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Synthesis and Ca^{2+}-desensitizing Activity of 2-methyl-7-aryl(α-furyl)-[1,3]thiazolo[4,5-*d*]pyridazin-4(5*H*)-ones

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Authors' contributions

This work was carried out in collaboration between all authors. Author AS gave the idea on vascular tone regulation due to change in myofilament calcium sensitivity, simultaneous measurements of smooth muscle contractile force and intracellular calcium concentration. Author SD performed the synthesis of research objects. Author LB performed the coordination of chemical and pharmaceutical research, summarized the results. Author VP performed search of the scientific literature and sorces; introduced corrections within the title of the article. Author SZ did the simultaneous measurements of smooth muscle contractile force and intracellular calcium concentration. Author AD wrote the protocol and the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Search of new chemical compounds for pressure decrease in accordance with new mode of action.
Study Design: Novel 2-methyl-7-aryl(α-furyl)-[1,3]thiazolo[4,5-d]pyridazin-4(5*H*)-ones were synthesized in 67-84% yields via reaction of 2-methyl-5-benzoyl(furoyl)-thiazol-4-carboxylates with hydrazines.

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Place and Duration of Study: Synthesis of new compounds has been performed at National T. Shevchenko University "Chernihiv Collegium" and Institute of Pharmacology and Toxicology, National Academy of Medical Sciences. Physiological studies have been conducted at Institute of Pharmacology and Toxicology, National Academy of Medical Sciences of Ukraine.
Methodology: Design of chemical structures which relax vascular smooth muscle cells without significant decreasing in intracellular calcium concentration, [Ca2+]i.
Results: These new compounds have been successfully tested for the aim to search so-called calcium desensitizers, i.e. compounds which possess the ability to decrease Ca^{2+}-sensitivity of myofilaments and therefore, vascular smooth muscle cells (VSMc) contractility and, hence, vascular tone.
Conclusion: 2-Methyl-7-aryl(α-furyl)-[1,3]thiazolo[4,5-d]pyridazin-4(5*H*)-ones may be used in future as safety and good therapeutic approach under arterial hypertension and local vasospasm development.

Keywords: Myofilaments; arterial hypertension; vascular tone; calcium desensitizers; hydrazine.

1. INTRODUCTION

In short, contractile activity in vascular smooth muscle cells (VSMc) is initiated by a Ca^{2+}-calmodulin interaction to stimulate phosphorylation of the light chain of myosin. Ca^{2+} sensitization of the contractile proteins in VSMc is signalled by the protein kinase C/Rho kinase's pathways to inhibit the dephosphorylation of the light chain by myosin phosphatase, thereby maintaining force generation. Removal of Ca^{2+} from the cytosol and stimulation of myosin phosphatase or decrease the sensitivity of myofilaments to Ca^{2+} initiate the process of VSMc relaxation.

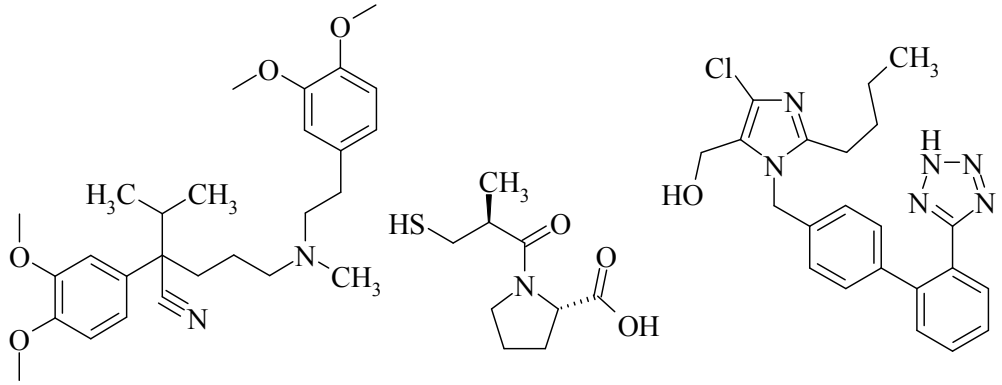
The twofold aim of the study is: (i) to design chemical structures which possess Ca^{2+}-desensitizing activity, i.e. possess the potential ability to produce VSMc relaxation due to dissociation between $[Ca^{2+}]_i$ andcontractile force development; and (ii) to get an experimental evidence for its ability to relax VSM without significant decreasing in intracellular calcium concentration, $[Ca^{2+}]_i$.

It is well known that the major types of commonly prescribed antihypertensive cardiovascular medications are as follows (**Sch. 1**): calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, beta-blockers, peripheral blood vessel dilators (vasodilators), alpha blockers,alpha-2 receptor agonists, combined alpha and beta-blockers, central agonists, peripheral adrenergic inhibitors, i.e. compounds with different mechanism of action but having the common final target for the majority of them to decrease $[Ca^{2+}]_i$ in VSMc cytoplasm.

Thus, it is well known that $[Ca^{2+}]_i$ in smooth muscle cells (SMCs) is one of the main factors determining the contractile force development. However, the data obtained allow suggesting that the contractile activity of SMCs and, hence, the vascular tone may also vary over a wide range without significant changes in $[Ca^{2+}]_i$. Thus, alterations in the myofilament Ca^{2+}-sensitivity may be very important in the regulation of muscle activity in both health and disease.

This conclusion is based on several lines of experimental evidence: at first, hypoxia shifts the "pCa-tension'' curves in chemically permeabilized SMCs to the right and decreases the maximal developed SMC force, indicating that myofilamentCa^{2+}-sensitivity and responsiveness have decreased [1]; secondly, leftward shift of the "pCa-tension" relationship as well as dissociation between contractile force and $[Ca^{2+}]$ I in SMCs obtained from spontaneously hypertensive [2,3]; and irradiated rats [4] as compared to normotensive rats clearly indicate an increased calcium sensitivity of myofilaments; and finally, the nitric oxide (NO) – induced reduction in $[Ca^{2+}]$ I was transient whilst vascular relaxant response was sustained [5].

Despite intensive research, only a few really effective antihypertensive drugs are in everyday use in clinical medicine, and all of them manifest unwanted side effects. That is why the task of discovering a new and effective types of hypertensive drugs directed to really established pharmacological targets is now becoming challenging especially given to the wide occurrence of cardiovascular diseases.



Verapamil **Captopril** **Losartan**

Sch. 1. Well known drugs that are widely used for antihypertensive therapy

For instance, that Ca^{2+} channel blockers do not completely inhibit agonist-induced VSMc contraction as well as this type of contraction could be observed even in Ca^{2+}– free solution [6].

Well known adverse effects of captopril and losartan include a cough due to increase in the plasma levels of bradykinin, angioedema, agranulocytosis, proteinuria, hyperkalemia, taste alteration, teratogenicity, postural hypotension, acute renal failure, and leukopenia. Some side effects intrinsic to losartan – abdominal or stomach pain, anxiety, bladder pain, bloody or cloudy urine, blurred vision, chills, cold sweats, etc. [7].

Interesting to note that some of the hypertensive drugs, such as peripheral vasodilators hydralazine chloride (expression) and motazipone contain pyridazin component in their molecules [8].

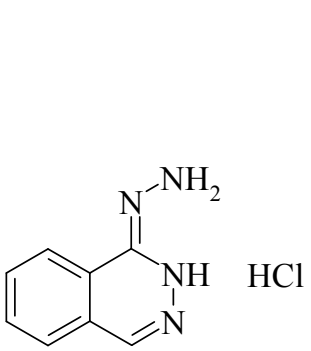
Apressin (hydralazine hydrochloride), for instance, reduces the tone of the walls of the arterioles by unclarified yet mechanism, causing the increase in peripheral circulation, reduction of high blood pressure due to an improvement of renal blood flow and decreases the load on the left ventricle of the heart. This relaxing effect is probably related to the presence in the press molecule of hydrazine group, capable of retaining the inactivation of endogenous vasodilator factors, including oxide (oxide) oxide (NO). Hydralazine requires the endothelium to provide nitric oxide, thus only causes vasodilation *in vivo* with functional endothelium. Hydralazine will not cause vasodilation *in vitro* in an isolated blood vessel. As for phosphodiesterase (PDE) inhibitor motapizone its therapeutic effect in hypertension treatment is due to a dose-dependent reduction in diastolic blood pressure and its antiplatelet action (**Sch. 2**).

That is why we decided to sensitize newly condensed pyridazines with SH fragment in a molecule.

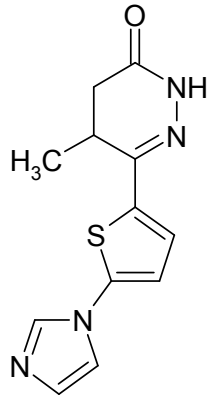
2. MATERIALS AND METHODS

2.1 Chemistry

The starting compounds for the synthesis of 2-methyl-7-aryl and (α-furoyl)-[1,3]thiazolo[4,5-d] pyridine-4(5*H*)-ones **7 a-f, 12** were methyl esters of 2,4-dioxo-4-aryl butyric acid **three a-f** (**Sch. 3**) and methyl ester 2,4-dioxo-4-(α-furyl)butyric acid **9** (**Sch. 4**). The letters were produced using well known Claisen condensation from substituted acetophenones**one a-f** and diethyl ester of oxalic acid in the presence of sodiummethoxide. Thus, compounds **3 a** and **3 c** were obtained by a known method [9] with 68% and 73% respectively. Getting of **3 b** (R1 = CH_3) are given in [10]. Synthesis of methyl ester 2,4-dioxo-4-(3^1-methoxy)butyric acid was given in [11]. Isomeric **three f** was obtained in accordance with [12] using 3-methoxyacetophenon interaction with diethyl oxalate in toluene in the presence of sodium hydride. Ester **3d** was obtained in 76% yield through the reaction of p-chloroacetophenone with dimethyl ester of oxalic acid in methanol medium with sodium methoxide added described in the article [13].

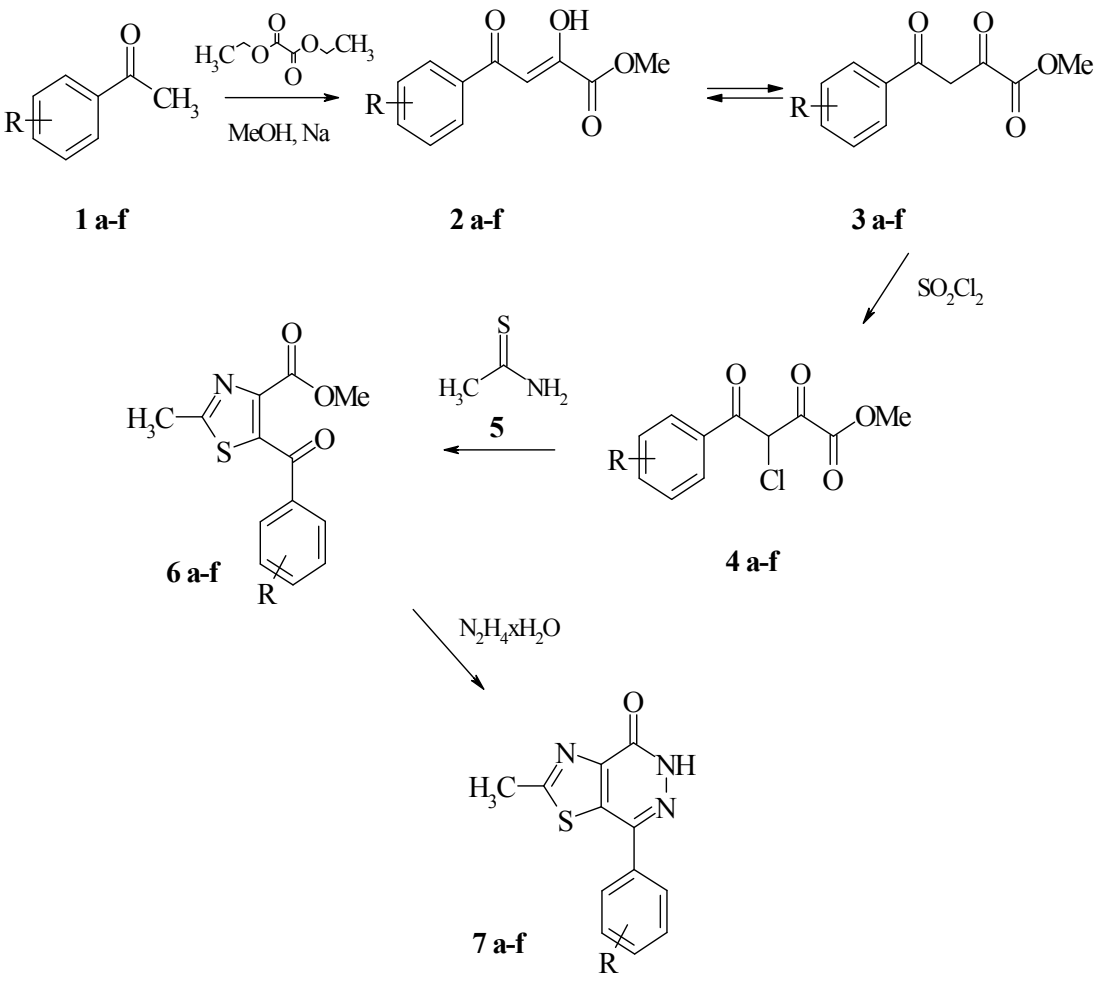


Hydralazine hydrochloride

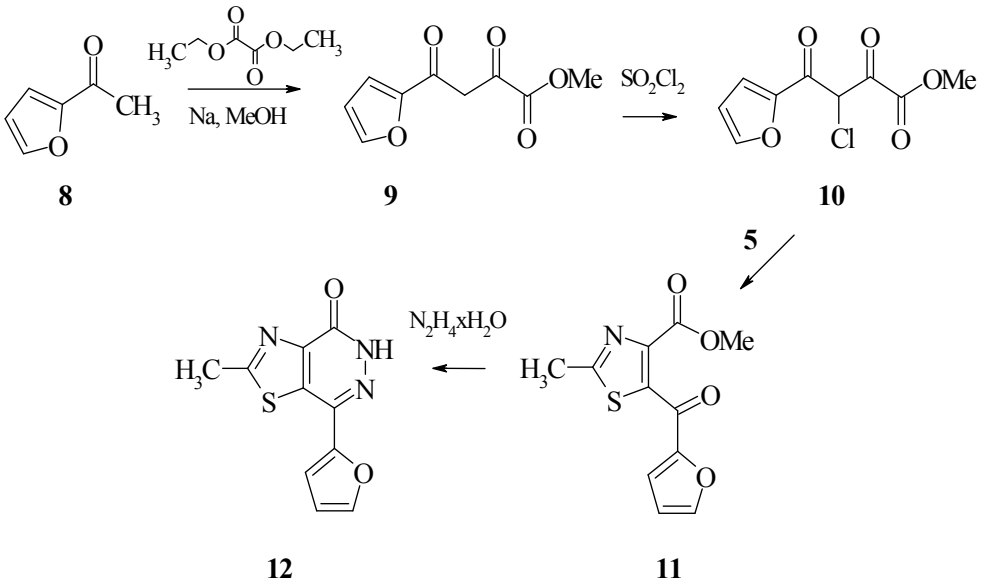


Motapizone

Sch. 2. Antihypertensive drugs which contain pyridazine fragment in



Sch. 3. Synthesis of 2-methyl-7-aryl-[1,3]thiazolo[4,5-d]pyridazin-4(5*H*)-ones 7 a-f. R = a) H, b) 4 CH_3, c) 4 F, d) 4 Cl, e) 4 OCH_3, f) 3 OCH_3.



Sch. 4. Synthesis of 2-methyl-7-(α-furyl)-[1,3]thiazolo[4,5-d]pyridazin-4(5*H*)-ones 12

Methods of obtaining methyl ester 2,4-dioxo-4-(α-furyl)-butyric acid nine were given in the patent [14].

Esther 2,4-dioxo-4-aryl(hetero)butyric acid and **3-f** were chlorinated using sulfuryl chloride in dry chloroform at boiling 1.5-2 hours before the termination of allocation gases by the general procedure given in the paper [15]. The solvent was evaporated in a vacuum jet pump, and the resulting methyl esters 3-chloro-2,4-dioxo-4-aryl(hetero)butyric acid and **4-f** were used without further purification.

Methyl esters of 5-benzoyl (α-furoyl)-2-methyl thiazol-4-carboxylic acid and **six a-f, 11** were synthesized using three hours boiling of equimolar amounts of chloresters**four a-f, 10** of thioacetamide in benzene by the method described in the [16]. After cooling the reaction, mixture benzene was evaporated, and the residue was ground with 10% aqueous sodium bicarbonate, filtered, washed with water and dried. Boiling methyl esters of 5-benzoyl-(α-furoyl)-2-metyltiazol-4-carboxylic acids 6 a-f, 11 with a double 80% hydrazine hydrate excess ethanol for 3-4 hours results in intra molecular cyclization with the formation of 2-methyl-7-aryl-(α-furyl)-*5H*-thiazolo[4,5-d]pyridazin-4-ones 7 a-f, 12. 1HNMR spectra in cyclization is accompanied by disappearance of three proton singlet of methyl carboxylate group and appearance of advanced one-proton singlet NH-group pyridazine ring of heterocyclic system in the 13.1 - 13.3 ppm. Re-registration of NMR spectra of compounds 7-f and adding drops of D_2O results in the disappearance of signals due to NH-group because of deuterium exchange. Interestingly, these signals are usually observed in the area 13,1-13,4 ppm. This indicates that the amide proton in [1,3]thiazolo[4,5-d]pyridazin-4(5*H*) cycle is sufficiently acidic.

All solvents were purified before use. Ethyl chloroformate, pyrrolidine, morpholine, piperidine, acetophenone, ethyl oxalate, 2-acetylthiophene, 2-acetylfurane were purchased from Acros Organics and use without purification. The reactions were monitored by thin-layer chromatography (TLC) using Fluka silica gel (60 F 254) plates (0.25 mm). Visualization was made with UV light. Melting points of synthesized compounds were taken on a melting point tube. ^{1}H-NMR spectra were recorded on the Varian Gemini 400 MHz (Germany) in DMSO-d6 using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm units with use of the d scale. The mass spectra were recorded on an Agilent LC/MSD SL 1100 instrument (USA).

2.2 General Procedure of Synthesis 2-methyl-7-aryl(furan-2-yl)-*5H*-thiazolo[4,5-d]pyridazin-4-ones 7 a-f, 12

A mixture of appropriate 3-chloro-2,4-dioxo-4-benzenebutanoic acid methyl ester **4 a-f** or 3-

chloro-4-furan-2-yl-2,4-dioxo-butyric acid methyl ester **10** and (0.01 mol) thioacetamide was refluxed for 4 h in 50 ml of benzene and left overnight at room temperature. Reaction mixture was diluted with 200 ml of distilled water and 10% aqueous sodium bicarbonate was added till pH 8. The obtained solid products were collected by filtration, washed with water, dry and use without additional purification and identification. 0.01 Mole of corresponding compounds **6 a-f, 11** were suspended in 100 ml of ethanol, twofold excess of 80% hydrazine hydrate was added to the mixture. The mixture was refluxed for 4 hours. The reaction mixture was cooled. The precipitate was filtered off and crystallized from methanol.

2-methyl-7-phenyl-*5H*-thiazolo[4,5-d]pyridazin-4-on (**7 a**).

Yied 72%, mp 244-245^{0}C.^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.86 (s, 3H, CH_3), 7.56 (m, 3H, Ph), 7,78 (d, 2H, J=6.6 Hz, Ph), 13.3 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d6) δ: 19.4, 126.7, 126.8, 128.9, 129.0, 135.2, 136.5, 140.7, 148.1, 155.6, 171.2. Anal.Calcd for $C_{12}H_9N_3OS$, % N, 17.3; S 13.2.Found, % N 17.4; 13.7. MS m/z: 244.1 [(M+H)+].

2-methyl-7-p-tolyl-*5H*-thiazolo[4,5-d]pyridazin-4-on (**7 b**).

Yied 71%, mp 259-261^{0}C.^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 2.86 (s, 3H, CH_3), 7.38 and 7.67 (d-d, 4H, J=7.6 Hz, C_6H_4), 13.1 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d6) δ: 19.4, 20.9, 126.6, 129.4, 132.4, 136.4, 139.4, 140.6, 148.1, 155.6, 171.1. Anal.Calcd for $C_{13}H_{11}N_3OS$, % N, 16.3; S 12.5. Found,% N 16.5; S 12.3.

2-methyl-7-(4^1-fluorophenyl)-*5H*-thiazolo[4,5-d]pyridazin-4-on (**7 c**).

Yied 68%, mp 252-254^{0}C.^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.86 (s, 3H, CH_3), 7.40 – 7.84 (m, 4H, C_6H_4), 13.3 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d6) δ: 19.4, 108.8, 115.8, 129.1, 131.7, 136.4, 139.8, 148.0, 155.6, 161.4, 163.9, 171.2. Anal.Calcd for $C_{12}H_8FN_3OS$, %N 16.3; S 12.3. Found,% N 16.1; S 12.3. 262.0 [(M+H)+].

2-methyl-7-(4^1-chlorophenyl)-*5H*-thiazolo[4,5-d]pyridazin-4-on (**7 d**)

Yied 64%, mp 238-239^{0}C.^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.86 (s, 3H, CH_3), 7.62 and 7.90 (d-d, 4H, J=8.8 Hz, C_6H_4), 13.4 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d6) δ: Anal. Calcd for $C_{12}H_8ClN_3OS$, % N 15.2; S 11.5.Found, % N 15.5; S 11.6.

2-methyl-7-(4^1-methoxyphenyl)-*5H*-thiazolo[4,5-d]pyridazin-4-on (**7 e**).

Yied 66%, mp 284-286^{0}C.^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.85 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.09 and 7.71 (d-d, 4H, J=8.6, Hz, C_6H_4), 13.2 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d6) δ: 19.4, 55.3, 114.2, 114.3, 127.6, 128.2, 136.5, 140.5, 148.0, 155.6, 160.2, 171.1. Anal.Calcd for $C_{13}H_{11}N_3O_2S$, % N 15.4; S 11.7. Found, % N 15.3; S 11.9. 274.1 [(M+H)+].

2-methyl-7-(3^1-methoxyphenyl)-*5H*-thiazolo[4,5-d]pyridazin-4-on (**7 f**).

Yied 59%, mp 223-224^{0}C.^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.86 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 7.09 - 7.47 (m, 4H, C_6H_4), 13.6 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d6) δ: 19.4, 55.5, 112.2, 115.4, 118.9, 119.0, 130.1, 136.5, 140.5, 148.0, 155,7, 159.4, 171.3. Anal.Calcd for $C_{13}H_{11}N_3O_2S$, % N 15.4; S 11.7.Found, % N 16.2; S 11.5.

2-methyl-7-(furan-2-yl)-*5H*-thiazolo[4,5-d]pyridazin-4-on (**12**).

Yied 58%, mp 298-300^{0}C.^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.86 (s, 3H, CH_3), 6.71 (m, 1H, 4^{1}H), 7.02 (d, 1H, J=3.4 Hz, 3^{1}H), 7.94 (m, 1H, 5^{1}H), 13.2 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d6) δ: 19.3, 108.8, 112.5, 132.7, 144.5, 147.9, 148.4, 155.6, 172.2. Anal.Calcd for $C_{10}H_7N_3O_2S$, % N 18.0; S 13.8.Found, % N 17.8; S 13.9. 234.0 [(M+H)+].

2.3 Animals

All animal studies were performed in accordance to the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Institutional Animal Care and Use Committees. Experiments were performed on 6-8 weeks male Wistar rats (weight 250–300 g) housed under controlled environmental conditions (21^{0}C, 12 h-12 h light-dark cycle) and free access to water and standard rodent chow.

2.4 Simultaneous Measurement of $[Ca^{2+}]_i$ and Contractile Force

Segments of thoracic aorta (1.5 cm-long) were obtained as described above, cleaned of both connective and adipose tissue, and cut into 1 to

1.5 mm width rings. All procedures were performed at room temperature in a nominally Ca^{2+}-free physiological salt solution.

Experiments for the simultaneous measurement of $[Ca^{2+}]_i$ and contractile force were carried out in a 500 µl tissue chamber mounted on the stage of a fluorescence microscope LUMAM-2 (Russian Federation) equipped with epifluorescence collection equipment. The aortic rings were mounted isometrically between a stationary stainless steel hook and a force transducer (AE 801, SensoNor A/S, Norten, Norway). Except for during the Fura-2AM loading procedure, the rings were continuously perfused with Krebs solution preheated to 35° C at a rate of 2.0 ml/min; Ca^{2+} measurements tended to be more stable at 35° as compared to 37° C. The rings were loaded with 10 µM Fura 2-AM in a physiological solution of the following composition (mM): 122 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 11.6 HEPES, 11.5 glucose, and a pH of 7.3-7.4. The loading solution also contained 2.5% DMSO and 5 mg/ml Pluronic F-127. Loading continued for 2 hours at room temperature. The tissues were then allowed to equilibrate in normal physiological salt solution for at least 30 minutes. Following the equilibration period, the tissues were exposed several times to phenylephrine (0.1 µM), high KCl (60 mM), or caffeine (20 mM) until reproducible contractile responses were obtained. High KCl solution was prepared by equimolar replacement of NaCl with KCl in order to avoid a change in osmolarity of the solution.

Fura-2 fluorescence was excited at 340 and 380 nm wavelength (λ) and recorded at 510 nm emission wavelength from a central region (approximately 0.5 mm in diameter) on the blood surface of the aortic ring. The fluorescence emitted from the tissue was collected by a photomultiplier through a 510 nm filter. The results of $[Ca^{2+}]_i$measurements are presented as the ratio (R) of the 510 nm emission fluorescence intensity $[I_{510}(\lambda)]$ at λ=340 nm and λ=380 nm excitation signals: $R = I_{510}(340)/I_{510}(380)$. At the end of the experiment, the maximum fluorescence ratios were determined. The maximum fluorescence was determined in a phosphate-free, bicarbonate-free, 120 mMKCl, five mM $CaCl_2$, salt solution containing ten µMionomycin and 50 µM phenylephrine. The minimum fluorescence ratio was determined by adding ten mM ethylene glycol-bis(β-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA). $[Ca^{2+}]_i$ was determined as generally accepted using the formula $[Ca^{2+}]_i$ (nM) = $K_d \times [(R - R_{min})/(R_{max} - R)] \times (S_{f2}/S_{b2})$, where: K_d (224 nM) is the dissociation constant of Fura-2 for Ca^{2+}; R is the ratio of fluorescence of the sample at 340 nm to that at 380 nm; R_{min} and R_{max} represent the ratios of fluorescence at the same wavelengths in the presence of zero and saturating Ca^{2+} respectively; S_{f2}/S_{b2} is the ratio of fluorescence of Fura-2 at 380 nm in zero Ca^{2+} to that in saturating Ca^{2+} respectively. Preliminary experiments indicated that contractions induced by high KCl, phenylephrine, and caffeine were not significantly affected by Fura-2 AM loading.

We have synthesized and carried out the screening of a number biologically active compounds that could work, at least from a theoretical point of view, as compounds that really possess the ability to decrease Ca^{2+} - sensitivity of contractile proteins in SMCs directly and definitely. For instance, using simultaneous measurements of contractile force and $[Ca^{2+}]_i$,we have shown that some of the synthesized compounds 7 a-f and 12, being added to the tissue bath solution at concentration, produced pronounced relaxation without visible changes in $[Ca^{2+}]_i$,i.e. may be classified as a calcium desensitizers. These types ofchemical compounds may be a good therapeutic approach under generalized (arterial hypertension) or local (cardiac ischemia) vasospasm development.

2.5 Statistics and Analysis

The data are shown as means ± S.E.M., and *n* indicates the number of cells, preparations or animals tested. Data analysis was performed using Origin 8.0 (OriginLab Corporation, Northampton, MA, USA) software. Half-maximally effective concentration (EC_{50}) values were expressed as pD_2 ($-log\ EC_{50}$). Concentration-response curves were fit to the Hill equation ($F=F_{max}/[1+exp(logEC_{50}-logs)^h]$, were *F* and F_{max} are actual and maximal relaxation (constriction) responses, *h* is the Hill coefficient, C is actual concentration) to determine the maximal response and EC_{50} value. Multiple comparisons were made using one-way analysis of variance (ANOVA), and if any significant difference was found, the Tukey's multiple comparison post hoc tests were performed. Differences were considered to be statistically significant when *P* was less than 0.05.

3. RESULTS AND DISCUSSION

It is generally accepted that both increased Ca^{2+} influx and intracellular Ca^{2+} release have been proposed to be involved in VSM

hypercontractility in both health and disease. However, one controversy here is that voltage- and store-operated Ca^{2+} entry channels can be, in fact, even inhibited, for instance, in diabetes [17]. It means. Alternatively, the protein kinase C-dependent Ca^{2+}-sensitization of the contractile proteins has been proposed as a more general mechanism of elevated vascular contractility. One striking example supporting this view is the enhanced contractile response to noradrenaline in mesenteric arteries from diabetic rats which are not associated with the enhancement of the corresponding $[Ca^{2+}]$ I responses [18].

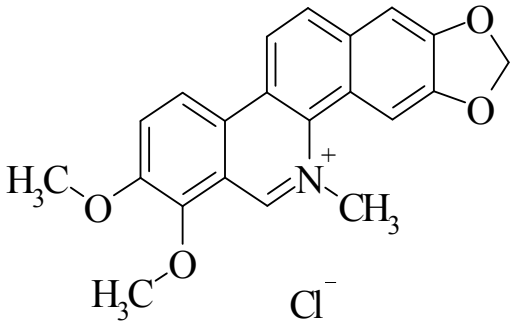
The increase in $[Ca^{2+}]$ I activate calmodulin-dependent myosin light chain (MLC) kinase which catalyzes the phosphorylation of MLC leading to actin-myosin interaction and VSM contraction. In addition to this primary regulatory pathway, several modulatory pathways exist in smooth muscle that can alter the magnitude of the force that is developed for any given level of $[Ca^{2+}]$ I [19,20]. Alterations in myofilament Ca^{2+} sensitivity can be either positive or negative depending on the pathways stimulated. For increases in myofilament Ca2+ sensitivity, two primary hypotheses have been proposed: G-protein dependent activation of Rho kinase and protein kinase C (PKC) [21].

This phenomenon, which is known as calcium sensitization of smooth muscle, may thereby maintain vascular contraction [19,20]. The opposing effect, calcium desensitization, is mediated by cGMP-kinase but plays a relatively minor role in the regulation of VSM contractility, although it can be more important in vascular hyporeactivity elicited by haemorrhagic and septic shock [22] (Xu and Liu, 2005).

Thus, upregulation of PKC activity appears to play a key role in a number diseases including hypertension, diabetes and even cancer protection – so-called PKC syndrome [23]. It is known that signalling pathways involving protein kinase C/Rho- kinase activation may increase the myofilament Ca^{2+} sensitivity, and thereby support high vascular tone in hypertension [21].

These data were used as the theoretical basis to design a new generation of ideal vasoactive drugs, so-called calcium desensitizers, which possess the ability directly affect *Ca*$^{2+}$-sensitivity of myofibrils and therefore, SMCs contractility and vascular tone.

First of all, we have to study the effect on vascular tone and $[Ca^{2+}]_I$, well known but rather toxic chemical compound, chelerythrine chloride (**Sch. 5**), a potent protein kinase C inhibitor.



Sch. 5. Structural formula of Chelerythrine chloride

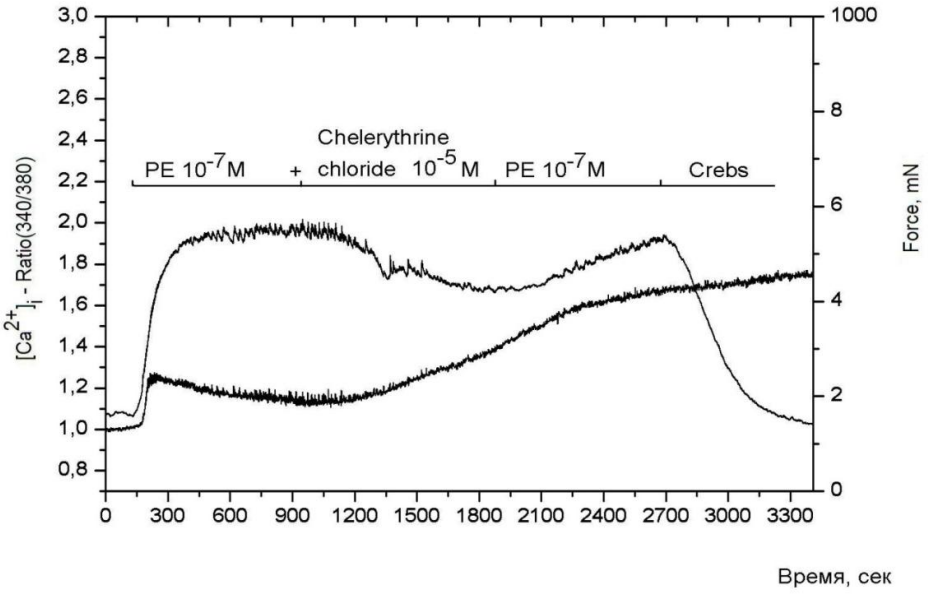
It has been shown that chelerythrine chloride (10^{-5} M) effectively relaxed VSMc but surprisingly increased $[Ca^{2+}]_i$ appeared to be absolutely non-acceptable for clinical application (**Sch. 6**).

Table shows the effects of synthesized compounds on maximal amplitude of relaxation (Rmax) and a related decrease in $[Ca^{2+}]$ I, ($\Delta[Ca^{2+}]_i$) in smooth muscles obtained from rat thoracic aorta.

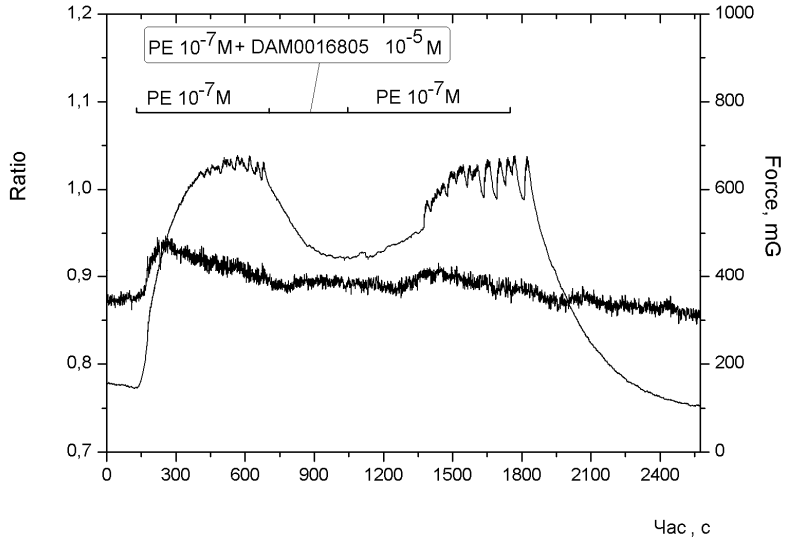
Table. Comparative effects of 2-methyl-7-aryl(α-furyl)-[1,3]thiazolo[4,5-d]pyridazin-4(5*H*)-ones at submaximal concentration of 10^{-5} M on maximal amplitude of relaxation in smooth muscles (Rmax) obtained from rat thoracic aorta and a related decrease in $[Ca^{2+}]_i$, ($\Delta[Ca^{2+}]_i$)

Items	Rmax, %	$\Delta[Ca^{2+}]_I$, %
7a	No relaxation	-
7b	97.4±15.1	-
7c	2.7±2.0	-
7d	30.6±6.4	-
7e	No relaxation	-
Seven f	9.6±2.2	-
12	41.4±9.2	4.2.2±1.0
Chelerythrine chloride	30.0±6.3	-

This compound effectively and reversibly relaxed VSMc while $[Ca^{2+}]_I$ remains unchanged (**Sch. 7**).



Sch. 6. Original traces of contractile force development (Force) and$[Ca^{2+}]_i$ in VSMc from denuded rat thoracic aorta pre-activated with phenylephrine (PE, 10^{-7}M) under potent protein kinase C inhibitor, chelerythrine chloride (10^{-5} M), action



Sch. 7. Original traces of contractile force development (Force) and$[Ca^{2+}]$ l in VSMc from denuded rat thoracic aorta pre-activated with phenylephrine (PE, 10^{-7}M) under compound12 (10^{-5} M) action

4. CONCLUSION

In conclusion, we have synthesized a series of 2-methyl-7-aryl(fyril)-[1,3]thiazolo[4,5-d]pyridazin-4(5*H*)-ones. Using simultaneous measurements of contractile force and $[Ca^{2+}]_i$, we have shown that some of the synthesized compounds, being added to the tissue bath solution at a concentration at 10^{-5} M, produced pronounced relaxation without visible changes in $[Ca^{2+}]_i$,i.e. may be classified as a calcium desensitizers. These types of chemical compounds may be a good therapeutic approach under generalized (arterial hypertension) or local (cardiac ischemia) vasospasm development. It is important to note that unlike the aforementioned antihypertensive

drugs, synthesized two methyl-7-aryl(α-furyl)-[1,3]thiazolo[4,5-*d*] pyridazine-3 4(5*H*)-ones possesses the unique ability to decrease vascular tone without corresponding lowering in intracellular calcium concentration, i.e, that it does not affect vital calcium metabolism.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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