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A search for cardiotropic biologically active substances among new derivatives of R- phenylimin-1,3-thiazole

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ABSTRACT

The purpose of our work was the search for safe and effective biologically active substances of cardiotropic action among 1,3- thiazole derivatives. New derivatives of 1-[(2Z)-2-[R-imin]-4-methyl-3-R^1-2,3-dihydro-1,3-thiazole-5-yl]ethan-1-one and ethyl-(2Z)-2-[R-imin]-4-methyl-3-R^1-2,3-dihydro-1,3-thiazole-5-carboxylate were synthesized by the Hantzsch reaction. The structure of the compounds obtained was confirmed by ^{1}H NMR spectroscopy and by the elemental analysis. The pharmacological screening showed that the obtained substances possess cardiotropic activity. The cardiotropic properties of the new 1,3-thiazole derivatives were studied on the isolated rings of the thoracic aorta of laboratory rats. A prospective substance 1-[(2Z)-2-[(4-methoxyphenyl)imin]-4-methyl-3-(4-methylpiperazin-1-yl) -2,3-dihydro-1,3-thiazol-5-yl]ethan-1-one hydrochloride, which exhibits cardiotropic activity exceeding the activity of L-carnitine and meldonia has been revealed during pharmacological researches of the substances obtained.

Key words: synthesis, Hantzsch reaction, 1,3-thiazole derivatives, cardiotropic activity.

INTRODUCTION

In the modern world, cardiovascular diseases are one of the main causes of mortality among the population. The purpose of our work was the search for safe and effective biologically active substances of cardiotropic action among 1,3- thiazole derivatives. Scientists who have been working in this direction have received great

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results [1,2]. But despite a large number of publications, there is practically no information in the literature on the physical, chemical and biological properties of compounds that have piperazine, morpholine and 1,3-thiazole cycles together in their structure. Since in modern medicine there are some examples of the successful use of medications based on these heterocycles, we considered it expedient to combine these known pharmacophores in one molecule [3, 4, 5].

The presence of several heterocycles in the structure of the planned compounds can lead either to synergism of the biological effect or to the appearance of new pharmacological effects In addition, in order to determine the structural and biological patterns, it was planned to introduce thiazole ring radicals with different alkyl chain lengths into the 3rd position between the nitrogen of the 1,3-thiazole, morpholine and piperazine cycles.

MATERIALS AND METHODS

In the course of the work, the required reagents were obtained and purified by standard techniques. 3-chlorpentane-2,4-dione, ethyl- 2-chlor-3-oxobutanoate, ethyl 2-bromo-3-oxobutanoate, piperazine ethylamine, 4-methylpiperazinamine, 3,4-dimethoxyphenylethylamine and morpholinethylamine were purchased from Acros Organics and used without further purification. R-phenyl isothiocyanates were synthesized by aromatic amines treatment using tetramethylthiuram disulfide followed by destruction of transition product of $N_{(1)}$-aryl-N,N-dimethylthiourea under the action of concentrated HCl [6]. The elemental analysis of the nitrogen content was performed using the Duma's method. ^{1}H NMR spectra were recorded on a Varian Gemini 400 MHz spectrometer; tetramethylsilane (TMS) was used as an internal standard.

The studies were performed on mature female rats weighing 267 ± 11.2 g, which were on the standard PC "Biomodel Service" diet at a free access to food and water. All experimental studies were carried out accordingly to the European Convention on Animals Protection (1986), the "Regulations on the Use of Animals in Biomedical Research" (1989).

Before the experiment starting, the animals were set apart without access to food and water for an hour to standardize the stress state in experimental studies. Then, the

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animals were weighed and euthanized by decapitation under light ether anesthesia accordingly to the European Convention on Animals Protection.

The cardiotropic properties of the new 1,3-thiazole derivatives were studied on the isolated rings of the thoracic aorta of laboratory rats. The target organ was removed immediately from the dead animal and was placed in Krebs solution of the following composition (in mmol / l): NaCl-132; KCl-4.7; $NaH_2PO_4 \cdot 2H_2O$-1.4; $NaHCO_3$ – 16.3; $CaCl_2$-2.5; $MgCl_2 \bullet 2H_2O$-1.05; glucose-6.5. Aeration of the solution was carried out with carbogen (gas mixture 5% CO_2 / 95% O_2) [7].

Further, the biological material was placed on a paraffinic operating table in the Krebs solution at a room temperature. After fixation with hooks, the biological material was cleaned from fat and connective tissues. The smooth muscle preparations purified were cut into rings with width of 1 mm at an angle of 45 °. The isolated and purified isolated rings of the thoracic aorta of rats were secured in a flow chamber (myographic unit) on two steel hooks with a previous load of 1.5 g. A 0.5 ml chamber was perfused with Krebs solution of the following composition in mmol/l: NaCl - 132; KCl - 4.7; $NaH_2PO_4 \bullet 2H_2O$ - 1.4; $NaHCO_3$ - 16.3; $CaCl_2$ - 2.5; $MgCl_2 \bullet 2H_2O$ - 1.05; glucose - 6.5, at a rate of 1.5 ml/min at a stable temperature of about 37 ± 0.5 °C. The initial tonic contraction of the isolated rings of the thoracic aorta of rats was caused by the hyper-potassium solution (KCl - 60 mmol/l). The test compounds were dissolved in dimethylsulphoxide followed by diluting in Krebs solution at the examined concentration of 100 μmol/l. The force of contractile response was measured in isometric mode using capacitive strain gauges (FTK-0.1; “Miosensor” Ltd). The contractions were recorded on a personal computer using DataTrax2 software by means of a Lab-Trax-4/16 analog-to-digital converter (World Precision Instruments). After stabilizing the isolated aortic rings for periodic stimulation using Krebs hyper-potassium solution (KCl - 60 mmol/l) for 50 min (2 times - 10 min stimulation using the hyper-potassium solution followed by washing with Krebs solution for 15 min), the test compounds were applied at the given concentration for 20 minutes. Further, a model of hypoxia was simulated by aerating Krebs solution with nitrogen for 40 min. The experiment was completed by monitoring the contractile activity of the isolated rings of

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the thoracic aorta, by treating them with Krebs solution with phenylephrine (10^{-6} mol/l) for 10 to 15 minutes to achieve the constriction plateau, following which Krebs solution was perfused, and the level of relaxation was observed. The mechanogram recorded whether the isolated vascular tone changed in under application of the test compounds, the normalized maximum rate of the contraction phase (Vnc) was calculated for hypoxia, the presence of any contraction in case of phenylephrine, and the level of relaxation a were analyzed t the end of the experiment [7, 8, 9, 10], were chosen as reference products.

RESULTS AND DISCUSSIONS

As initial substances to synthesize new derivatives of 1-[(2Z)-2-[R-imin]-4-methyl-3-R^1-2,3-dihydro-1,3-thiazol-5-yl-ethan-1-one and ethyl-[(2Z)-2-[R-imin]-4-methyl-3-R^1-2,3-dihydro-1,3-thiazole-5-carboxylate the asymmetrical thioureas (**3a-d**) were used, which were synthesized by reaction between R-phenyl isothiocyanates and arylamines substituted by piperazine amines and morpholinamines in equimolar amounts in a dry dioxane medium [11, 12, 13]. The hydrochlorides 1-[(2Z)-2-[R-imin]-4-methyl-3-R1-2,3-dihydro-1,3-thiazol-5-yl] ethan-1-ones (**4a-c**) were obtained by unsymmetrical thioureas **(3a-c)** boiling in ethanol in equimolar amounts with 3-chlorpentane-2,4-dione (Hantzsch reaction). Ethyl-[(2Z)-2-[R-imin]-4-methyl-3-R^1-2,3-dihydro-1,3-thiazole-5-carboxylate hydrochloride and hydrobromide **(5a and 7d)** were obtained under similar conditions by action of ethyl 2-chlor-3-oxobutanoate and 2-bromo-3-oxobutanoate on the corresponding thioureas **3a and 3d**, respectively. Compounds **6a** and **8c** were isolated as bases by neutralizing the corresponding hydrochlorides with a 10% solution of NH_4OH. (Scheme 1).

The synthesized compounds (**4a-c, 5a, 7d**) are white crystalline substances highly soluble in water and most organic solvents such as dimethylformamide, acetone, propanol, isopropanol. They are poorly soluble in ethyl acetate, ethanol, methanol.

Synthesized compounds **6a, 8c** are white crystalline substances soluble in methanol, ethanol, propanol-2, insoluble in water.

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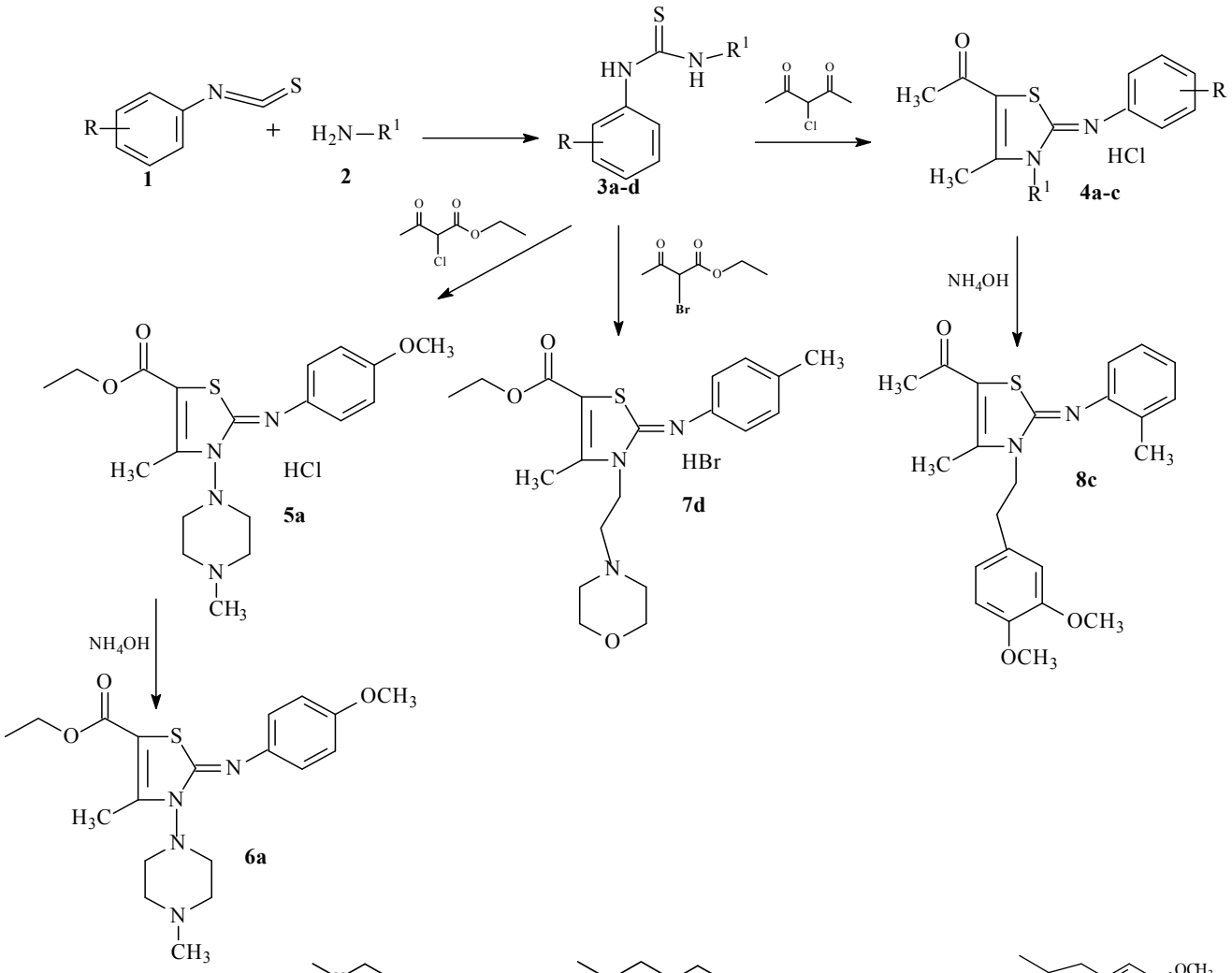
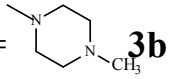
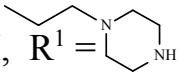
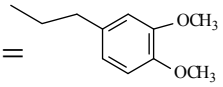
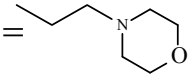
Scheme 1



3a R = 4-OCH_3, R^1 =  **3b** R = H, R^1 =  **3c** R = 2-CH_3, R^1 = 

3d R = 4-CH_3, R^1 = 

The structure of the synthesized compounds (**4a-b, 6a, 7d, 8c**) was confirmed by elemental analysis and by ^{1}H NMR spectroscopy.

Pharmacological screening has shown that the obtained substances possess cardiotropic activity. The indicator of cardiotropic activity was the normalized maximum rate of the contraction phase (Vnc).

The essence of the method is to standardize the analysis of the mechanogram "contraction-relaxation" of smooth muscle organs, which does not depend on the size of the isolated smooth muscle preparation used in the experiment [7].

During analysis of mechanokinetic curves, linearization of contraction phase was done in the coordinates {ln [(fm-f) / f]; ln t}, where f - instantaneous force (at

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time t), f_m - maximum force, t - characteristic time, and n - logarithmic coefficient of slope of the mechanokinetic curve (Figure 1.)

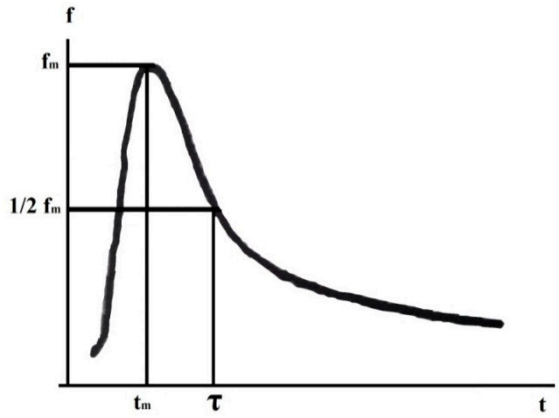

Figure 1. Graphical representation of the course ofthe smooth muscles contraction and relaxation phase.

This method allows to calculate indicators independent to the maximum force – standardized value of the maximum contraction-rate Vn:

$$V_n = \pm\left(\frac{1}{f_m}\right)\left(\frac{df}{dt}\right) = \left|\frac{(n-1)^{\frac{n-1}{n}}\cdot(n+1)^{\frac{n+1}{n}}}{4n\tau}\right| \quad (1)$$

The effect of compounds examined on the contraction of isolated aortic thoracic rings for hypoxia were analyzed using the calculated normalized maximum rate of the contraction phase (Vnc - from the start of the muscle preparation strain increase to the maximum) in accordance with Formula 1.

While *in vitro* experiments, it was established that when the test compounds have been applied to isolated rings of the thoracic aorta of laboratory rats they have not caused constrictor responses of the latter, and for the two compounds, the decrease in vascular tone have been inherent. Such results confirm that there is no possible effect on hemodynamic parameters of the cardiovascular system *in vivo* studies, namely, systolic and diastolic arterial pressure, which is one of the key requirements at a cardioprotective drug development.

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For cardiotropic drugs, the ability to influence energy processes in cardiomyocytes is of high importance. This directly depends on the rate of pathophysiologic myocardium damages development. In our experiment, we modeled the pathological state of hypoxia, in order to analyze the effect of the test compounds on the rate of hypoxic contraction development of the rings of the thoracic aorta of rats.

When carrying out the pharmacological studies of synthesized substances, a prospective substance 1-[(2Z)-2-[(4-methoxyphenyl)imin]-4-methyl-3-(4-methylpiperazine-1-yl)-2,3-dihydro-1,3-thiazole-5-yl]ethan-1-one hydrochloride **4a** was detected. Compound **4a** has delayed the development of constrictor responses of isolated rings of the thoracic aorta of rats and shows a well-pronounced cardiotropic effect and exceeds the activity of L-carnitine by 18.8% and meldonium by 12.8% (Figure 2, Table 1).

Compound **4b**, reduced the normalized maximum rate of the contraction phase for hypoxia on a par with the drugs of comparison, that from our point of view indicates the ability of this compound to realize a decrease in the energy potential of the cardiomyocyte damaged by hypoxia. Derivatives **6a, 7d, 8c** also did not decrease, but accelerated by 0.5 times the normalized maximum rate of the contraction phase for hypoxia, which indicates the energy-consuming mechanism of their action. (Figure 2, Table 1).

As can be seen from the results of pharmacological research, the cardiotropic activity of the obtained substances depends on the nature of the radicals at positions 3 and 5 of the thiazole cycle. The cardiotropic activity decreases with increase in the length of the aliphatic chain of substituents at position 3. The presence of alkyl radicals and a piperazine fragment in the structure of the 1,3-thiazole derivatives positively affects the activity index. The replacement of ethane-1-one with carboxylate at position 5 of the thiazole cycle resulted in a cardiotropic effect decrease.

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Table 1.

The normalized maximum rate of the contraction phase for hypoxia for the test compounds 4a-b, 6a, 7d, 8c

Compound	Vnc (M±m)
4a	0,02670 ± 0,00350
4b	0,03525 ± 0,00645
6a	0,09325 ± 0,01235
7d	0,08080 ± 0,00680
8c	0,08680 ± 0,01370
L-Carnitine	0,03285 ± 0,00515
Meldoney	0,03064 ± 0,00477
Negative control value	0,06243 ± 0,01928

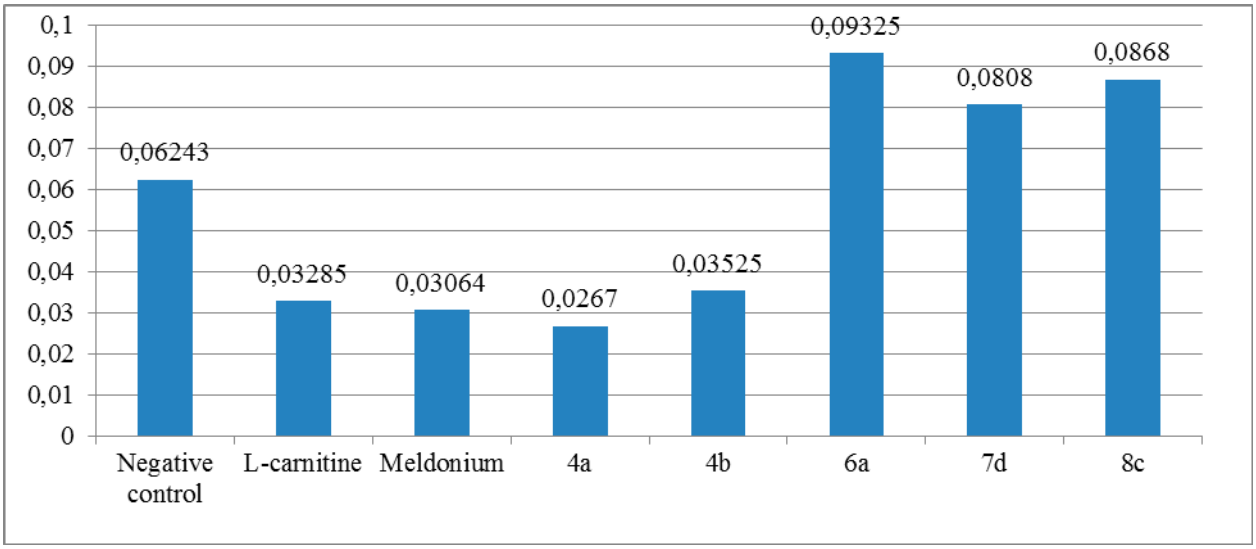

Figure 2. Diagram of changes in the normalized maximum rate of the contraction phase for hypoxia for **4a-b, 6a, 7d, 8c** derivatives.

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1-[(2Z)-2-[(4-methoxyphenyl)imin]-4-methyl-3-(4-methylpiperazine-1-yl)-2,3-dihydro-1,3-thiazole-5-yl]ethan-1-one hydrochloride 4a

To the 1.40 g of solution (0.005 mol) 1-(4-methoxyphenyl)-3-(4-methylpiperazine-1-yl)thiourea in 40 ml of ethanol a solution of 0.67 g (0.005 mol) of 3-chlorpentane-2,4-dione was added with stirring and boiled under reflux for 2 hours. After cooling, the solid that precipitated out was filtered off and crystallized from 2-propanol.

Yield: 67%, (2-propanol). ^{1}H NMR (400 MHz, TMS) δ: 2.16 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 2.82 - 2.95 (m, 4H, CH_2–CH_2 (piperazine)), 3.05 - 3.15 (m, 4H, CH_2–CH_2 (piperazine)), 7.31 - 7.56 (m, 4H, Ar-H), 11.0 (s, 1H, NH^+). Calculated for $C_{18}H_{25}ClN_4O_2S$ N 14.11%. Found, %: N 14.30

1-[(2Z)-2- (phenylimin)-4-methyl-3-[2-(piperazine-1-yl)ethyl]-2,3-dihydro-1,3-thiazole-5-yl]ethan-1-one hydrochloride 4b

Compounds 4b was obtained similarly.

Yield: 75%, (2-propanol), ^{1}H NMR (400 MHz, TMS) δ: 2.18 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 5.72 (s, 4H, CH_2–CH_2), 2.98 - 3.15 (m, 4H, CH_2–CH_2 (piperazine)), 3.37 - 3.48 (m, 4H, CH_2–CH_2 (piperazine)), 6.35 (s, 1H, NH (piperazine)), 7.35 - 7.55 (m, 5H, Ar-H), 11.01 (s, 1H, NH^+). Calculated for $C_{17}H_{25}ClN_4OS$ N 15.19 %. Found, %: N 15.54.

1 - [(2Z) -2 - [(2-methylphenyl) imin] -4-methyl-3- [2- (3,4-dimethoxyphenyl) ethyl] -2,3-dihydro-1,3-thiazole-5 -yl-ethan-1-one 8c

0.67 g (0.005 mol) of 3-chlorpentane-2,4-dione was added with stirring to a solution of 1.65 g (0.005 mol) of 1- (4-methylphenyl) -3- (3,4-dimethoxyphenylethyl-1-yl) thiourea in 40 ml of ethanol and boiled under reflux for 2 hours. After cooling, 10% ammonia solution was added. The solid that precipitated out was filtered off and crystallized from 2-propanol.

Yield: 79%, (propanol-2). ^{1}H-NMR (400 MHz, TMS) δ: 2.05 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 3.65 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.90-5.10 (m, 4H, CH_2-CH_2), 7.28-7.65 m, 7H, Ar-H). Calculated for $C_{22}H_{26}N_2O_3S$ N 6.44%.

Found: %: N 6.45.

Ethyl - [(2Z) -2- [(4-methoxyphenyl) imin] -4-methyl-3- (4-methylpiperazin-1-yl) -2,3-dihydro-1,3-thiazole-5-carboxylate 6a

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0.82 g (0.005 mol) of ethyl-2-chlor-3-oxobutanoate was added with stirring to a solution of 1.40 g (0.005 mol) of 1- (4-methoxyphenyl) -3- (4-methylpiperazin-1-yl) thiourea in 40 ml of ethanol and boiled under reflux for 2 hours. After cooling, 10% ammonia solution was added. The solid that precipitated out was filtered off and crystallized from 2-propanol.

Yield: 76%, (propanol-2). ^{1}H NMR (400 MHz, TMS) δ: 2.07 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 2.25-2.38 (m, 2H, CH_2CH_3), 1.27 (t, 3H, CH_2CH_3), 2.75-2.83 (m, 4H, CH_2-CH_2 (piperazine)), 3.05-3.23 (m, 4H, CH_2-CH_2 (piperazine)), 7.30-7.65 (m, 4H, Ar-H). Calculated for $C_{19}H_{26}N_4O_3S$ N 14.35%. Found: %: N 14.80.

Ethyl (2Z) -2- [4-methylphenyl] imin] -4-methyl-3- (morpholinethyl) -2,3-dihydro-1,3-thiazole-5-carboxylate hydrobromide 7d

1.05 g (0.005 mol) of ethyl 2-bromo-3-oxobutanoate was added with stirring to a solution of 1.39 g (0.005 mol) of 1- (4-methylphenyl) -3- (morpholinethyl) -1-yl) thiourea in 40 ml of ethanol and boiled under reflux for 2 hours. After cooling the solid that precipitated out was filtered off and crystallized from 2-propanol.

Yield: 73%, (propanol-2). ^{1}H ЯМР (400 MHz, TMS) δ: 2.07 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.48-2.75 (m, 2H, $C\underline{H_2}$ CH_3), 1.35 (t, 3H, $CH_2C\underline{H_3}$) 5.15 (s, 4H, CH_2–CH_2), 2.95-3.08 (m, 4H, CH_2–CH_2 (morpholine)), 3.30-3.42 (m, 4H, CH_2–CH_2 (morpholine)), 7.30-7.52 (m, 5H, Ar-H), 11.01 (s, 1H, NH^+). Calculated for $C_{20}H_{28}BrN_3O_3S$ N 8.93 %. Found: %: N 8.58.

CONCLUSIONS

1. A preparative method was developed for obtaining new derivatives of 1- (2Z) -2- [R-imin] -4-methyl-3-R^1-2,3-dihydro-1,3-thiazol-5-yl-ethan-1-one and ethyl- (2Z) - 2- [R-imin] -4-methyl-3-R^1-2,3-dihydro-1,3-thiazole-5-carboxylate under the Hantzsch reaction.
2. The structure of the obtained compounds was confirmed by ^{1}H NMR spectroscopy and by elemental analysis.
3. The pharmacological screening showed that the obtained substances possess high cardiotropic activity. A prospective substance 1-[(2Z)-2-[(4-methoxyphenyl)imin]-4-methyl-3-(4-methylpiperazine-1-yl)-2,3-dihydro-1,3-

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thiazole-5-yl]ethan-1-one hydrochloride **4a** was detected, it shows cardiotropic activity which exceeds the activity of L-carnitine by 18.8% and meldonium by 12.8%. The lead compound may be proposed as a potential cardiotrophic agent for in-depth pharmacological studies.

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