-6-methyl-4-phenyl-5-acetyl-3-cyano-1,4-dihydropyridines (IIa-d) were obtained in 83-93% yields by alkylating piperidinium 6-methyl-4-phenyl-5-acetyl-3-cyano-1,4-dihydropyridine-2-thiolate [1]. The 2-alkylthio-6-methyl-4-o-chlorophenyl-5-acetyl-3-cyano-1,4-dihydropyridines (IIe-g) were obtained in 77-88% yields by condensing acetylacetone with 3-(o-chlorophenyl)-2-cyanothioacrylamide in the presence of piperidine, followed by treatment with the alkyl halide. Isolation of the 2-methylthio- compounds (Ia-c) and (IIe) was facilitated by acidifying the reaction mixtures.

The structures of (I) and (II) were confirmed spectroscopically. In the IR spectra of crystalline samples of the dihydropyridines (I) and (II), the most characteristic absorption

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TABLE 1. Properties of 1,4-Dihydropyridines (I) and (II)

Compound	Yield, %	Mp, °C	UV spectrum, λ, nm	IR spectrum, ν, cm⁻¹			Empirical formula
				CO	CN	NH, NH_2	
Ia	81	212—215	226, 273, 348	1646, 1657	2190	3218, 3346, 3452	$C_{15}H_{15}N_3OS$
Ib	73	226—228	227, 272, 352	1652, 1682	2184	3142, 3358, 3416	$C_{15}H_{14}N_3ClOS$
Ic	74	208—209	225, 273, 350	1637, 1670	2206	3080, 3195, 3252	$C_{16}H_{15}F_1N_3O_2S$
IIa	85	182—184	236 sh., 262, 284 sh., 370	1660	2204	3244	$C_{16}H_{16}N_2OS$
IIb	93	138—140	244, 262 sh., 288, 376	1660	2204	3250	$C_{17}H_{18}N_2OS$
IIc	85	162—164	247, 289, 378	1614, 1638 sh.,	2202	3160, 3244	$C_{18}H_{20}N_2OS$
IId	83	112—114	237, 268, 330, 374	1634 sh., 1732	2205	3164, 3218	$C_{19}H_{20}N_2O_3S$
IIe	88	185—187	244, 262, 288, 375	1682	2192	3316	$C_{16}H_{15}N_2ClOS$
IIf	77	124—125	244, 266, 376	1633, 1702	2204	3182, 3256	$C_{19}H_{19}N_2ClO_3S$
IIg	86	210—212	268, 378	1653, 1676 sh., 1693	2200	3190, 3338, 3438	$C_{17}H_{16}N_3ClO_2S$

TABLE 2. PMR Spectral Data for 1,4-Dihydropyridines (I) and (II)

Compound	Chemical shifts, δ, ppm (multiplicity) in DMSO-d_6						
	NH (br. s)	C_6H_4R (m)	$CONH_2$ (s)	4-H (s)	SCH_3 (or SR') (s or m)	6-CH_3 (s)	$COCH_3$ (s)
Ia	8,85	7,4—7,0	6,92	4,58	2,50	2,11	—
Ib	8,88	7,5—7,2	6,98	5,12	2,47	2,04	—
Ic[a]	8,88	7,4—7,1	7,04	4,94	2,48	2,07	—
IIa[b]	6,02	7,4—7,1	—	4,62	2,44	2,37	2,06
IIb[b]	6,32	7,3—7,1	—	4,63	2,90; 2,82 and 1,18	2,39	2,06
IIc[b]	6,24	7,4—7,2	—	4,65	3,47 and 1,21; 1,18	2,38	2,06
IId	8,57	7,4—7,1	—	4,64	3,54[c] and 3,50[c]; 4,26 and 1,30	2,42	2,05
IIe[b]	6,20	7,4—7,1	—	5,28	2,44	2,36	2,04
IIf	8,60	7,4—7,1	—	5,28	3,53, 4,27 and 1,30	2,38	2,02
IIg	10,47	7,5—7,2	7,92 and 7,62	5,18	3,74[d] and 3,60[d]	2,34	2,02

[a] $OCHF_2$ signals at 7.08, J = 74 Hz.
[b] In $CDCl_3$.
[c] $^1J_{CH_2}$ = 13.0 Hz.
[d] $^2J_{CH_2}$ = 15.0 Hz.

was that for ν_{CN} at 2184-2206 cm^{-1} and ν_{CO} at 1633-1682 cm^{-1}. The PMR spectra of (I) and (II) showed singlet signals for the 4-H proton at 5.28-4.58 ppm, and in the case of (IId, f, g) the signals for the CH_2 protons of the SCH_2 group are noteworthy as a result of the presence of an asymmetric center at C(4).

The UV spectra of the 5-carbamoyldihydropyridines (I) show long wavelength absorption at 348-352 nm, whereas in the 5-acetyl compounds (II) these are shifted bathochromically to 374-378 nm, as a result of the different electron-acceptor properties of the carbamoyl and acetyl groups.

EXPERIMENTAL (CHEMISTRY)

IR spectra were obtained on a Perkin—Elmer 580B (UK) in Nujol mulls, and UV spectra on a Specord UV-Vis in ethanol. PMR spectra were recorded on a WH 90/DC instrument, operating frequency 90 MHz, internal standard TMS. The main physicochemical and pharmacological properties of the products are shown in Tables 1-5. The elemental analyses were in agreement with the calculated values.

2-Methylthio-6-methyl-4-phenyl-5-carbamoyl-3-cyano-1,4-dihydropyridine (Ia). A mixture of 1.0 g (10 mmole) of cyanothioacetamide, 1.0 ml (10 mmole) of benzaldehyde, and 1.0 g (10 mmole) of acetoacetamide in 10 ml of absolute ethanol and 1.0 ml (10 mmole) of piperidine was stirred for 30 min at room temperature, then 1.9 ml (30 mmole) of methyl iodide was added, and the mixture boiled for 10 min on the water bath. Stirring was continued for 3 h at room temperature, 10 ml of 1 N HCl added, cooled to 0°C, kept for 20 h, and the solid filtered off and washed with 20 ml of 50% ethanol and 20 ml of water to give 2.3 g (81%) of (Ia).

2-Methylthio-6-methyl-4-(o-chlorophenyl)-5-carbamoyl-3-cyano-1,4-dihydropyridine (Ib). A mixture of 1.0 g (10 mmole) of acetoacetamide and 2.23 g (10 mmole) of 3-(o-chlorophenyl)-2-cyanothioacrylamide in 10 ml of absolute ethanol and 1 ml (10 mmole) of piperidine was stirred for 1 h at room temperature, then 1.9 ml (30 mmole) of methyl iodide was added and the mixture treated as for (Ia) to give 2.33 g (73%) of (Ib).
Compound Ic was obtained analogously.

TABLE 3. Effects of Test Compounds in a Range of Doses on AlAT Activity in Blood Serum 24 h after Treatment with CCl_4

Test conditions	AlAT, μmole/g per ml					
	100 mg/kg		50 mg/kg		200 mg/kg	
	M±m	%	M±m	%	M±m	%
Control	1,56±0,11 (30)	—	—	—	—	—
CCl_4	4,54±0,10* (29)	—	—	—	—	—
CCl_4 + cysteamine	3,10±0,81** (13)	48,3	3,95±0,65* (5)	19,8	2,53±0,61** (5)	67,4
CCl_4 + vitamin E	3,90±0,46** (10)	21,5	4,18±0,39* (7)	12,0	3,66±0,54** (8)	29,5
CCl_4 + SKF-525A	3,40±0,25** (8)	38,2	3,78±0,53** (5)	25,5	2,86±0,84** (5)	56,4
CCl_4 + Ia	4,47±0,67* (5)	2,3	—	—	—	—
CCl_4 + Ib	3,97±0,26** (5)	19,1	4,46±0,85* (6)	2,7	3,56±0,53** (5)	32,9
CCl_4 + Ic	2,59±0,71** (5)	65,4	3,58±0,61** (5)	32,2	3,20±0,25** (6)	45,0
CCl_4 + IIa	4,57±0,47* (5)	—1,0	—	—	—	—
CCl_4 + IIb	4,41±0,52* (5)	4,4	—	—	—	—
CCl_4 + IIc	4,81±0,32* (5)	—9,1	—	—	—	—
CCl_4 + IId	3,66±0,71** (8)	29,5	4,24±0,69* (5)	10,1	3,37±5,1** (5)	39,3
CCl_4 + IIe	3,67±0,55** (7)	29,2	4,38±0,41* (7)	5,4	3,42±0,44** (7)	37,6
CCl_4 + IIf	4,70±0,56** (6)	—5,3	—	—	—	—
CCl_4 + IIg	4,00±0,24** (8)	18,1	4,42±0,33* (5)	4,0	3,85±0,41** (5)	23,2

Notes. Here and in Tables 4-5, one asterisk denotes significant difference ($p \leq 0.05$) from the control, two asterisks that there is a statistically significant difference ($p \leq 0.05$) from the group of animals receiving CCl_4 only. The numbers of animals in the groups are shown in brackets.

TABLE 4. Effects of the Test Compounds in a Range of Doses on AlAT Activity in Rat Blood Serum 24 h Following Administration of D-Galactosamine (D-gal)

Test conditions	AlAT, μmole/g per ml					
	100 mg/kg		50 mg/kg		200 mg/kg	
	M±m	%	M±m	%	M±m	%
Control	1,39±0,21 (5)	—	—	—	—	—
D-gal	3,59±0,33** (5)	—	—	—	—	—
D-gal + cysteamine	3,43±0,52* (6)	7,2	3,58±0,64* (5)	0,4	3,40±0,37* (5)	8,6
D-gal + vitamin E	3,02±0,19** (5)	25,9	3,34±0,51** (6)	11,4	2,91±0,25** (6)	30,9
D-gal + SKF-525A	3,57±0,61* (5)	0,9	3,55±0,31* (5)	1,8	3,66±0,49* (5)	—3,2
D-gal + Ib	3,35±0,41* (5)	10,9	3,71±0,32* (5)	—5,4	3,16±0,28* (5)	19,5
D-gal + Ic	3,19±0,26* (6)	18,2	3,34±0,21* (5)	11,4	3,02±0,22** (5)	25,9
D-gal + IId	3,37±0,18* (6)	10,0	3,55±0,29* (5)	1,8	3,66±0,51* (5)	—3,2
D-gal + IIe	3,51±0,23* (6)	3,6	3,56±0,44* (5)	1,3	3,65±0,15* (5)	—2,7
D-gal + IIg	3,42±0,24* (6)	7,7	3,66±0,18* (5)	—3,2	3,52±0,31* (5)	3,2

General Method of Preparation of 2-Alkylthio-6-methyl-4-phenyl-5-acetyl-3-cyano-1,4-dihydropyridines (IIa-d). A mixture of 10 mmole of piperidinium 6-methyl-4-phenyl-5-acetyl-3-cyano-1,4-dihydropyridine-2-thiolate (I) and 12-30 mmole of the alkyl halide in 20 ml of absolute ethanol was heated briefly on the water bath, filtered, cooled to 0°C, and the solid filtered off and washed with ethanol and water to give 83-93% of product.

General Method of Preparation of 2-Alkylthio-6-methyl-4-(o-chlorophenyl)-5-acetyl-3-cyano-1,4-dihydropyridines (IIe-g). A mixture of 1.02 ml (10 mmole) of acetylacetone and 2.23 g (10 mmole) of 3-(o-chlorophenyl)-2-cyanothioacrylamide in 10 ml of absolute ethanol and 1 ml (10 mmole) of piperidine was stirred for 1 h at room temperature. The alkyl halide (12-20 mmole) was then added, and the mixture heated briefly on the water bath, filtered, and cooled to 0°C. In the case of (IIe), the mixture was treated with 10 ml of 1 N HCl in ethanol. After 2-3 h, the solid was filtered off, and washed with ethanol and water to give 77-8% of product.

EXPERIMENTAL (BIOLOGY)

Tests were carried out on mongrel male white rats weighing 180-210 g and male mice weighing 20-22 g. Eighteen hours before the test was initiated, the animals were deprived of food. One group of rats received an intraperitoneal injection of CCl_4 in a dose of 0.5 ml/kg as a 10% solution in olive oil, and a second group, D-galactosamine (Fluka) in a dose of 400 mg/kg as an 8% solution in 0.9% sodium chloride solution. Mice were treated intraperitoneally with CCl_4 in a dose of 0.05 ml/kg as a 10% solution in olive oil. The test compounds were given orally in doses of 50, 100, and 200 mg/kg as aqueous suspensions in 0.1% Tween-80 24 and 1 h before treatment with CCl_4. The control rats received an intraperitoneal

TABLE 5. Effects of Test Compounds in a Range of Doses in Prolonging Hexenal Narcosis in Mice 24 h after Administration of CCl_4

Test conditions	Hexenal narcosis, min					
	100 mg/kg		50 mg/kg		200 mg/kg	
	$M \pm m$	%	$M \pm m$	%	$M \pm m$	%
Control	65,0±10,7 (10)	—	—		—	
CCl_4	135,5±23,8* (10)	—	—		—	
CCl_4+ cysteamine	63,0±27,1** (10)	102,8	78,2±28,1** (10)	81,3	65,3±28,2** (8)	99,6
CCl_4+ vitaminE	78,9±21,2** (10)	80,3	103,1±33,1* (8)	45,9	74,1±35,1** (8)	87,1
CCl_4+SKF-525A	86,7±32,1** (10)	69,2	97,8±15,6** (8)	53,5	72,5±38,2** (10)	90,0
CCl_4+Ib	110,0±45,6* (6)	36,2	114,0±36,4* (9)	30,5	100,2±31,6* (10)	50,1
CCl_4+Ic	91,7±20,1** (12)	62,1	100,5±44,3* (7)	49,6	73,4±36,1** (8)	88,1
CCl_4+IId	129,2±37,6* (7)	8,9	126,6±25,2* (8)	12,6	111,8±28,8* (8)	33,6
CCl_4+IIe	97,6±34,1* (9)	53,8	127,1±51,2* (7)	11,9	91,3±19,1** (7)	62,7
CCl_4+IIg	135,1±44,1* (7)	0,5	129,5±23,1* (8)	8,5	142,2±40,3* (6)	—9,5

dose of 4.5 ml/kg of olive oil and 5 ml/kg of 0.9% sodium chloride solution respectively, and the mice received 0.45 ml/kg of olive oil. All the control animals also received the appropriate amounts of Tween-80 solution.

Twenty-four hours following administration of CCl_4 or D-galactosamine, the rats were decapitated under light ether narcosis. Alanine aminotransferase (AlAT) activity was measured in the blood serum as described in [14]. Twenty-four hours after receiving CCl_4, the mice were given an intravenous dose of 70 mg/kg of Hexenal as an 0.4% aqueous solution, and the duration of Hexenal narcosis at 22-23°C measured.

The extent of improvement in these parameters resulting from treatment with the test compounds, in the presence of liver damage by CCl_4 or D-galactosamine, was calculated using the formula:

$$D = \frac{B-C}{B-A} \cdot 100\ \%,$$

where A is the value of the parameter in the control group of untreated animals, B the value in the control group receiving CCl_4 or D-galactosamine, C the value in the group receiving the test compound before treatment with CCl_4 or D-galactosamine, and D the percentage improvement in the parameter.

Significant differences between the experimental series were evaluated using the criteria of Wilcoxon, Mann, and Whiting. The control materials used were cystamine hydrochloride (Ferak), vitamin E (DL-α-tocopherol) (Serva), and SKF-525A (β-diethylaminoethyl 2,2-diphenylvalerate hydrochloride) (Smith, Kline and French, USA).

Table 3 shows the results of the primary selection of active compounds (in a dose of 100 mg/kg), and for other active compounds (in doses of 50 and 200 mg/kg). The ability of these compounds to reduce the increase in AlAT activity in rat serum 24 h after administration of CCl_4 was assessed. Under the test conditions, the activity of this enzyme, which normally resides mainly in the liver, rises by a factor of 2.9. The liberation of significant amounts of the enzyme into the blood indicates severe damage to the hepatocyte membranes. It is noteworthy that the optimum doses of CCl_4 and the other hepatotropic toxin used in the tests (D-galactosamine) were specially chosen when these models were designed [8]. The criterion for selection was a statistically significant reduction in blood serum AlAT. For the sake of convenience, the hepatoprotectant activity of the test compounds is expressed as a percentage.

The statistically significant effects of the control compounds cysteamine, vitamin E, and SKF-525A in a dose of 100 mg/kg show that this experimental model is satisfactory. Hepatoprotectant activity in the test compounds was shown by (Ic), (IId), (IIe), (Ib), and (IIg) (in order of decreasing activity). Most of the compounds showed greatest activity when given in a dose of 200 mg/kg, and at 50 mg/kg the only compound to show significant activity was (Ic). The activity of the latter compound (in a dose of 50 mg/kg) was substantially greater than that of the reference compounds.

Hepatocyte membrane damage was induced by both CCl_4 and D-galactosamine, but the mechanism of intoxication by D-galactosamine is different, since while the toxic effects of CCl_4 are due to development of free radical reactions in the membranes, D-galactosamine interferes with carbohydrate metabolism [7, 12]. Nevertheless, the ultimate result in both cases

is membrane damage, with the consequent liberation of enzymes localized in the liver, so that the AlAT blood levels 24 h after administration of D-galactosamine in a dose of 400 mg/kg are raised by a factor of 2.6 (Table 4).

In the liver damage model induced by D-galactosamine, significant hepatoprotectant activity is shown by vitamin E of the reference compounds, and by (Ic) of the test compounds.

Metabolic activity is one of the principal functions of the liver. Measurement of this activity was carried out in mice by assessment of the duration of Hexenal narcosis. It will be seen from Table 5 that the duration of Hexenal sleep is increased by a factor of 2.1 after 24 h following administration of CCl_4 in a dose of 0.05 ml/kg. The fact that the toxic effects of CCl_4 in this test are apparent at a much lower dose (by a factor of 10) than that used earlier indicates that the change in AlAT activity is due to the specific effect of CCl_4 on the enzyme responsible for the metabolism of Hexenal, namely cytochrome P-450 [7, 11]. Statistically significant hepatoprotectant activity in the Hexenal narcosis model (with liver damage by CCl_4) is shown by all the reference compounds (cysteamine, vitamin E, and SKF-525A) and two of the test compounds (Ic and IIe), although the activity of (Ic) is seen at doses of 100 and 200 mg/kg, while that of (IIe) is only apparent at 200 mg/kg. Generally speaking, however, the hepatoprotectant activity of (Ic) was of the same order as that of vitamin E, but was inferior to that of cysteamine and SKF-525A.

To summarize, compound (Ic) shows the greatest hepatoprotectant activity of the test compounds. By adding the percentage improvements in the factors in the different models and at differing concentrations, it will be seen that the activity of (Ic) is somewhat greater than that of vitamin E and SKF-525A, but somewhat lower than that of cysteamine. Hepatoprotectant activity among the other test compounds was inferior to that of the reference compounds, in the following order of decreasing activity: (IIe), (Ib), (IId), (IIg). Compounds (Ia), (IIa), (IIb), (IIc), and (IIf) showed no hepatoprotectant activity.

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