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SYNTHESIS OF N-ACETYL DERIVATIVES OF 5- AND 6-ETHOXY-2-METHYLTHIOBENZIMIDAZOLE AND THEIR CARDIOTONIC ACTIVITY

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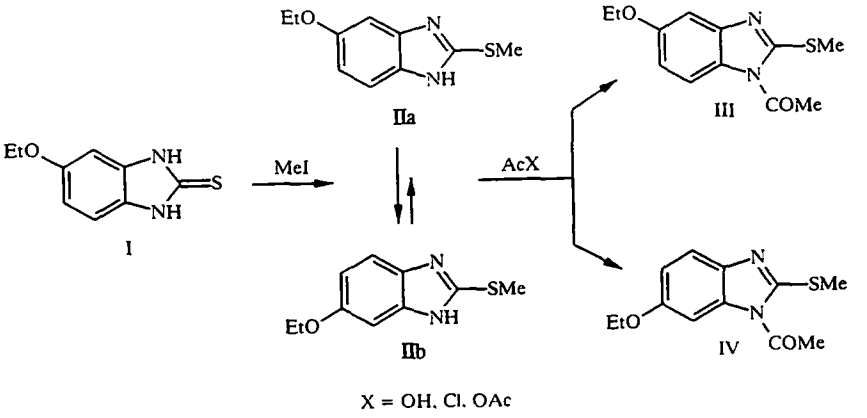
The regioselectivity of the N-acetylation of 5- and 6-ethoxy-2-methylthiobenzimidazole for various acetylating agents under various conditions has been investigated. The cardiotonic activity of the 1-acetyl-5-ethoxy- and 1-acetyl-6-ethoxy-2-methylthiobenzimidazoles synthesized and of their 5,6-diethoxy analog has been studied.

The 1-acyl-2-alkylthio-5,6-dialkoxybenzimidazoles synthesized by us previously displayed good cardiotonic activity [1, 2]. As a continuation of investigations on the relationship of structure and biological activity among benzimidazoles it seemed expedient to us to synthesize 1-acetyl-2-methylthio-5(and 6)-ethoxybenzimidazoles, which are unknown at present. Synthesis of the desired compounds may be affected by two alternative approaches, either cyclization of 2-amino-4(or 5)-ethoxyacetanilides with carbon disulfide and subsequent alkylation of the resulting benzimidazoline-2-thiones with iodomethane or acetylation of 5(6)-ethoxy-2-methylthiobenzimidazole. The use of the latter approach involves the regioselectivity of the acetylation of 5(6)-monosubstituted benzimidazoles, since they may exist in two tautomeric forms (Scheme 1).

Scheme 1



We have found no data in the literature on the acylation of 5(6)-alkoxy-2-alkylthiobenzimidazoles. Only in [3] was the acylation of 5-ethoxybenzimidazoline-2-thione reported as a result of which 1-acetyl-5-ethoxybenzimidazoline-2-thione was formed. In the same study an attempt was made to obtain this compound by cyclization of 2-amino-4-ethoxyacetanilide with carbon disulfide but only deacylated product, viz. 5-ethoxybenzimidazoline-2-thione, was obtained. Other authors [4] have obtained 5-substituted 1-acetylbenzimidazoline-2-thiones by cyclization with carbon disulfide in dimethylformamide at room temperature.

There are also some data on the synthesis of acyl derivatives close in structure such as thiazolobenzimidazoles. It was shown in [5] that cyclization of 5(6)-methoxybenzimidazole-2-thioacetic acid in a mixture (3:1) of pyridine and acetic anhydride leads to the formation of 6-methoxythiazolo[3,2-a]benzimidazol-3(2H)-one as cyclization product. Later, other

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TABLE 1. Conditions for Acetylating 5(6)-Ethoxy-2-methylthiobenzimidazole (II) and Ratios of the Isomers (III) and (IV) Obtained

Method	Ratio of isomers	
	III	IV
A (acetic acid with dicyclohexyl carbodiimide)	1,2	1
B (acetic anhydride with pyridine in dioxan)	1,3	1
C (acetic anhydride in acetic acid at room temperature)	3,3	1
D (acetic anhydride in boiling acetic acid)	1,5	1
E (acetyl chloride in the presence of triethylamine with cooling)	1	1,2
F (acetyl chloride in the presence of triethylamine at room temperature)	1	1,5
G (acetyl chloride in boiling pyridine)	1	1,8

TABLE 2. Effect of Compounds (III), (IV), and (XIII) on the Strength of Contractions of Guinea Pig Atria (as a percentage of the initial level taken as 100)

Compound	Compound concentration, M		
	$1 \cdot 10^{-5}$	$1 \cdot 10^{-4}$	$5 \cdot 10^{-4}$
Control *	$92,2 \pm 4,5^{a}$	$96,4 \pm 3,5^{b}$	$100,7 \pm 3,5^{c}$
III	$114,9 \pm 1,1$	$130,6 \pm 9,8$	$124,5 \pm 13,4$
IV	$134,0 \pm 6,0$	$150,3 \pm 10,7$	$110,4 \pm 8,6$
XIII	$100,2 \pm 7,0$	$111,6 \pm 11,2$	$128,0 \pm 15,0$
Milrinone	$132,2 \pm 4,6$	$156,8 \pm 10,2$	$162,4 \pm 11,7$

*Physiological saline containing the appropriate quantity of dimethylacetamide [concentration: a) $5 \cdot 10^{-4}$ M; b) $5 \cdot 10^{-3}$ M; c) $5 \cdot 10^{-2}$ M].

authors reported [6] that cyclization of 5(6)-substituted benzimidazole-2-thioacetic acids by heating at 200°C in Dowtherm or by reaction in a mixture of pyridine and acetic anhydride leads to the formation of a mixture of 6- and 7-substituted thiazolo[3,2-*a*]benzimidazol-3(2H)-ones and the result of the reaction depended little on the nature of the substituent at position 5(6).

Since the results of the acylation of unsymmetrical benzimidazoles are ambiguous and in certain cases contradictory we decided to study the regioselectivity of the acetylation of 5(6)-ethoxy-2-methylthiobenzimidazole (II). Compound (II) was obtained by the S-methylation of 5-ethoxybenzimidazoline-2-thione (I) with iodomethane.

It is known that various acylating agents react by various mechanisms and acylate pyrrole or pyridine nitrogen atoms selectively [7, 8]. In addition, according to [9] the tautomers with protons on the nitrogen atom subject to the minimal influence of an electron-donating substituent and maximal influence of an electron acceptor must be energetically more stable (Scheme 1). Various reagents and conditions were therefore used in the reaction. The mixture of isomeric acetylation products (III) and (IV) was analyzed by PMR spectroscopy since we failed to obtain satisfactory results using GLC. The ratio of isomers (III) and (IV) was calculated from the integrated intensity of the signals of the aromatic protons and the acetyl group protons.

As is evident from the data (Table 1) the use of acetic anhydride as acetylating agent leads to the formation of a large quantity of 1-acetyl-5-ethoxy-2-methylthiobenzimidazole (III). Little regioselectivity was observed for the reaction at room temperature, the ratio of (III):(IV) was 3.3:1. However the 6-ethoxy isomer (IV) predominated in the product mixture on acetylating compound (II) with acetyl chloride. This may be explained in the following way.

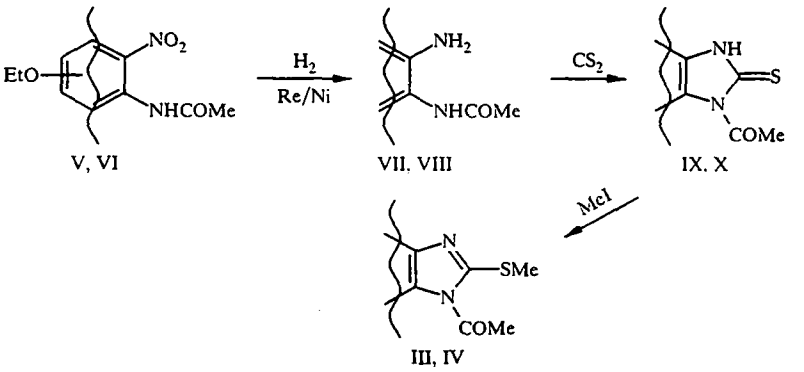
As acetylation with acetic anhydride proceeds at the pyridine nitrogen atom by an S_E2' mechanism [7] and tautomer (IIb) predominates in the reaction mixture, then a larger quantity of 5-ethoxy isomer (III) is formed on acetylation. Acetylation with acetyl chloride occurs preferably at the pyrrole nitrogen atom according to a S_E2cB mechanism [8]. Since the electrical charge on the anion of the 6-ethoxy tautomer (IIb) is less subject to the influence of the electron-donating group then it also predominates in the reaction mixture, which leads to the formation of a larger amount of the 6-ethoxy isomer (IV).

When acetylating with acetic anhydride the regioselectivity increases on reducing the temperature probably due to the slowing of tautomer inversion. However acetylation with acetyl chloride is more selective at higher temperatures which is probably linked with acceleration of the reaction forming the anion.

Since the ratio of isomers is displaced significantly in the direction of isomer (III) in the synthesis with acetic anhydride in acetic acid at room temperature, we succeeded in isolating pure isomer (III) by repeated crystallization from acetone.

An alternative route was developed for the synthesis of individual isomers (III) and (IV) which were needed in the pure state to study their cardiotonic activity and to interpret the PMR spectra of mixtures.

Scheme 2



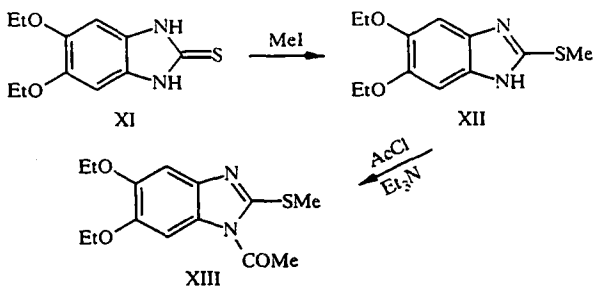
V, VI — VII, VIII — IX, X — III, IV

V, VII = 4-OEt; III, VI, VIII, IX = 5-OEt; IV, X = 6-OEt

Compounds (III) and (IV) were obtained by the reduction of the corresponding 4- and 5-ethoxy-2-nitroacetanilides (V) and (VI) with hydrogen in the presence of Raney nickel to the 2-aminoacetanilides (VII) and (VIII). These were then cyclized with carbon disulfide in dimethylformamide at room temperature and the resulting 1-acetylbenzimidazolin-2-thiones (IX) and (X) were S-methylated with iodomethane.

We also synthesized 1-acetyl-5,6-diethoxy-2-methylthiobenzimidazole (XIII) from 5,6-diethoxybenzimidazoline-2-thione (XI) by S-methylation with iodomethane and subsequent acetylation of the resulting 2-methylthiobenzimidazole (XII) with acetyl chloride in the presence of triethylamine to establish the link between chemical structure and cardiotonic activity.

Scheme 3



XI — XII — XIII

An absorption band for a carbonyl bond was observed in the IR spectra of the isomeric compounds (III) and (IV) at 1740 cm^{-1}. In the PMR spectra the acetyl singlet of the 5-ethoxy isomer (III) was located at slightly higher field (2.78 ppm) compared with the 6-ethoxy isomer (IV) (2.80 ppm). Signals of the ethoxy groups in the PMR spectra coincided completely but the disposition of the aromatic proton signals differed very markedly. The signals of the aromatic protons were arranged in the sequence $H_{(6)}$, $H_{(4)}$, and $H_{(7)}$ towards lower field for isomer (III) and $H_{(5)}$, $H_{(7)}$, and $H_{(4)}$ for isomer (IV). It is interesting that in thiones (IX) and (X) the aromatic proton signals were arranged in a different sequence than in their methylthio derivatives (III) and (IV). The aromatic proton signals were arranged $H_{(4)}$, $H_{(6)}$, and $H_{(7)}$ in the direction of lower field for the 5-ethoxy isomer (IX) and $H_{(5)}$, $H_{(4)}$, and $H_{(7)}$ for the 6-ethoxy isomer (X). The singlets of the acetyl group protons in compounds (IX) and (X) were located very close to one another at 3.02 and 3.03 ppm respectively, but in the IR

TABLE 3. Effect of Compounds (III), (IV), and (XIII) on the Strength of Contractions of the Papillary Muscle of Guinea Pig Heart (as a percentage of the initial level taken as 100)

Compound	Compound concentration, M		
	$1 \cdot 10^{-5}$	$1 \cdot 10^{-4}$	$5 \cdot 10^{-4}$
Control *	100,0 ± 0,2[a]	102,0 ± 2,0[b]	101,3 ± 1,4[c]
III	116,2 ± 6,7	116,7 ± 7,8	89,3 ± 3,9
IV	133,7 ± 2,7	160,5 ± 6,9	84,9 ± 15,6
XIII	149,0 ± 22,5	181,0 ± 19,8	239,0 ± 36,9
Milrinone	141,2 ± 14,2	181,7 ± 15,6	260,6 ± 19,6

*Physiological saline containing the appropriate quantity of dimethylacetamide [concentration: a) $5 \cdot 10^{-4}$ M; b) $5 \cdot 10^{-3}$ M; c) $5 \cdot 10^{-2}$ M].

spectra the absorption bands of the carbonyl group differed markedly at 1680 for (IX) and 1716 cm^{-1} for (X). The absorption band of the carbonyl group for compound (XIII) was at 1720 cm^{-1}.

The cardiotonic activity of compounds (III), (IV), and (XIII) was investigated in the present study.

It was established that compound (III) had almost no effect on the strength of contractions of the atrium or the papillary muscle of guinea pig heart. Compounds (IV) and (XIII) displayed positive inotropic activity but the 1-acetyl derivative of 5,6-diethoxybenzimidazole (XIII) was more active than the 6-ethoxybenzimidazole derivative (IV). Probably both alkoxy groups are necessary for the display of a large positive inotropic effect which indicates the promising nature of continuing the search for new cardiotonic agents in the area of 1-acyl derivatives of 5,6-dialkoxybenzimidazoles.

EXPERIMENTAL

The IR spectra were drawn on a Specord M 80 instrument in Nujol. The PMR spectra were obtained on a Tesla BS 587 A instrument (80 MHz), internal standard was TMS. A check on the progress of reactions and the purity of compounds was carried out by TLC on Silufol UV 254 plates, visualizing with UV light and iodine vapor.

5(6)-Ethoxy-2-methylthiobenzimidazole (II). A solution of iodomethane (1.56 g, 90 mmole) in methanol (10 ml) was added at room temperature with stirring to a solution of 5-ethoxybenzimidazoline-2-thione (I) [3] (16.5 g, 90 mmole) and sodium hydroxide (4.6 g, 115 mmole) in water (100 ml). The mixture was brought carefully to boiling and boiled for 1 h, then cooled. The precipitate which separated was filtered off, recrystallized, and compound (II) was obtained (Tables 3 and 4).

Acetylation of 5(6)-Ethoxy-2-methylthiobenzimidazole (II). A. A mixture of benzimidazole (II) (0.5 g, 2.4 mmole), dicyclohexylcarbodiimide (1.2 g, 5.8 mmole), and acetic acid (0.28 ml, 4.7 mmole) in anhydrous chloroform (40 ml) was kept at room temperature for 2 h. The mixture was then boiled under reflux for 5 h, water (2 ml) added, and the mixture boiled 0.5 h further. After cooling, the precipitate of dicyclohexylurea was filtered off, the filtrate was washed with dilute (1:1) hydrochloric acid, with aqueous sodium bicarbonate solution, and dried over sodium sulfate. A mixture (0.3 g, 50%) of isomers (III) and (IV) was obtained after evaporation of the solvent (Table 1).

B. Benzimidazole (II) (1 g, 4.8 mmole) was dissolved with heating in a mixture of dioxan (20 ml) and pyridine (7 ml). After cooling to 13°C, acetic anhydride (2 ml, 21 mmole) was added in portions. The mixture was kept for 48 h at room temperature, then poured into water (100 ml), the crystals were filtered off, and washed with water. A mixture (0.75 g, 63%) of isomers (III) and (IV) was obtained (Table 1).

C. A mixture of benzimidazole (II) (0.5 g, 2.4 mmole), acetic acid (5 ml), and acetic anhydride (2.5 ml) was kept at room temperature for 72 h. The resulting crystals were filtered off and washed with water. A mixture (0.55 g, 92%) of isomers (III) and (IV) was obtained (Table 1).

D. A mixture of benzimidazole (II) (1 g, 4.8 mmole), glacial acetic acid (10 ml), and acetic anhydride (5 ml) was boiled under reflux for 1.5 h. The solvents were distilled off in vacuum and the residue washed with water. A mixture (0.6 g, 50%) of isomers (III) and (IV) was obtained (Table 1).

TABLE 4. Characteristics of Compounds (II)-(IV), (VIII)-(X), (XII), and (XIII)

Compound	Empirical formula	Found, % / Calculated, %			mp, °C (solvent)	IR spectrum, ν, cm^{-1}	PMR spectrum, δ, ppm	Yield, %
		C	H	N				
II	$C_{12}H_{14}N_2O$	58,05 / 57,66	6,01 / 5,81	14,85 / 13,45	136...137 (EtOAc)	3200...2600 (NH)	1.37 (3H, t, J = 6 Hz, CH_3); 2.70 (3H, s, SCH_3); 4.01 2H, q, J = 6 Hz, CH_2); 6.88 (1H, d.d, J = 8 and 2.2 Hz, ArH); 6.96 (1H, d, J = 2.2 Hz, ArH); 7.32 (1H, d, J = 8 Hz, ArH)	66
III	$C_{12}H_{14}N_2O$	57,85 / 57,58	5,80 / 5,64	11,28 / 11,19	163...164 (acetone)	1740 (CO)	1.37 (3H, t, J = 7 Hz, CH_3); 2.61 (3H, s, SCH_3); 2.78 (3H, s, $COCH_3$); 4.08 (2H, q, J = 7 Hz, CH_2); 6.84 (1H, d.d, J = 8 and 2.5 Hz, ArH-6); 7.08 (1H, d, J = 2.5 Hz, ArH-4); 7.66 (1H, d, J = 9 Hz, ArH-7)	95
IV	$C_{12}H_{14}N_2O_2$	57,60 / 57,58	5,82 / 5,64	11,26 / 11,19	118...119 (acetone)	1740 (CO)	1.38 (3H, t, J = 7 Hz, CH_3); 2.61 (3H, s, SCH_3); 2.80 (3H, s, $COCH_3$); 4.08 (2H, q, J = 7 Hz, CH_2); 6.91 (1H, d.d, J = 9 and 2.3 Hz, ArH-5); 7.31 (1H, d, J = 2.3 Hz, ArH-7); 7.44 (1H, d, J = 9 Hz, ArH-4)	66
VIII	$C_{10}H_{14}N_2O_2$	61,50 / 61,83	7,38 / 7,26	14,23 / 14,43	119...120,5 (EtOAc)	3448 (NH), 3344 (NH), 3232 (NH), 1648 (CO)	1.32 (3H, t, J = 7 Hz, CH_3); 2.14 (3H, s, $COCH_3$); 2.53 (1H, s, NH); 3.93 (2H, q, J = 7 Hz, CH_2); 6.50-7.00 (3H, m, ArH)	38
IX	$C_{11}H_{12}N_2O_2$	56,03 / 55,91	5,20 / 5,12	12,00 / 11,86	194...195,5 (EtOAc)	3400 (NH), 3312 (NH), 1680 (CO)	1.35 (3H, t, J = 7 Hz, CH_3); 3.02 (3H, s, $COCH_3$); 4.07 (2H, q, J = Hz, CH_2); 6.77 (1H, d, J = 2.5 Hz, ArH-4); 6.83 (1H, d.d, J = 9 and 2.5 Hz, ArH-6); 7.99 (1H, d, J = 8 Hz, ArH-7)	31
X	$C_{11}H_{12}N_2O_2$	56,10 / 55,91	5,20 / 5,12	11,92 / 11,86	160...162,5 (EtOAc)	3392 (NH), 3288 (NH), 1716 (CO)	1.36 (3H, t, J = 7 Hz, CH_3); 3.03 (3H, s, $COCH_3$); 4.03 (2H, q, J = 7 Hz, CH_2); 6.88 (1H, d.d, J = 8 and 2.5 Hz, ArH-5); 7.13 (1H, d, J = 8 Hz, ArH-4); 7.68 (1H, J = 2.5 Hz, ArH-7)	47
XII	$C_{12}H_{16}N_2O_2$	57,30 / 57,12	6,12 / 6,39	11,10 / 11,10	151...153 (EtOAc)	3200...2600 (NH)	1.32 (6H, t, J = 5 Hz, CH_3); 2.63 (3H, s, SCH_3); 4.00 (4H, q, J = 5 Hz, CH_2); 6.98 (2H, s, ArH)	83

E. A solution of acetyl chloride (0.3 ml, 3.8 mmole) in chloroform (5 ml) was added dropwise with stirring to a cooled (−14°C) solution of benzimidazole (II) (0.5 g, 2.4 mmole) in anhydrous chloroform (35 ml) and triethylamine (0.6 ml, 4.3 mmole) checking that the temperature did not rise above −11°C. The reaction mixture was kept at −18°C for 24 h. The solvent was the distilled off in vacuum and the residue washed with water. A mixture (0.5 g, 83%) of isomers (III) and (IV) was obtained (Table 1).

F. A solution of acetyl chloride (0.6 ml, 7.6 mmole) in chloroform (10 ml) was added dropwise with stirring at room temperature to a solution of benzimidazole (II) (1 g, 4.8 mmole) and triethylamine (1.2 ml, 8.6 mmole) in anhydrous chloroform (50 ml). The mixture was stirred for 1 h, the solvent distilled off in vacuum, and the residue washed with water. A mixture (1.1 g, 92%) of isomers (III) and (IV) was obtained (Table 1).

G. Acetyl chloride (0.6 ml, 7.6 mmole) was added to a hot (105°C) solution of benzimidazole (II) (0.5 g, 2.4 mmole) in pyridine (5 ml). After a minute the reaction mixture was poured into cold water (100 ml), the resulting crystals were filtered off, and washed with water. A mixture (0.4 g, 67%) of isomers (III) and (IV) was obtained (Table 1).

1-Acetyl-5-ethoxy-2-methylthiobenzimidazole (III). A mixture of thione (IX) (0.1 g, 0.42 mmole), K_2CO_3 (0.1 g, 0.72 mmole), and iodomethane (0.08 ml, 0.12 mmole) in acetone (4 ml) was kept at room temperature for 48 h, then heated at 40°C for 1 h, and cooled. The inorganic solid was filtered off and washed with acetone. The filtrate was concentrated to dryness in vacuum, the solid washed with water, recrystallized, and compound (III) obtained (Tables 3 and 4).

1-Acetyl-6-ethoxy-2-methylthiobenzimidazole (IV) was obtained from thione (X) by the procedure for compound (III) (Tables 3 and 4).

2-Amino-4-ethoxyacetanilide (VII). Hydrogen was passed into a mixture of 4-ethoxy-2-nitroacetanilide (V) (4 g, 20.6 mmole) and freshly prepared Raney nickel (3 g) in methanol (100 ml) at room temperature and atmospheric pressure until the solution was colorless. The catalyst was filtered off, the solvent distilled off in vacuum, the residue was recrystallized, and compound (VII) (Tables 3 and 4) was obtained.

2-Amino-5-ethoxyacetanilide (VIII) was obtained from 5-ethoxy-2-nitroacetanilide (VI) by the procedure for the synthesis of compound (VII) (Tables 3 and 4).

1-Acetyl-5-ethoxybenzimidazoline-2-thione (IX). A solution of acetanilide (VII) (0.8 g, 4.1 mmole) and CS_2 (4 ml) in dimethylformamide (6 ml) was stirred at room temperature for 72 h and the reaction mixture then poured into water (300 ml). The solid was filtered off, washed with water, recrystallized, and compound (IX) obtained (Tables 3 and 4).

1-Acetyl-6-ethoxybenzimidazoline-2-thione (X) was obtained from acetanilide (VIII) by the procedure for synthesizing compound (IX) (Tables 3 and 4).

5,6-Diethoxy-2-methylthiobenzimidazole (XII) was obtained from the 5,6-substituted benzimidazoline-2-thione (XI) [10] by the procedure for synthesizing compound (II) (Tables 3 and 4).

1-Acetyl-5,6-diethoxy-2-methylthiobenzimidazole (XIII) was obtained by acetylation of compound (XII) by method F for the acetylation of the monoethoxy analog (II) (Tables 3 and 4).

Assessment of the inotropic activity of compounds was carried out on electrostimulated atria and papillary muscle of guinea pig heart at 36-37°C and a stimulation frequency of 1.0 Hz. The solution for perfusion had the following composition (mM): NaCl 114, KCl 4, $CaCl_2$ 1.8, tris-Cl 10, $MgCl_2$ 1, glucose 5, pH 7.3-7.4. Oxygenation was carried out with pure oxygen. The compounds being investigated were first dissolved in dimethylformamide (0.3 ml). This solvent does not affect inotropic function in the concentrations $5 \cdot 10^{-4}$ to $3 \cdot 10^{-2}$ M used. The dimethylformamide solution was then diluted with physiological saline to the concentration required and the solution introduced after a 60 min control period. Five experiments were performed at each concentration. The effectiveness of the test compounds was compared with the effectiveness of milrinone [11] (Table 2).

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