SYNTHESIS OF 4-SUBSTITUTED PYRIDIN-2(1H)-ONES, PYRIDINE-2(1H)-THIONES, RELATED DERIVATIVES AS ANALOGUES OF CARDIOTONIC DRUG MILRINONE

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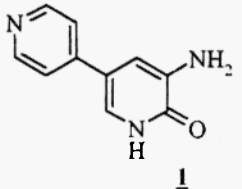
Abstract: A convenient method of synthesis of 4-substituted 5-(4'-pyridyl)pyridine-2(1H)-ones **9**-**12** and pyridine-2(1H)-thione **18** was elaborated by a Michael reaction of 4-pyridylacetone **3** and 2-cyano-3-R-acrylamides (thioamides) **4**-**6**, **13**-**14** with subsequent heterocyclization, dehydration and dehydrogenation. The sodium salt of thione **19** as a water-soluble compound was obtained by treatment of thione **18** with sodium methylate. Alkylation of the salt of 1,4-dihydropyridine-2(3H)-thione **16** and pyridinethione **18** yielded the corresponding 2-alkylthioderivatives **21** and **23**-**24**. By treatment of 2-carbamoylmethylthio-1,4-dihydropyridine **21** with acetic acid 7H-thiazolo[3,2-a]pyridin-3(2H)-one **22** was obtained, but treatment of pyridine **24** with sodium methylate gave thieno[2,3-b]pyridine **25**. The synthesized compounds were screened in vitro for cardiotonic activity on spontaneously contracted rabbit atria and guinea pig papillary muscle.

Introduction

Congestive heart failure (CHF) is a widespread and highly malignant disease. Among the most promising recent developments of treatment of CHF are the non-glycoside, non-sympathomimetic positive inotropic (cardiotonic) agents. The principal mechanism of these agents is elevation of cAMP levels in the myocardial cell by inhibition of cyclic nucleotide phosphodiesterase [1-3]. 3,4'-Dipyridyls have been of interest as cardiotonic agents for more than 10 years. Among them amrinone [3-amino-5-(4'-pyridyl)pyridine-2(1H)-one] **1** [1, 4, 5] and milrinone [3-cyano-6-methyl-5-(4'-pyridyl)pyridine-2(1H)-one] **2** [1, 6-10] have been discovered.



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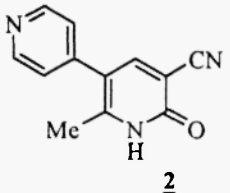

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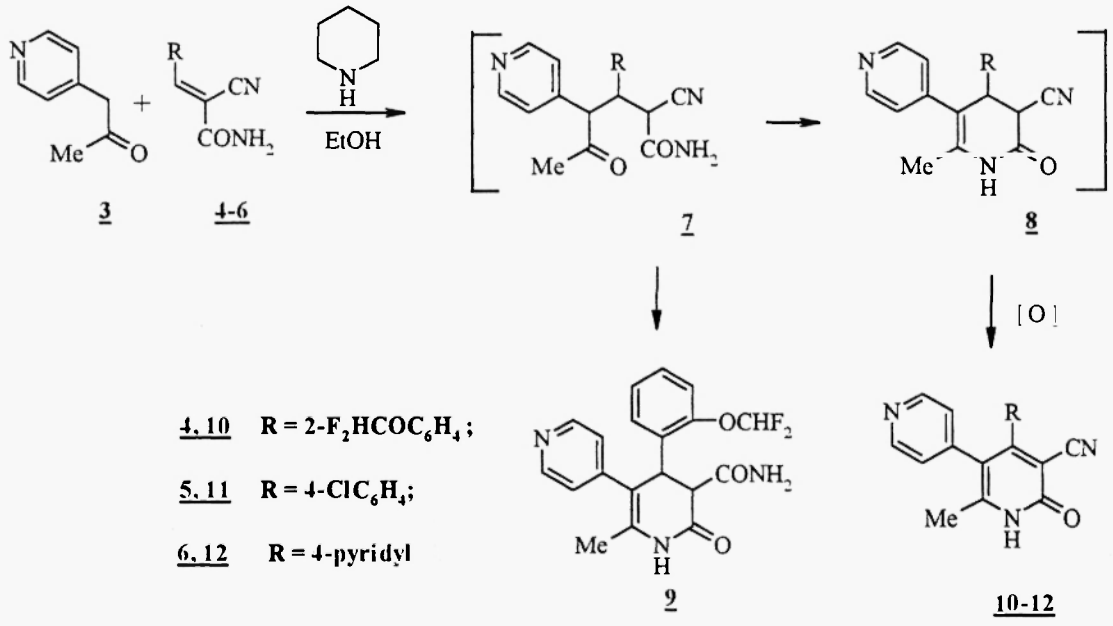
The sulphur analogue of milrinone [11] and its 2-alkylthioderivatives [12] have also exhibited cardiotonic activity. Hitherto only pyridin-2(1H)-ones and pyridine-2(1H)-thiones unsubstituted at position 4 have been investigated for cardiotonic activity.

In continuation of our research on the synthesis of substituted pyridine-2(1H)-ones and -2(1H)-thiones with potential cardiotonic activity [13-15] we have elaborated methods which allow modification of milrinone: the introduction of pharmacophore groups at position 4 and study of their effect on structure-activity relationships.

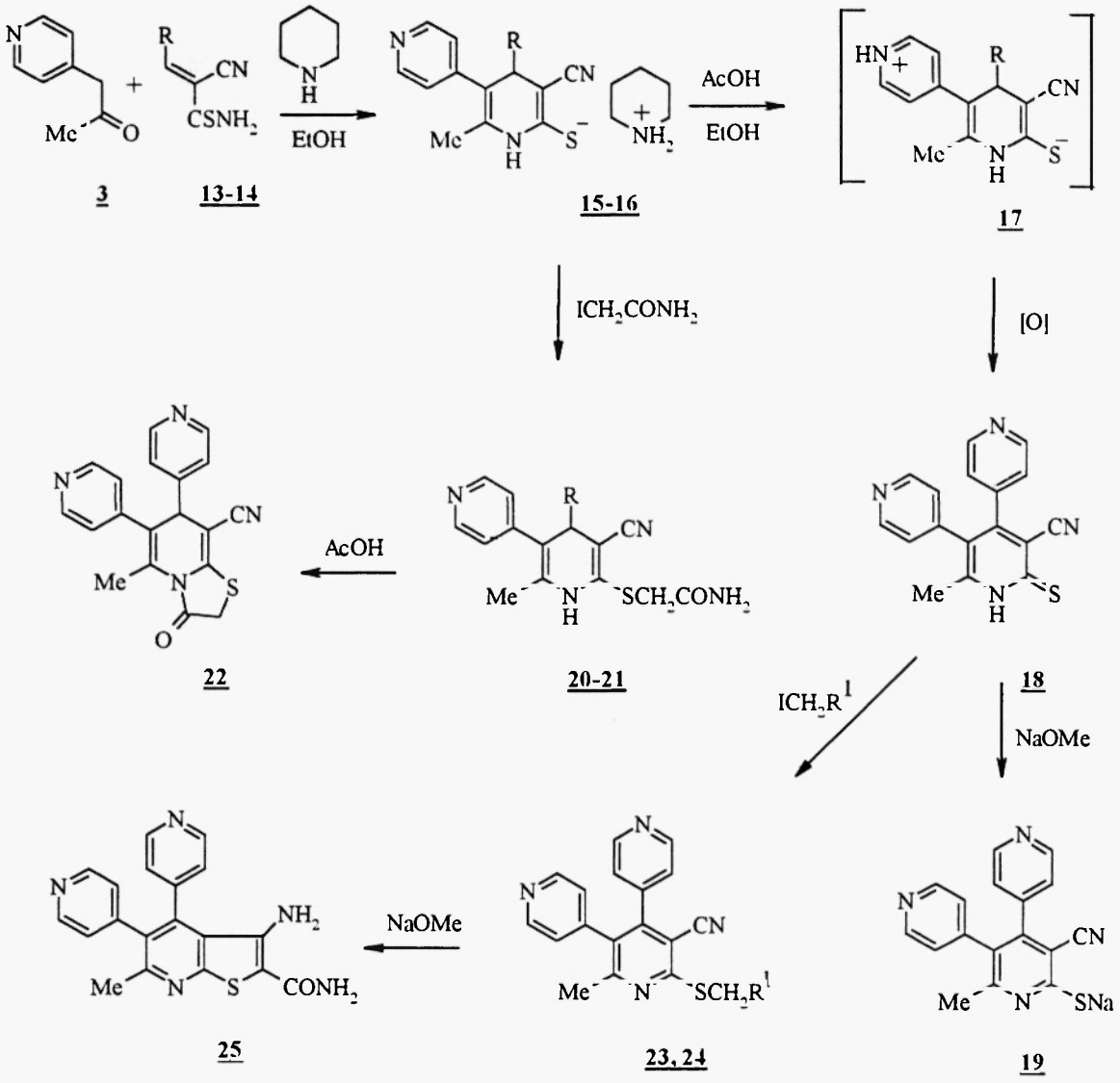
Results and discussion

Pyridine-2(1H)-ones **10**-**12** were obtained by a Michael reaction of 4-pyridylacetone **3** and 2-cyano-3-R-acrylamide **4**-**6** with subsequent heterocyclization, dehydration and dehydrogenation. The low yield of products **10**-**12** is due to competitive reactions occurring at the stage of heterocyclization of 4-acetyl-2-cyanobutyramides **7**. The reaction mixture after separation of the less soluble target pyridine-2(1H)-ones **10**-**12** contains a mixture of the corresponding 3-cyano- and 3-carbamoyl-1,4-dihydropyridine-2(3H)-ones **8** and **9**, which follows from checking of ^{1}H NMR spectra of the latter and is in good agreement with [16]. Separation of **8** and **9** is difficult both by fractional crystallization and column chromatography. Only 4-(2-difluoromethoxyphenyl)-3,4-dihydropyridin-2(1H)-one **9** has been isolated.
3-Cyanoderivatives **8** air oxidize to **10**-**12** during reaction and isolation. (Scheme 1).



Scheme 1.

By the treatment of 4-pyridylacetone **3** with 2-cyano-3-R-acrylthioamides **13**-**14** in the presence of piperidine piperidinium 1,4-dihydropyridine-2-thiolates **15**, **16** were formed (yield 82 % of **16**). On heating of thiolate **16** in acetic acid pyridine-2(1H)-thione **18** was obtained in 83 % yield. The intermediate - betaine of 5-pyridyl-1,4-dihydropyridine-2-thione **17** - in comparison with betaines of 4-pyridyl-1,4-dihydropyridine-2-thiones [17] is less stable and it undergoes dehydration in the course of the reaction. The sodium salt of thione **19** was obtained as water-soluble compound by treatment of **18** with sodium methylate (Scheme 2).



3 13-14 15-16 17 20-21 22 18 23, 24 25 19

13, 15, 20 R = 2-ClC$_6$H$_4$; 14, 16, 21 R = 4-pyridyl; 23 R^1= H; 24 R^1= CONH$_2$

Scheme 2

Alkylation of thiolate 16 with iodoacetamide yielded the corresponding 2-carbamoylmethylthio-1,4-dihydropyridine 21, but alkylation of thione 18 in the presence of triethylamine with alkyl halides gave the corresponding 2-alkylthiopyridines 23, 24. In the case of the 4-(2-ClC$_6$H$_4$)substituent isolation of salt 15 and thione of type 18 is rather complicated, but treatment of reaction mixture containing 15 with iodoacetamide gave 2-alkylthio-1,4-dihydropyridine 20 in 53% total yield. By refluxing of 2-carbamoylmethylthio-1,4-dihydropyridine 21 for 1 h with excess acetic acid 7H-thiazolo[3,2-a]pyridin-3(2H)-one 22 was formed, but by treatment of pyridine 24 with sodium methylate thieno[2,3-b]pyridine 25 has been obtained.

The structure of the synthesized compounds was proved by spectroscopic methods. In the IR spectra absorption bands of $\nu_{C\equiv N}$ for 16 at 2160 cm^{-1}, for 20 – 21 at 2186–2198 cm^{-1}, for 10 – 12, 18, 23, 24 at 2216- 2234 cm^{-1}, characterizing the hydrogenation degree of the pyridine ring and the type of conjugation of C≡N group, are observed. The doublets in

the case of ^{1}H NMR spectrum of **9** with $J_{3,4}$ = 2.6 Hz according to [18] cofirm a trans-diequatorial arrangement of the 3-H and 4-H protons and therefore a trans-diaxial arrangement of 3-$CONH_2$ and 4-(2-F_2HCO-C_6H_4) substituents. In the case of **16, 20 and 21** the characteristic 4-H proton signals at 4.23, 5.12 and 4.66 ppm, respectively, are observed, which support the dihydro structure. In the case of **20** the ^{1}H NMR signal is shifted downfield due to α-substituent in the 4-phenyl group.

The studied compounds showed lower cardiotonic activity than milrinone in spontaneously contracted rabbit atria and guinea pig papillary muscle models. Unfortunately the solubility of the investigated compounds was not sufficient to carry out detailed screening. In comparison with milrinone (positive inotropic activity on spontaneously beating atria EC_{50} = 6.5 ± 2.9 μM; on papillary muscle EC_{50} = 37.9 ± 13.8 μM, positive chronotropic activity EC_{30} = 1.3 μM, 44 % decrease of blood pressure, duration 62 min in anaesthetized rats, LD_{50} 55 – 117 mg/kg, i.p. in mice) the investigated compounds showed smaller effects on blood pressure and their LD_{50} were more than 1000 mg/kg. The most pronounced positive inotropic effects were induced by water-soluble sodium thiolate **19** (EC_{50} = 854.6 ± 270.2 μM on papillary muscle, no chronotropic activity, no changes of blood pressure) and pyridin-2(1H)-one **12** (EC_{50} = 612.6 ± 196.8 μM on spontaneously beating atria, positive chronotropic activity EC_{30} = 0.5 μM, 23 % decrease of blood pressure, duration 3 min).

In conclusion, a convenient method of synthesis of 4-substituted 5-(4'-pyridyl)pyridine-2(1H)-ones **9**-**12** and pyridine-2(1H)-thione **18** has been elaborated. The introduction of sterically bulky aryl or pyridyl groups in position 4 of pyridin-2(1H)-one and pyridine-2(1H)-thione did not enhance cardiotonic activity, but caused rather tangible decrease of toxicity and diminished the cardiotonic side effects. In continuation of research of compounds exerting cardiotonic activity, the functional groups enhancing solubility and lipophilicity have to be introduced in pyridin-2(1H)-one and pyridine-2(1H)-thione skeleton.

Experimental

Melting points were determined on a Boetius apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 580 B spectrometer (in nujol) and peak positions ν_{max} were expressed in cm^{-1}. ^{1}H NMR spectra were recorded on a Bruker WH-90 spectrometer and chemical shifts are reported as δ values (ppm) relative to tetramethylsilane. The synthesis of compound **2** is described in [19].

3-Carbamoyl-4-(2'-difluoromethoxyphenyl)-6-methyl-5-(4'-pyridyl)-1,4-dihydropyridin-2(3H)-one hydrochloride hydrate 9.

A mixture of 4-pyridylacetone **3** (4.06 g; 0.03 mol), 2-cyano-3-(2'-difluoromethoxyphenyl)acrylamide **4** (7.15 g; 0.03 mol) and piperidine (2 ml, 0.02 mol) in 40 ml of ethanol was stirred at room temperature for 24 h. Then 3 ml of acetic acid was added and reaction mixture after 6 h stirring was poured into 300 ml of water. After 2 h stirring, the crude precipitate was separated by filtration and refluxed for 15 min in 50 ml of 60 % ethanol – water solution and chilled to 0°C. The precipitate that separated during 2 days was combined and recrystallized from 100 ml 0.1 M HCl solution in ethanol to give 2.18 g (17 %) of **9** as colourless crystals, mp 190 – 193°C; IR: 1668, 1680 (C=O); 3160, 3238, 3456 (NH, NH_2); ^{1}H NMR (DMSO-d_6): 2.02 (3H, s, 6-Me); 3.36 (1H, d, J = 2.6 Hz, 3-H); 4.68 (1H, d, J = 2.6 Hz, 4-H); 7.0 –

7.3 (4H, m, 4-C_6H_4); 7.14 (1H, t, J = 74 Hz., $OCHF_2$); 7.64 and 8.62 (4H, d and d, J = 7 Hz, 5-C_5H_4N); 10.12 (1H, s, NH). Anal. calcd. for $C_{19}H_{17}F_2N_3O_3$ x HCl x H_2O: C 53.33; H 4.71; N 9.82. Found: C 53.14; H 4.58; N 9.64.

3-Cyano-4-(2'-difluoromethoxyphenyl)-6-methyl-5-(4'-pyridyl)pyridin-2(1H)-one 10.

After separation of crude 9 from reaction mixture, the filtrate was evaporated to dryness. The residue was dissolved in 20 ml of acetic acid, 3 g of sodium nitrite was added and after evolution of NO_2 ceased, the reaction mixture was cooled and poured into 200 ml of water. The precipitate was separated by filtration and then recrystallized from 50 ml of ethanol to give 1.1 g (10.4 %) of 10 as colourless crystals , mp > 310°C (decomp); IR: 1660 (C=O); 2230 (C≡N); 3150 (NH); ^{1}H NMR (DMSO-d_6): 2.15 (3H, s, 6-Me); 7.0 and 8.35 (4H, d and d, J = 7 Hz, 5-C_5H_4N); 7.1 – 7.4 (4H, m, 4-C_6H_4); 7.14 (1H, t, J = 74 Hz., $OCHF_2$); 12.48 (1H, s, NH). Anal. calcd. for $C_{19}H_{13}F_2N_3O_2$: C 64.59; H 3.71; N 11.89. Found C 64.56; H 3.65; N 11.82.

3-Cyano-4-(4'-chlorophenyl)-6-methyl-5-(4'-pyridyl)pyridine-2(1H)-one 11.

A mixture of 4-pyridylacetone 3 (4.06 g; 0.03 mol), 2-cyano-3-(4'-chlorophenyl)acrylamide 5 (6.20 g; 0.03 mol) and piperidine (1.5 ml; 0.015 mol) in 20 ml of ethanol was refluxed for 15 min and stirred at ambient temperature for 24 h. Then 1.5 ml of acetic acid was added and chilled to 5°C. The crude precipitate was collected during 2 days, combined and recrystallized from 60 % ethanol – water solution to give 2.80 g (29 %) of 11 as colourless crystals, mp >350°C (decomp) (from acetic acid-ethanol), IR: 1676 (C=O); 2224 (C≡N); 3090 (NH). ^{1}H NMR (DMSO-d_6): 2.10 (3H, s, 6-Me); 7.0-8.4 (8H, dd and dd, C_6H_4Cl and C_5H_4N); 12.80 (1H, br.s, NH). Anal. calcd. for $C_{18}H_{12}ClN_3O$: C 67.19; H 3.76; N 13.06; Found C 67.14; H 3.64; N 12.93.

3-Cyano-6-methyl-4,5-di(4'-pyridyl)pyridine-2(1H)-one 12.

Compound 12 was prepared in the same manner as 11 (the small excess of acetic acid was neutralized with 5 % sodium carbonate solution) from 4-pyridylacetone 3 and 2-cyano-3-(4'-pyridyl)acrylamide 6. Yield 23 % of 12 as colourless crystals, mp > 300°C (decomp) (from ethanol). IR: 1680 (C=O); 2218 (C≡N); 3290 (NH). ^{1}H-NMR (DMSO-d_6): 2.12 (3H, s, 6-Me); 7.0-8.6 (8H, dd and dd, 2C_5H_4N); 13.00 (1H, br.s, NH). Anal. calcd. for $C_{17}H_{12}N_4O$: C 70.82; H 4.20; N 19.43; Found: C 70.56; H 4.07; N 19.42.

Piperidinium 3-cyano-6-methyl-4,5-di(4'-pyridyl)-1,4-dihydropyridine-2-thiolate 16.

A mixture of 4-pyridylacetone 3 (1.35 g; 0.01 mol), 2-cyano-3-(4'-pyridyl)acrylthioamide 14 (1.89 g; 0.01 mol) and piperidine (1 ml; 0.01 mol) in 10 ml of ethanol was briefly heated on water bath and stirred for 1 h at ambient temperature. Then 20 ml of ether was added gradually and the reaction mixture was cooled to 0°C. The precipitated crystals were filtered and washed with 20 ml of ether and 10 ml of hexane to give 3.21 g (82 %) of 16 as yellow crystals, mp > 180°C (decomp.). IR: 2160 (C≡N), 2430,2520 (+NH_2); 3162, 3206 (NH). ^{1}H NMR (DMSO-d_6): 1.60 [4H, m, $N(CH_2)_2$]; 1.90 (3H, s, 6-Me); 3.00 [6H, m,$(CH_2)_3$]; 4.23 (1H, s, 4-H); 6.9-8.4 (8H, dd and dd, 2 C_5H_4N); 7.82 (1H, s, NH). Anal. calcd. for $C_{22}H_{25}N_5S$: C 67.49; H 6.44; N 17.89; S 8.19. Found: C 67.35; H 6.54; N 17.77; S 8.04.

3-Cyano-6-methyl-4,5-di-(4'-pyridyl)pyridine-2(1H)-thione 18.

A sample of thiolate **16** (3.92 g, 0.01 mol) was refluxed in 10 ml of acetic acid and 10 ml of ethanol for 30 min on a water bath, stirred at room temperature for 6 h and cooled to 0°C. The crystals that precipitated during 3 days were filtered off, combined and washed with 50 ml of ethanol and 20 ml of water to give 2.56 g (83 %) of **18** as yellow crystals, mp > 320°C (decomp.). IR: 2234 (C≡N), 3186 (NH). ^{1}H-NMR (DMSO-d_6): 2.16 (3H, s, 6-Me); 7.1-8.4 (8H, dd and dd, $2C_5H_4N$); 14.22 (1H, br.s, NH). Anal. calcd. for $C_{17}H_{12}N_4N$: C 67.08; H 3.98; N 18.41; S 10.53. Found: C 66.98; H 3.79; N 18.19; S 10.51.

Sodium 3-cyano-6-methyl-4,5-di-(4'-pyridyl)pyridine-2-thiolate 19.

A mixture of thione **18** (3.04 g, 0.01 mol) and 25 ml of 0.5 N sodium methylate was briefly heated and filtered. After 2 h 20 ml of dry ether was added, the reaction mixture was kept 1 h at 0°C, the precipitated crystals were filtered and washed with 20 ml of dry ether to give 2.67 g (82 %) of **19** as yellow crystals, mp > 330°C (decomp). IR : 2226 (C≡N). ^{1}H NMR (DMSO-d_6): 1.98 (3H, s, 6-Me); 7.0-8.4 (8H, dd and dd, $2C_5H_4N$). Anal. calcd.for $C_{17}H_{11}N_4NaS$: C 62.56; H 3.40; N 17.17. Found C 62.33; H 3.21; N 16.96.

2-Carbamoylmethylthio-4-(2'-chlorophenyl)-3-cyano-5-(4'-pyridyl)-6-methyl-1,4-dihydropyridine 20.

A mixture of 4-pyridylacetone **3** (1.35 g; 0.01 mol), 2-cyano-3-(2'-chlorophenyl)acrylthioamide **13** (2.23 g; 0.01 mol) and piperidine (1 ml; 0.01 mol) in 10 ml of ethanol was briefly heated untill dissolution and stirred 1 h at ambient temperature for 2 h. Then iodoacetamide (2.03 g , 0.011 mol) was added, shortly heated untill dissolution, filtered and chilled to 0°C. The precipitated crystals were removed by filtration, washed with 20 ml of 50 % ethanol and 20 ml of water to give 2.1 g (53 %) of **20** as yellow crystals, mp 193-195°C (from ethanol). IR: 1680 (C=O); 2198 (C≡N); 3160, 3230 (NH). ^{1}H NMR (DMSO-d_6): 1.83 (3H, s, 6-Me); 3.64 and 3,74 (2H, d and d, J = 14.4 Hz, SCH_2); 5.12 (1H, s, 4-H); 7.0–8.9 (8H, complex, 4-C_6H_4 and 5-C_5H_4N); 9.97 (1H, s, NH). Anal. calcd. for $C_{20}H_{17}ClN_4OS$: C 60.53; H 4.32; N 14.12; S 8.08. Found: C 60.31; H 4.29; N 13.98; S 7.95.

2-Carbamoylmethylthio-3-cyano-4,5-di(4'-pyridyl)-6-methyl-1,4-dihydropyridine 21.

A mixture of thiolate **16** (3.92 g, 0.01 mol) and iodoacetate (2.22 g, 0.012 mol) in 30 ml of ethanol was briefly heated and filtered. The reaction mixture was kept for 2 h at 0°C, the precipitated crystals were filtered and washed with 10 ml of ethanol and 20 ml of water to give 2.76 g (76 %) of **21** as yellow crystals, mp 192-194°C (from ethanol). IR: 1682 (C=O), 2186 (C≡N); 3146, 3200sh, 3350 (NH). ^{1}H NMR (DMSO-d_6): 1.91 (3H, s, 6-Me); 3.62 and 3.78 (2H, d and d, J=15.0 Hz, SCH_2);); 4.66 (1H, s, 4-H); 7.12 and 8.48, 7.28 and 8.38 (8H, dd and dd, dd and dd, 4,5-C_5H_4N); 7.60 and 7.90 (2H, s and s, NH_2); 10.06 (1H, s, NH). Anal. calcd. for $C_{19}H_{17}N_5OS$: C 62.79; H 4.72; N 19.27; S 8.82. Found: C 62.52; H 4.57; N 19.12; S 8.75.

8-Cyano-5-methyl-6,7-di(4'-pyridyl)-7H-thiazolo[3,2-a]pyridin-3(2H)-one 22.

A sample of 1,4-dihydropyridine **21** (0.37 g, 0.001 mol) in 10 ml of acetic acid was refluxed for 1 h. Then the reaction mixture was evaporated under reduced pressure to 1/5 of its initial volume, cooled to room temperature and 20 ml of 50 % ethanol solution was added gradually. The precipitated crystals were filtered off and washed with 5 ml of ethanol to

give 0.21 g (61 %) of **22** as yellow crystals, mp 246-248°C (from ethanol). IR: 1726 (C=O); 2194 (C≡N). ^{1}H NMR (DMSO-d_6): 2.12 (3H, s, 5-Me); 4.16 (2H, s, 2-CH_2); 4.82 (1H, s, 7-H); 7.1 – 8.6 (8H, dd and dd, 4,5-C_5H_4N). Anal. calcd. for $C_{19}H_{14}N_4OS$: C 65.88; H 4.07; N 16.17; S 9.26. Found C 65.66; H 4.14; N 16.14; S 9.31.

3-Cyano-4,5-di-(4'-pyridyl)-6-methyl-2-methylthiopyridine 23.

A mixture of thione **18** (0.91 g; 0.003 mol), methyl iodide (1.25 ml; 0.01 mol) and triethylamine (0.7 ml, 0.005 mol) in 10 ml of ethanol was refluxed for 5 min and stirred for 2 h at the ambient temperature. Then under stirring 20 ml of water was added gradually and the reaction mixture was cooled to 0°C. After 12 h the precipitate was filtered to give 0.65 g (68 %) **23** as colourless crystals, mp 183-185°C (from ethanol). IR: 2216 (C≡N). ^{1}H NMR ($CDCl_3$): 2.42 (3H, s, 6-Me); 2.68 (3H, s, SMe); 6.92 and 8.56, 7.00 and 8.52 (8H, dd and dd, dd and dd, 4,5-C_5H_4N). Anal. calcd. for $C_{18}H_{14}N_4S$: C 67.90; H 4.43; N 17.60; s 10.07. Found C 67.81; H 4.41; N 17.71; S 10.22.

2-Carbamoylmethylthio-3-cyano-4,5-di-(4'-pyridyl)pyridine 24.

A mixture of thione **18** (1.52 g, 0.005 mol) and triethylamine (1.4 ml, 0.01 mol) in 15 ml of ethanol was briefly heated, then iodoacetamide (1.11 g, 0.006 mol) was added, reaction mixture refluxed for 15 min, cooled to room temperature and the precipitated crystals were filtered, washed with 20 ml of cooled (~ 0°C) ethanol and 20 ml of water to give 1.52 g (84 %) of **24** as colourless crystals, mp 223-225°C. IR: 1680 (C=O); 2220 (C≡N); 3160, 3280, 3360 (NH_2). ^{1}H NMR (DMSO-d_6): 2.36 (3H, s, 6-Me); 4.06 (2H, s, SCH_2); 7.0 – 8.6 (12H, complex, 2 C_5H_4N and NH_2). Anal. calcd. for $C_{19}H_{15}N_5OS$: C 63.14; H 4.18; N 19.38; S 8.87. Found C 62.87; H 4.32; N 19.23; S 8.95.

3-Amino-2-carbamoyl-4,5-di(4'-pyridyl)thieno[2,3-b]pyridine 25.

A mixture of pyridine **24** (0.72 g, 0.002 mol) and 2 ml 2N potassium hydroxide solution in 10 ml of ethanol was shortly heated and stirred at ambient temperature for 2 h. The precipitated crystals were filtered, washed with 10 ml of ethanol and 10 ml of water to give 0.56 g (78 %) of **25** as yellow crystals, mp 325-327°C. IR: 1658 (C=O); 3160, 3300, 3470 (NH_2). ^{1}H NMR (DMSO-d_6): 2.38 (3H, s, 6-Me); 5.60 (2H, s, 3-NH_2); 7.2- 8.8 (10H, complex, 2 C_5H_4N and $CONH_2$). Anal. calcd. for $C_{19}H_{15}N_5OS$: C 63.14; H 4.18; N 19.38; S 8.87. Found C 62.92; H 4.25; N 19.39; S 9.04.

Determination of cardiotonic activity (in vitro) and acute toxicity.

Inotropic and chronotropic activity was studied using spontaneously contracted rabbit atria. Male Wistar rats (body weight 330-380 g) were sacrificed by cervical dislocation, and the heart was rapidly removed, placed in oxygenated nutritient solution (mmol/l: NaCl 137; KCl 2.7; $CaCl_2$ 1.9; $MgCl_2$ 1.0; $NaHCO_3$ 11.3; NaH_2PO_4 0.4; glucose 5.5), pH 7.4. The atria were separated and placed into a 20-ml glass bath filled with nutritient solution aerated with oxygen at 30.0 ± 0.5 °C. The atria was connected to a force-displacement transducer TB-612T (Nihon Kohden, Japan) with a resting tension 1 g. Spontaneous contractions of atria were registered on physiograph RM-6000 (Nihon Kohden, Japan). To stabilize rhythm and contraction force the atria was kept under these conditions for 30-60 min.

Guinea pig right ventricle papillary muscle was mounted in an organ bath with a circulation of PSS (mM/l: NaCl 144; KCl 4; $CaCl_2$ 1.8; tris.Cl 10; $MgCl_2$ 1; glucose 5; pH 7.3-7.4; 36-37°C), continuously aerated with oxygen and 0.5 Hz stimulation frequency was used. After 60 min equilibration period perfusion with substances was performed and

studied. From concentration-response curves (2-4) EC values were determined. Statistical analyses were performed using the Student's *t*-test.

In vivo influence on blood pressure and heart rate was studied in anaesthetized Wistar rats (body weight 250-300 g). Systemic arterial pressure (pressure transducer P 23ID. Statham) and transthoracal electrocardiogram (ECG) were recorded on a Polygraph. The substances were studied at doses of 0.1 mg/kg intravenously.

Intraperitonial toxicity was studied on adult mice. After administration of a single dose the animals were observed for 10 days. LD_{50} was calculated according to Litchfield and Wilcoxson.

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