SYNTHESIS OF 4,5-DIPYRIDYLPYRIDIN-2(1H)-ONES, PYRIDINE-2(1H)-THIONES AND RELATED DERIVATIVES AS ANALOGES OF CARDIOTONIC DRUG MILRINONE

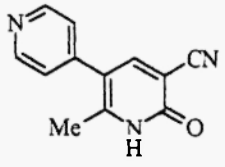
A.Krauze*[a], V.Garalene[b], R.Vītoliņa[a], G.Duburs[a]

[a]Latvian Institute of Organic Synthesis, Riga, Latvia LV-1006
[b]Lithuanian Institute of Cardiology, Kaunas, Lithuania LT-3007

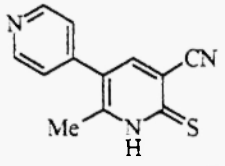
Abstract: 4,5-Dipyridyl substituted pyridine-2(1H)-one 8 and pyridine-2(1H)-thione 12 have been prepared by Michael reaction of 4-pyridylacetone 3 and 2-cyano-3-(3-pyridyl)acrylamides (thioacrylamides) (4, 9) with subsequent heterocyclization, dehydration and dehydrogenation. Sodium salt of thione 14, as water soluble compound has been obtained by treatment of thione 12 with sodium methylate. Oxidation of 12 yielded 2,2-bispyridyldisulfide 13, but methylation - 2-methylthiopyridine 15.

Introduction

Congestive heart failure (CHF) is a widespread and highly malignant disease. Among the most promising 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 15 years. Among them milrinone 1 [1, 4-8] has been discovered.



1



2

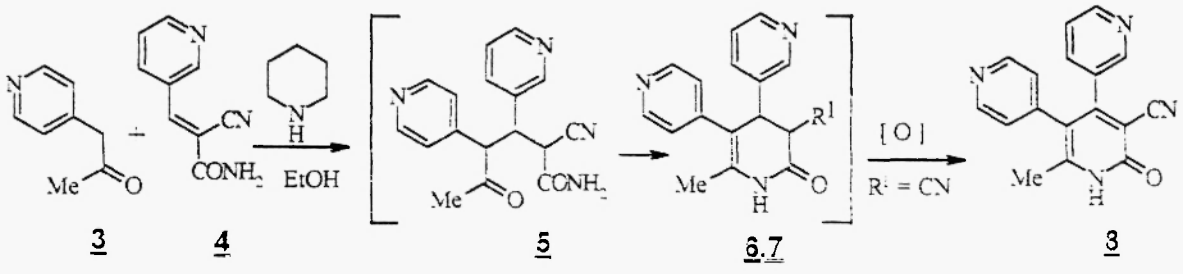
The sulphur analogue of milrinone – the thione 2 [9] and its 2-alkylthioderivatives [10] have also exhibited cardiotonic activity.

In continuation of our research on the synthesis of substituted pyridine-2(1H)-ones and -2(1H)-thiones with potential cardiotonic activity [11-14] we have synthesized 4,5-dipyridyl substituted pyridin-2(1H)-one 8, pyridine-2(1H)-thione 12, corresponding sodium salt 14, 2,2-

bispyridyldisulfide **13** and 2-methylhiopyridine **15** , and studied structure-activity relationships of these compounds.

Results and discussion

3-Cyano-6-methyl-4-(3'-pyridyl)-5-(4'-pyridyl)pyridine-2(1H)-one (**8**) was obtained by Michael reaction of 4-pyridylacetone (**3**) and 2-cyano-3-(3'-pyridyl)acrylamide (**4**) with subsequent heterocyclization, dehydration and dehydrogenation (Scheme 1). The 27 % yield of pyridin-2(1H)-one **8** is due to competitive reactions proceeding in the stage of heterocyclization of 4-acetyl-2-cyanobutyramide **5**. The reaction mixture after separation of desirable less soluble pyridine-2(1H)-one **8** contains mixture of corresponding 3-carbamoyl- and 3-cyano-1,4-dihydropyridine-2(3H)-ones **6** and **7**, which follows from checking of ^{1}H NMR spectra of the latter. Their separation is



3 **4** **5** **6,7** **8**

6 R^1 = CN; **7** R^1 = $CONH_2$

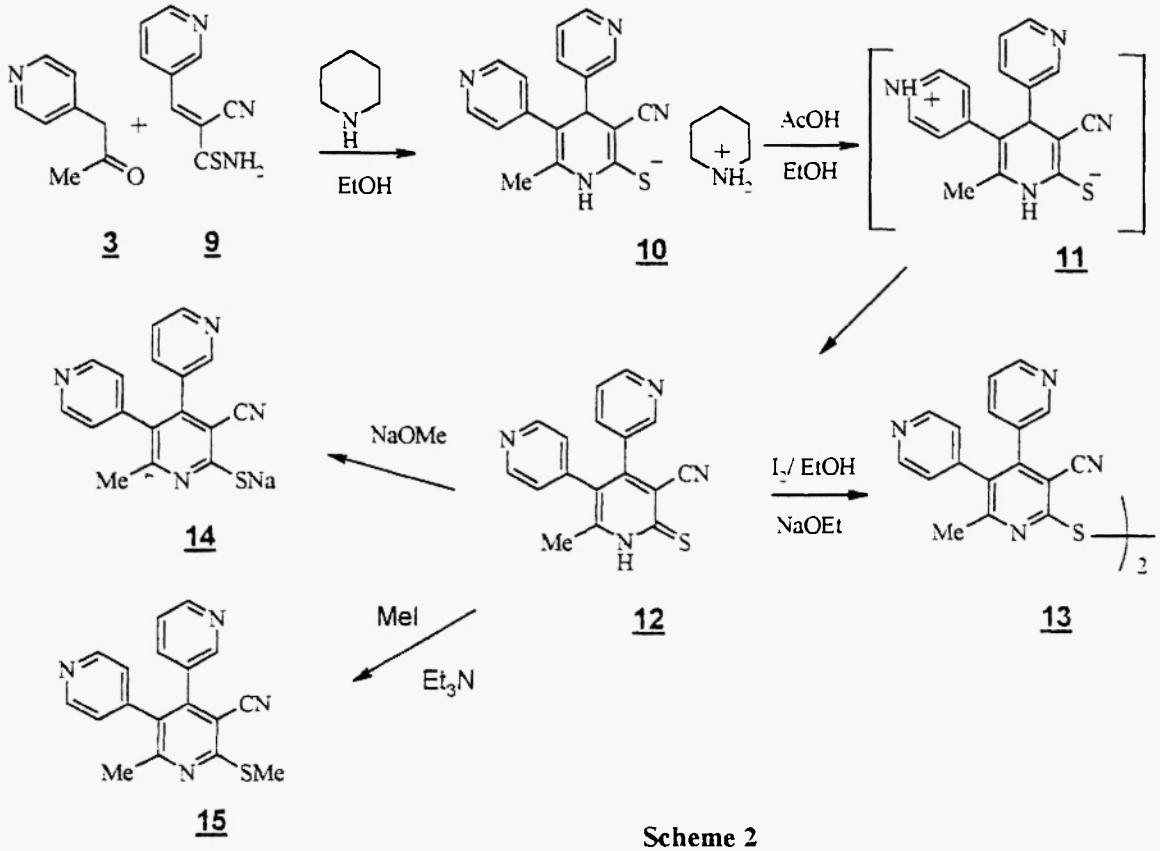
Scheme 1.

complicated both on fractionate crystallization and column chromatography. 3-Cyanoderivative of compounds of type **7** is unstable to oxidation [14] and on standing gives rise to **8**.

By the treatment of 2-cyano-3-(3'pyridyl)thioacrylamide (**9**) with 4-pyridylacetone **3** in the presence of piperidine piperidinium 1,4-dihydropyridine-2(3H)thiolate **10** was obtained in 81 % yield. On heating of **10** in acetic acid pyridine-2(1H)-thione **12** was obtained in 85 % yield. The betaine of 1,4-dihydropyridine-2-thione **11**, obviously, similarly to **6** in the course of reaction oxidizes to **12**. Sodium salt of thione **14** as water soluble compound was obtained by treatment of **12** with sodium methylate. The oxidation of thione **12** with iodine in presence of sodium methylate gave disulfide **13**. The alkylation of thione **12** with iodomethane in presence of Et_3N yielded 2-methylthiopyridine **15** (Scheme 2).

The structure of synthesized compounds was proved by spectroscopic methods and elemental analysis. In the IR spectra characteristic absorbtion bands of C≡N group for compounds **8** and **12** –

16 at 2214 – 2240 are observed. The singlet in case of ^{1}H NMR spectrum of thiolate **10** at 4.26 ppm, characterising hydrogenated structure, is observed.



Scheme 2

The studied 4-(3-pyridyl)derivatives of milrinone (both O and S analogues) showed lower cardiotonic activity than milrinone in spontaneously contracted rat 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} 53 – 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 pyridin-2(1H)-one **8** (EC_{50} = 231.8 ± 53.1 μM on papillary muscle. No chronotropic activity and no changes of blood pressure was observed.

Disulfide **13** (35 - 38 % increasing of activity at concentration 0.01 – 0.1 μm) and sodium salt **14** (30 % effect at 10 μm) seemed perspective at low concentrations, unfortunately the solubility of these and other investigated compounds was not sufficient to carry out detailed screening.

The introduction of sterically bulky pyridyl groups in position 4 of pyridin-2(1H)-one and pyridine-2(1H)-thione ring (milrinone structure) did not enhance cardiotonic activity, but caused rather tangible decrease of toxicity and diminished the cardiotonic side effects.

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} are expressed in cm^{-1}. ^1H NMR spectra were recorded on a Bruker WH-90 spectrometer and chemical shifts are reported as δ values (ppm) relative to tetramethylsilane.

3-Cyano-6-methyl-4-(3'-pyridyl)-5-(4'-pyridyl)pyridine-2(1H)-one (8). A mixture of 4-pyridylacetone **4** (4.06 g; 0.03 mol), 2-cyano-3-(3'-pyridyl)acrylamide **5** (5.20 g; 0.03 mol) and piperidine (0.85 g; 0.01 mol) in 50 ml of ethanol was refluxed for 30 min. Then 2 ml of acetic acid were added and the reaction mixture was stirred 5 h at room temperature, neutralized with 30 ml of 5 % sodium carbonate and left for 12 h. The crude precipitate was removed by filtration, refluxed 15 min in 30 ml of 50 % ethanol and cooled to 0°C. The precipitated crystals were filtered and washed with 10 ml of cold 20 % ethanol to give 2.36 g (27 %) of **8** as colourless powder, mp 163-165°C (from ethanol). IR: 1660, 1685 (C=O); 2240 (C≡N); 3200 (NH). ^1H-NMR (DMSO-d_6): 13.16 (1H, br.s, NH); 8.6 - 7.0 (8H, complex, 4,5-C_6H_4N); 2.12 (3H, s, 6-Me). Anal.calc. for $C_{37}H_{12}N_4O \times H_2O$: C 66.66; H 4.61; N 18.29. Found: C 66.85; H 4.62; N 18.23.

Piperidinium 3-cyano-6-methyl-4-(3'-pyridyl)-5-(4'-pyridyl)-1,4-dihydropyridine-2-thiolate (10). A mixture of 4-pyridylacetone **3** (1.35 g; 0.01 mol), 2-cyano-3-(3'-pyridyl)acrylthioamide **9** (1.89 g; 0.01 mol) and piperidine (0.85 g; 0.01 mol) in 10 ml of ethanol was briefly heated on water bath and stirred 1 h at ambient temperature. Then gradually 20 ml of ether were added and 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 3.19 g (81 %) of **10** as grey powder, mp >150°C (decomp.). IR: 2146, 2172 (C≡N); 2430, 2526 ($^+NH_2$); 3160, 3214 (NH). ^1H NMR (DMSO-d_6): 8.4 - 7.0 (8H, complex, 4,5-C_5H_4N); 4.26 (1H, s, 4-H); 2,98 [6H, m, $(CH_2)_3$]; 1.90 (3H, s, 6-Me); 1.60 [4H, m, $N(CH_2)_2$]. Anal.calc. for $C_{27}H_{25}N_5S$: C 67.50; H 6.44; N 17.90; S 8.20. Found: C 67.32; H 6.26; N 17.79; S 7.98.

3-Cyano-6-methyl-4-(3'-pyridyl)-5-(4'-pyridyl)pyridine-2(1H)-thione (12). A sample of thiolate **10** (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 precipitated during 3 days crystals were filtered, combined and washed with 50 ml of ethanol and 20 ml of water to give 2.85 g (85 %) of **12** as yellow powder, mp > 270°C (decomp) (from acetic acid-ethanol). IR: 2228 (C≡N); 3176 (NH). ^{1}H NMR (DMSO-d_6): 14.34 (1H, br.s, NH); 8.4 - 7.0 (8H, complex, 4,5-C_5H_4N); 2.15 (3H, s, 6-Me). Anal.calc. for $C_{17}H_{12}N_4S$ x H_2O: C 63.34; H 4.38; N 17.38; S 9.94. Found: C 63.63; H 4.26; N 17.19; S 9.95.

2,2'-Bis-[3-cyano-6-methyl-4-(3'-pyridyl)-5-(4'-pyridyl)pyridine]disulfide (13). 10 ml of 0.5 N Iodine solution in etanol was added gradually to the mixture of thione **3** (1.52 g, 0.005 mol) in 15 ml of 0.4 N sodium methylate solution and stirred for 1 h. The reaction mixture was poured in 50 ml of cold water, the precipitate was separated by filtration, recrystallized from ethanol to give 0.96 g (63 %) of **13** as grey powder, mp > 160°C (decomp). IR: 2218 (C≡N). ^{1}H NMR (DMSO-d_6): 8.6 – 7.2 (8H, complex, 4,5-C_5H_5N); 2.37 (3H, s, 6-Me). Anal.calc. for $C_{34}H_{22}N_8S_2$: C 67.31; H 3.65; N 18.47; S 10.57. Found: C 67.09; H 3.77; N 18.23; S 10.49.

Sodium 3-cyano-6-methyl-4-(3'-pyridyl)-5-(4'-pyridyl)pyridine-2-thiolate (14). A mixture of thione **12** (3.04 g, 0.01 mol) and 25 ml of 0.5 N sodium methylate was briefly heated and filtered. The reaction mixture was kept 1 h at 0°C, the precipitate was filtered and washed with 20 ml of dry ether to give 2.19 g (67 %) of **14** as yellow powder, mp > 330°C (decomp), IR: 2225 (C≡N). ^{1}H NMR (DMSO-d_6): 8.3 - 6.9 (8H, complex, 4,5-C_5H_4N); 2.00 (3H, s, 6-Me). Anal.calc. for $C_{17}H_{11}N_4NaS$ x 0.5 H_2O: C 60.88; H 3.61; N 16.71. Found: C 61.09; H 3.46; N 16.78.

3-Cyano-6-methyl-2-methylthio-4-(3'-pyridyl)-5-(4'-pyridyl)pyridine (15). A mixture of thione **12** (0.91 g; 0.003 mol), iodomethane (1.25 ml; 0.02 mol) and 1 ml of triethylamine in 10 ml of ethanol was refluxed for 5 min and stirred for 1 h at room temperature. Then under stirring 20 ml of water were added gradually and reaction mixture was cooled to 0°C. After 12 h the precipitate was removed by filtration to give 0.49 g (52 %) of **15** as slightly yellow powder, mp 153-155°C (from ethanol). IR: 2214 (C≡N). ^{1}H NMR ($CDCl_3$): 8.6-6.9 (8H, complex, 4,5-C_5H_5N); 2.70 (3H, s, SMe); 2.42 (3H, s, 6-Me). Anal.calc. for $C_{18}H_{14}N_4S$: C 67.90; H 4.43; N 17.60; S 10.07. Found: C 67.59; H 4.48; N 17.79; S 10.02.

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

Inotropic and chronotropic activities were 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 231D, 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 Wilcocson.

References

1 B.Wetzel, N.Hauel, Trends in Pharmacol Sci., 91, 166 (1988).

2 H.Landmann, H.Lowe, Pharmazie, 41, 169 (1986).

3 W.H.Moos, C.C.Humblet, I.Sircar, C.Rithner, R.E.Weishaar, J.A.Bristol, A.T. McPhail, J. Med. Chem., 30, 1963 (1987).

4 A.A.Alausi, J.M.Canter, M.J.Montenaro, D.J.Fort, R.A.Ferrari, J.Cardiovasc.Pharmacol., 5, 792 (1983).

5 A.A.Alausi, D.C.Johnson, Circulation, 73, 10 (1986).

6 R.K.Goyal, J.H.McNeill, Eur.J.Pharmacol., 120, 267 (1986).

7 C.Q.Earl, J.Linden, J.Weglicki, J. Cardiovasc. Pharmacol., 8, 864 (1986).

8 P.G.Fitzpatrick, M.P.Cinquegrani, A.R.Vakiener, J.G.Baggs, T.L.Biddle, C.S.Liang, W.P.Hood, M.D. Rochester, Amer. Heart J., 114, 97 (1987).

9 V.Hagen, A.Rumler, G.Reck, A.Hagen, D.Labes, S.Heer, Pharmazie, 44, 809 (1989).

10 A.Rumler, V.Hagen, A.Hagen, Pharmazie, 45, 657 (1990).

11 E.V.Narushavicius, V.N.Garalene, A.A.Krauze, G.Ya.Dubur, Khim-Farm.Zh (in Russian), 23,1459 (1989); CA 112: 131894y (1990).

12 A.A.Krauze, V.N.Garalene, G.Ya.Dubur, Khim-Farm. Zh. (in Russian), 26, 40 (1992); CA 118: 38791b (1993).

13 A.A.Krauze, V.N.Garalene, G.Y.Dubur, Khim-Farm. Zh (in Russian), 27, 39 (1993); CA 121: 300729s (1993).

14 A.Krauze, R.Vitolina, V.Garaliene, H.-J.Jänsch, G.Dubur, Heterocycl. Commun., 5, 569 (1999).

Received on June 21, 2001