SYNTHESIS, PROPERTIES, AND CARDIOTONIC ACTIVITY OF SOME 2-CARBAMOYLMETHYLTHIO-6-PHENYL-5-ETHOXYCARBONYL-3-CYANO-4-[PYRID-3-YL]PYRIDINES AND THEIR HYDROGENATED ANALOGS

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3,4'-Bipyridines are attracting increasing interest as cardiotonic drugs for the treatment of cardiac insufficiency. These include amrinone and milrinone [6-9, 13, 14, 16-19], which have a considerable positive inotropic effect on the heart, while simultaneously showing vasculodilatory activity. On the other hand, some partially hydrogenated 4,3'-bipyridines show coronary dilating activity [4].

In continuation of work on the synthesis, properties, and biological activity of pyridine-2(1H)-thiones [5] and 1,4-dihydropyridine-2(3H)-thiones [3, 4], we have now obtained some 4-(pyrid-3'-yl)-2-carbamoylmethylthio-6-phenyl-5-ethoxycarbonyl-3-cyanopyridines (4,3'-bipyridines) and their hydrogenated analogs, and examined them for cardiotonic activity.

Condensation of ethyl benzoylacetate with 2-cyano-3-(pyrid-3'-yl)-acrylothioamide in the presence of base has given 70% of 6-hydroxy-6-phenyl-5-ethoxycarbonyl-3-cyano-4-(pyrid-3'-yl)-1,4,5,6-tetrahydropyridine-2-thiolates (I). These compounds crystallize with one molecule of water. The sodium salt (Ib), unlike the piperidinium salt (Ia), is soluble in water, but it is extremely hygroscopic and is unstable to dehydration. Brief heating of salt (Ia) in acetic acid–ethanol solution gives 80% of the betaine (II).

Alkylation of (Ia) with betaine II iodoacetamide affords high yields of 6-hydroxy-2-carbamoylmethylthio-1,4,5,6-tetrahydropyridine (III) and 2-carbamoylmethylthio-1,4-dihydropyridine (IV) respectively. It is noteworthy that the latter route is preferable for the preparation of the 1,4-dihydropyridine (IV), since the alternative method involving dehydration of the 6-hydroxy-tetrahydropyridine (III) in acid media under the conditions usual for this reaction [2] gives a complex mixture of products.

Oxidation of the 1,4-dihydropyridine (IV) with an excess of sodium nitrite in acetic acid affords 45% of the bipyridine (VI).

Thorpe cyclization of the 2-carbamoylmethylthio-3-cyano-1,4-dihydropyridine (IV) and the corresponding bipyridine (VI) with KOH gave respectively 81% and 77% of the 4,7-dihydrothieno-[2,3-b]pyridine (V) and the thieno[2,3-b]pyridine (VII).

The structures of (I-VII) were proved spectroscopically. The IR spectra of the salts of (I) and the betaine (II) showed characteristic absorption for cyano-group stretching vibrations at 2160-2178 cm^{-1} (Table 1), indicating a very high level of conjugation between the cyano-group and the anion, and that (II) has the betaine structure [3]. In the case of the tetra and dihydropyridines (III) and (IV), ν_{CN} increased to 2192-2204 cm^{-1}, while in the bipyridine (VI) this absorption was seen at 2222 cm^{-1}.

Absorption for the ester group when this group was unconjugated (I and III) was seen at ν_{CO} 1700-1738 cm^{-1}, while in the dihydro-compounds (II, IV, and V) ν_{CO} decreased to 1628-1672 cm^{-1}, which is typical of β-aminovinylcarbonyl systems.

In the IR spectra of 4,7-dihydrothieno[2,3-b]pyridine (V) and thieno-[2,3]-bipyridine (VII), absorption for the conjugated cyano-group which is present in (IV) and (VI), was absent while several new bands were present at 3122-3484 cm^{-1} due to stretching vibrations of the 3-amino-group (Table 1).

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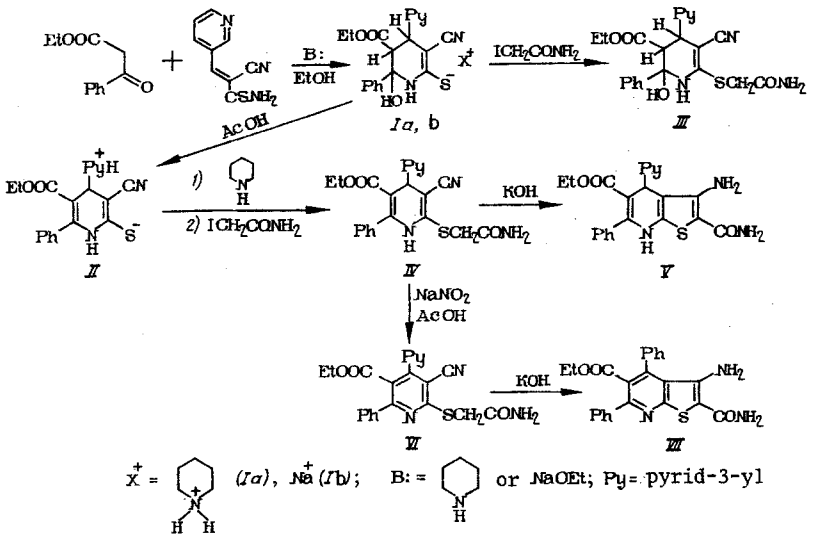
TABLE 1. Properties of Synthesized Compounds (I-VII)

Compound	Yield, %	Mp, °C	Empirical formula	IR spectra, ν, cm^{-1}			UV spectra, λ_{max}, nm
				C=O	C≡N	H, NH_2, OH	
Ia[a]	72	101—105[b]	$C_{25}H_{30}N_4O_3S \cdot H_2O$	1700, 1728	2160	3130, 3252 n. 3520, 3600	237, 297
Ib[b]	72	170[b]	$C_{20}H_{18}N_3NaO_3S \cdot H_2O$	1718	2176	3228, 3420	237, 297
II	80	157—160	$C_{20}H_{17}N_3O_2S$	1672	2178	3158	256, 320, 360n.
III	82	170—172	$C_{22}H_{22}N_4O_4S$	1670, 1738	2192	3166, 3340	286
IV	79	183—185	$C_{22}H_{20}N_4O_3S$	1628, 1673	2204	3142, 3374	258, 360
V	81	206—208	$C_{22}H_{20}N_4O_3S$	1637, 1667	—	3122, 3298, 3400, 3424, 3480	253, 290, 382
VI	67	167—169	$C_{22}H_{18}N_4O_3S$	1698, 1727	2222	3110, 3270, 3460	276, 337
VII	77	205—207	$C_{22}H_{18}N_4O_3S$	1670, 1722	—	3166, 3284, 3320, 3340, 3380, 3484	256, 302, 390

[a] $^{+}H_2$ = 2425, 2520 cm^{-1}.
[b] With decomposition.



The UV spectra of the tetrahydropyridines (I) and (III) show absorption at 286-298 nm, and of the dihydropyridines (II) and (IV) and the dihydrothieno[2,3-b]pyridine (V) at 360-382 nm, i.e., as conjugation increases, the long wavelength maximum undergoes a bathochromic shift.

The PMR spectra of (I) and (III) (Table 2) show doublets characteristic of the 4-H and 5-H protons at 4.12-4.06 and 2.98-2.77 ppm, $^3JH_4H_5$ = 12 Hz, indicating that the 4-H and 5-H protons are trans-diaxially oriented, so that the 4-(pyrid-3'-yl) and 5-COOEt groups must be trans-equatorial.

The most characteristic signals in the PMR spectra of (II) and (IV) are those for the 4-H proton at 4.62-4.58 ppm. In the case of the dihydropyridine (IV), the chemical shifts for the SCH_2 group are seen as an AB-quartet, showing that the CH_2 protons are nonequivalent owing to the presence of an asymmetric center in the molecule at C(4). In the PMR spectrum of the 4,7-dihydrothieno[2,3-b]pyridine (V), no signals for the SCH_2 group are seen, and the signal for the 4-H proton is shifted to lower field by 0.52 ppm as a result of the increased molecular conjugation.

Pharmacological examination of (III-VII) was carried out using amrinone as the reference standard. Unlike the latter, the 4,3'-bipyridines showed a twofold effect on the contractile strength of the papillary muscles and the auricles of guinea pigs. For example, in the concentration range 10^{-8}-10^{-5} M, the test compounds exerted a negative inotropic effect, since the contractile force was considerably reduced, in some instances to the extent of 50% (compound (II), Figs. 1 and 2). At concentrations of 10^{-5}-$5 \cdot 10^{-4}$ M, with the exception of (II) the compounds had the opposite activity; i.e., the contractile force of both the auricles and the papillary muscles began to rise, with (V) and (VI) giving rise to 25-120 and 7-30% increases over the initial levels. It is noteworthy that in these tests the auricles were more sensitive than the papillary muscles (Figs. 1, 2). The frequency of stimulation is also important.

TABLE 2. PMR Spectra of Compounds (I-VII) in DMSO-d_6

Compound	Chemical shifts (δ), multiplicity							
	NH (s)	NH_2 (s)	OH (s)	aromatic protons (m)	4-H (s or d)	5—H (d)	$S-CH_2$ (s or d)	OC_2H_5 (q and t)
Ia[a]	5,66	—	5,45	8,4—7,2	4,06[b]	2,78	—	3,38 and 0,44
Ib	5,90	—	5,40	8,4—7,2	4,07[b]	2,77	—	3,30 and 0,44
II	8,04	—	—	8,7—7,3	4,58	—	—	3,66 and 0,68
III	9,23	Under aromatic protons	6,98	8,6—7,2	4,12[b]	2,98	3,60	3,41 and 0,45
IV	10,78	7,86 and 7,54	—	8,5—7,3	4,62	—	3,85 3,63[c]	3,67 and 0,70
V	9,98	6,50 and 6,42	—	8,1—7,2	5,14	—	—	3,62 and 0,66
VI	—	7,60 and 7,22	—	8,7—7,4	—	—	4,00	3,78 and 0,70
VII	—	7,28 and 5,58	—	8,7—7,4	—	—	—	3,72 and 0,68

[a]Signals for $C_5H_{12}\overset{+}{N}$ protons were present at 3.00 and 1.62 ppm.
[b]$^3J_{H_4H_5}$ = 12.0 Hz.

According to literature reports, the inotropic activity of cardiotropic drugs increases as the frequency of stimulation of the test subject increases [12]. With respect to structural transformations in these 4,3'-bipyridines, progressive oxidation of the pyridine ring (III—IV—VI) slightly enhances activity, although no such effect is seen in the differently hydrogenated thienopyridines (V) and (VII). It has also been reported [1] that on oxidation of 1,4-dihydropyridines (calcium antagonists), which strongly inhibit the contractile activity of cardiomyocytes, negative inotropic activity is lost.

The principal mode of action of most nonglycosidic cardiotropic drugs is their ability to selectively inhibit cardiomyocyte phosphodiesterase, resulting in a rise in intracellular cAMP concentrations and an increase in extra- and intracellular Ca^{2+} transport [10, 11, 15].

In our tests, amrinone in concentrations of $5\cdot10^{-4}$-10^{-3} M increased the T''_{min}/T''_{max} ratio (the tension weakening factor) on average by 11-16.7% ($p < 0.05$), an effect which was not observed with the other compounds. It is however possible that at higher concentrations this ratio would change in one direction or the other, but the limited solubility made this difficult. In the opinion of Skomedal et al., [19], a decrease in the tension weakening factor indicates that the mode of action of amrinone involves cAMP. Our results are in accordance with this view.

EXPERIMENTAL (CHEMICAL)

IR spectra were obtained on a Perkin-Elmer 580B (UK) in vaseline grease (Nujol), and UV spectra on a Specord UV-VIS (Germany) in ethanol. PMR spectra were obtained on a WH 90/DC instrument (Germany), internal standard TMS. The elemental analyses were in agreement with the calculated values. The principal physicochemical and pharmacological properties of the compounds are shown in Tables 1 and 2.

Piperidinium 6-Hydroxy-6-phenyl-5-ethoxycarbonyl-3-cyano-4-(pyrid-3'-yl)-1,4,5,6-tetrahydropyridine-2-thiolate (Ia). A mixture of 1.89g (10 mmole) of 2-cyano-3-(pyrid-3'-yl)acrylothioamide and 1.92 g (10 mmole) of ethyl benzoylacetate was dissolved in 20 ml of ethanol and 1.2 ml (12 mmole) of piperidine with stirring and heating to 50°C. The mixture was stirred for 1 h at ambient temperature, cooled to 0°C, and the finely crystalline, colorless product filtered off and washed with 10 ml of ethanol to give 3.5 g (72%) of (Ia).

Sodium 6-Hydroxy-6-phenyl-5-ethoxycarbonyl-3-cyano-4-(pyrid-3'-yl)-1,4,5,6-tetrahydropyridine-2-thiolate (Ib). A mixture of 1.89 g (10 mmole) of 2-cyano-3-(pyrid-3'-yl)acrylothioamide and 1.92 g (10 mmole) of ethyl benzoylacetate was dissolved in 10 ml of absolute ethanol and 10 ml of 1.0 N NaOEt solution and stirred for 30 min at ambient temperature, then cooled to 0°C. After 3 h, 10 ml of ether was added, then 1 h later the precipitated solid was filtered off and washed with 10 ml of cold ethanol to give 3.04 g (72%) of (Ib).

6-Phenyl-5-ethoxycarbonyl-3-cyano-4-(pyrid-3'-yl)-1,4-dihydropyridine-2-thione Betaine (II). Salt (Ia) (4.85 g, 10 mmole) was dissolved in 20 ml of ethanol and 20 ml of glacial acetic acid with heating on the water bath, then filtered and cooled to 0°C. The solid which separated was filtered off after 1 h, and washed with 5 ml of ethanol and 10 ml of water to give 2.91 g (80%) of (II).

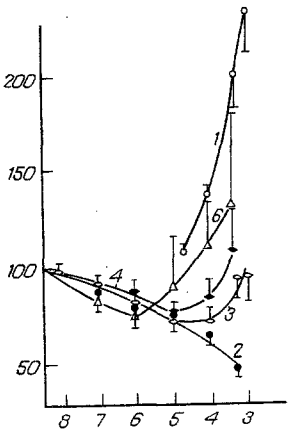


Fig. 1

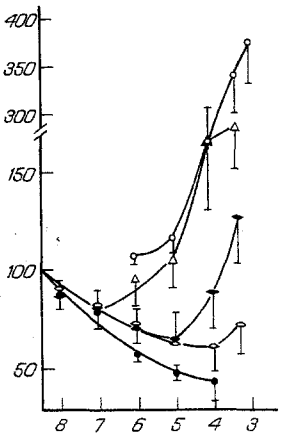


Fig. 2

Fig. 1. Plots of contractile force of guinea pig papillary muscles against concentration of the test compounds. 1) Amrinone, 2) (II), 3) (IV), 4) (V), 5) (VI). Here and in Fig. 2, the horizontal axis represents log(compound), M, and the horizontal axis, the contractile force, %.

Fig. 2. Plots of the contractile force of guinea pig auricles against concentration of the test compounds. Designations at in Fig. 1.

6-Hydroxy-2-carbamoylmethylthio-6-phenyl-5-ethoxycarbonyl-3-cyano-4-(pyrid-3'-yl)-1,4-5,6-tetrahydropyridine (III). A mixture of 4.85 g (10 mmole) of (Ia) and 2.04 g (12 mmole) of iodoacetamine in 200 ml of abs. ethanol was heated briefly until the starting materials had dissolved, then cooled to 0°C, and the solid which separated was washed with 10 ml of ethanol and 20 ml of water to give 3.60 g (82%) of (III).

2-Carbamoylmethylthio-6-phenyl-5-ethoxycarbonyl-3-cyano-4-(pyrid-3'-yl)-1,4-dihydropyridine (IV). To a mixture of 3.64 g (10 mmole) of (II) in 20 ml of absolute ethanol and 1.2 ml (12 mmole) of piperidine was added with stirring 2.04 g (12 mmole) of iodoacetamide, and the mixture stirred for a further hour. After cooling to 0°C, the mixture was kept for 20 h, the solid was filtered off, and washed with 10 ml of ethanol and 10 ml of water to give 3.33 g (79%) of (IV).

3-Amino-6-phenyl-5-ethoxycarbonyl-2-carbamoyl-4-(pyrid-3'-yl)-4,7-dihydrothieno[2,3-b] pyridine (V). A mixture of 4.2 g (10 mmole) of (IV) in 20 ml of ethanol and 2 ml of 2 N KOH was heated briefly on the water bath, and cooled to 0°C. After 2 h, the solid was filtered off and washed with 10 ml of ethanol to give 3.4 g (81%) of (V).

2-Carbamoyl-6-phenyl-5-ethoxycarbonyl-3-cyano-4-(pyrid-3'-yl)pyridine (VI). Compound (IV) (4.2 g, 10 mmole) was dissolved with heating in 10 ml of glacial acetic acid, and 2.1 g (30 mmole) of sodium nitrite added. When evolution of oxides of nitrogen had ceased, the mixture was poured into water, washed well and the liquid decanted. The residue was dissolved with heating in 20 ml of 50% ethanol, filtered, and cooled to 0°C. After 20 h, the solid was filtered off to give 2.70 g (65%) of (VI).

3-Amino-6-phenyl-5-ethoxycarbonyl-2-carbamoyl-4-(pyrid-3'-yl)thieno-[2,3-b]pyridine (VIII). A mixture of 0.84 g (2 mmole) of (VI), 10 ml of ethanol, and 1.0 ml of 2 N KOH was heated briefly on the water bath, then cooled to 20°C, 20 ml of water added, and after 1 h the solid was filtered off to give 0.65 g (77%) of (VII).

EXPERIMENTAL (BIOLOGICAL)

Inotropic activity was determined in rhythmically stimulated guinea pig papillary muscles and auricles at frequencies of 0.5 and 1 Hz respectively. The stimuli were 1.2-1.5 times greater than the threshold values. The saline solution had the following composition (in mmoles): NaCl 144, KCl 4, $CaCl_2$ 1.8, Tris-HCl 10, $MgCl_2$ 1, glucose 5, at 36-37°C and pH 7.3-7.4. Oxygenation was carried out with pure oxygen. The test compounds were dissolved in dimethylacetamide, then diluted with the saline solution, the concentrations used being determined by the solubilities of the compounds.

The dose-dependent changes in the contractile force of rhythmically stimulated papillary muscles and auricles were measured. The 'weakening factor' (T''_{max}/T'_{min}) was calculated, where T'_{max} and T''_{min} are the maximum and minimum terms of the first and second derivatives of the tension force [19].

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