ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Platelet aggregation under the influence of some dithiocarbamate derivatives of 9, 10-anthracenedione

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ABSTRACT

The antiplatelet activity of dithiocarbamate derivatives of 9,10-anthracenedione was studied. The activity of the compounds was tested *in vitro* using platelet-rich plasma. Two dithiocarbamates (compounds **11** and **12**) with high anti-aggregative activity have been established as a result of the screening test. The concentration-dependent inhibitory effects on platelet aggregation induced by adenosine-5'-diphosphoric acid (ADP) and arachidonic acid have been determined. IC50 values of 30±3 for dithiocarbamate 11 and 15±2 µM for dithiocarbamate 12 have been established for ADP-dependent aggregation. IC50 values of 10±2 for dithiocarbamate 11 and 18±2 µM for dithiocarbamate 12 have been identified for aggregation induced by arachidonic acid.

Keywords: 9,10-anthracenedione, dithiocarbamates, platelet aggregation, antiaggregant agents.

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ISSN: 0975-8585

INTRODUCTION

Blood platelets play important role in primary hemostasis, forming thrombi that prevent blood loss after vascular injury. However, hyperactivation of these cells may lead to formation of occlusive platelet-rich thrombi, resulting in the tissue ischemia and necrosis [1-3]. Risk of pathologic thrombus formation very increased in the past decades. And nowadays, thrombotic disease is recognized as one of the most common pathology underlying ischemic heart disease and ischemic stroke, which together caused one in four deaths worldwide. One of the most perspective approaches for the preventing of thrombotic diseases or lowering the risk for the development of their complications is pharmacological modification of platelet function. Unfortunately, using of modern anti-platelet drugs is often accompanied by side effects such as resistance to their action, an increased risk of uncontrolled bleeding or the development of serious systemic complications that along with the high costs of these medications suggest the need for further search for new, more efficient and safer anti-platelet drugs.

The natural and synthetic derivatives of 9,10-anthracenedione have a wide range of pharmacological effects [4]. In recent years, they have been widely studied as biologically active compounds with antitumor, antiviral, antidiabetic, antibacterial, antifungal activities [5]. Compounds with anti-platelet activity are also known among anthracenedione derivatives [6-13]. Different mechanisms of their inhibitory effects on platelet activation and aggregation have been described. For example, it was shown that amino-9,10-anthracenedione derivatives (mitoxantrone and bisantrene) inhibit platelet aggregation induced by both ADP and collagen [6]. Several derivatives of anthra[2,1-d]isothiazol-3,6,11-trione also were described as effective inhibitors of ADP-dependent platelet aggregation [9]. Selective inhibitors of cyclooxygenase, enzyme involving in the synthesis of one of the key mediators of platelet aggregation – thromboxane A2, was identified among 3-alkylaminopropoxy-9,10-anthracenedione derivatives [7]. Some glycoside derivatives of anthracenedione have antithrombotic properties [8]. Thus, 9,10-anthracenedione derivatives appears to be a perspective class of organic compounds in the search for the new effective inhibitors of platelet aggregation and in the creation of antithrombotic drug substances.

Taking into account the results of our previous study of anti-platelet activity of some 9,10-anthracenedione derivatives [10], the aim of this study was investigation of the platelet aggregation under the influence of newly synthesized dithiocarbamate derivatives of the 9,10-anthracenedione.

MATERIALS AND METHODS

The synthesis of test compounds (Fig.1) **1-13** has been reported previously [14]. The studied compounds were dissolved in pure dimethyl sulfoxide (DMSO) and their test concentrations were prepared using distilled water. The final DMSO concentration in all experiments was constant at 1%.

The blood was collected from rabbit ear artery into a polyethylene tube with 3.8% sodium citrate in ratio 9:1. Platelet-rich plasma (PRP) was obtained by centrifugation of stabilized blood at 300 g for 10 min at 20 °C. Platelet-poor plasma (PPP) was obtained by further centrifugation of PRP at 1500 g for 30 min at 20°C.

Platelet aggregation was assessed within the first 3 h after blood sampling using photo-optical aggregometer AT-02 (Medtech, Russia). The number of platelets in PRP was counted and adjusted using PPP to 230-250×10^3 cells/µl before the assessment.

Primary screening for anti-aggregation activity of dithiocarbamate derivatives of 9,10-anthracenedione was performed *in vitro*: PRP was incubated with studied compounds (final concentration was 50 µM) in a cuvette for 2 min at 37°C with constant stirring (500 rpm). PRP incubated with 1% DMSO was used as a control. Adenosine 5'-diphosphate (Renam, Russia; hereinafter ADP) at the final concentration of 5×10^{-6} M was used as an aggregation inducer. In this experiment we have studied the level of spontaneous aggregation induced by addition of the tested compounds to plasma, evaluated the degree of aggregation (the maximal level of light transmission of PRP after addition of inducer) and calculated the degree of inhibition of ADP-dependent aggregation under the action of dithiocarbamate derivatives relative to control, which was taken as 100%.

ISSN: 0975-8585

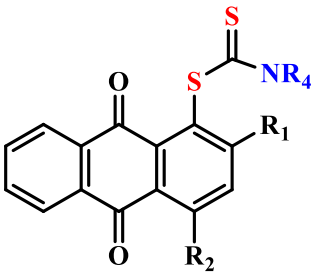
The IC_{50} value was determined for dithiocarbamate derivatives, which decreased the degree of ADP-dependent aggregation by more than 50% at concentration of 50 μM. For this, PRP was incubated with the studied compounds at concentration range from 5 to 100 μM for 2 min followed by addition of inducers such as ADP (5×10^{-6} M) or arachidonic acid (5×10^{-4} M, Renam, Russia). The IC_{50} value was defined as the concentration at which the compound inhibits platelet aggregation by 50%.

The experimental studies of the effect of dithiocarbamate derivatives on platelet aggregation were performed on three PRP samples from different rabbits for each compound. The experiment for each PRP sample was replicated three times. Statistical processing of the results was performed using software Statistica 7. Value changes was considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

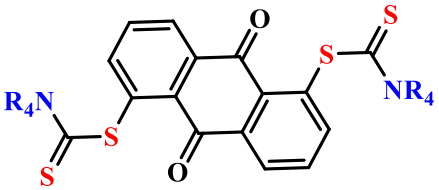
Platelets, due to their adhesive and aggregation functions, are an extremely important element, not only for normal hemostasis [15], but also for the process of pathological thrombus formation [16, 17]. Platelet membrane contains a large number of receptors, which provide a signal transmission from an inducer to intracellular messengers following cell activation and secretion of platelet prothrombotic mediators [15]. ADP is one of the most important platelet agonist that acts through the $P2Y_1$ and $P2Y_{12}$ receptors. Taking into account that ADP receptors are considered to be a key target of modern antithrombotic drugs (clopidogrel, prasugrel, ticlopiridin) which have benefit in the management of ischemic vascular disease [18] we also choose the process of ADP-dependent aggregation as an object for the study of the anti-aggregating properties of the synthesized dithiocarbamate derivatives of 9,10-anthracenedione.

In continuation of interest in the chemistry of new derivatives of 9,10-anthracenedione [14, 19-24] and discovery of biological properties, perspective mono- and bisdithiocarbamate derivatives of 9,10-anthracenedione **1-13** (Figure 1) were synthesized by consecutive two-stage refunctionalization of aminoanthracenediones [14] in the absence of any catalyst in mild temperature conditions in the aqueous medium with yields 50-96%. The structures of the obtained S-anthracenyl dithiocarbamates **1-13** were confirmed by the ^1H, ^{13}C NMR, IR spectra and elemental analysis data [14].



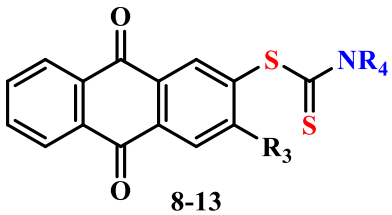
1-5

R_1=R_2=H, NR_4=NEt_2 (1); R_1=R_2=H, NR_4=N⟨O⟩(2);
R_1 = H, R_2 = 4-HN-C_6H_4Me, NR_4 = NEt_2 (3);
R_1 = Cl, R_2 = 4-HN-C_6H_4Me, NR_4 = NEt_2 (4);
R_1 = Br, R_2 = 4-HN-C_6H_4Me, NR_4 = NEt_2 (5);



6, 7

NR_4 = NEt_2 (6); N⟨O⟩(7)



8-13

R_3=H, NR_4= N⟨O⟩(8); R_3=H, NR_4=N⟨⟩(9);
R_3=H, NR_4=N⟨⟩(10); R_3=Cl, NR_4=NEt_2 (11);
R_3=Cl, NR_4=N⟨⟩(12); R_3=Cl, NR_4=N⟨⟩(13)

Fig.1. The structures of dithiocarbamate derivatives of 9,10-anthracenedione

ISSN: 0975-8585

According to the results the degree of aggregation in the control PRP, after its incubation for 2 min with 1% DMSO, in response to 5×10^{-6} M ADP was 56±4%. The effects of the dithiocarbamate derivatives of 9,10-anthracenedione on the process of ADP-induced platelet aggregation are presented in the Figure 2.

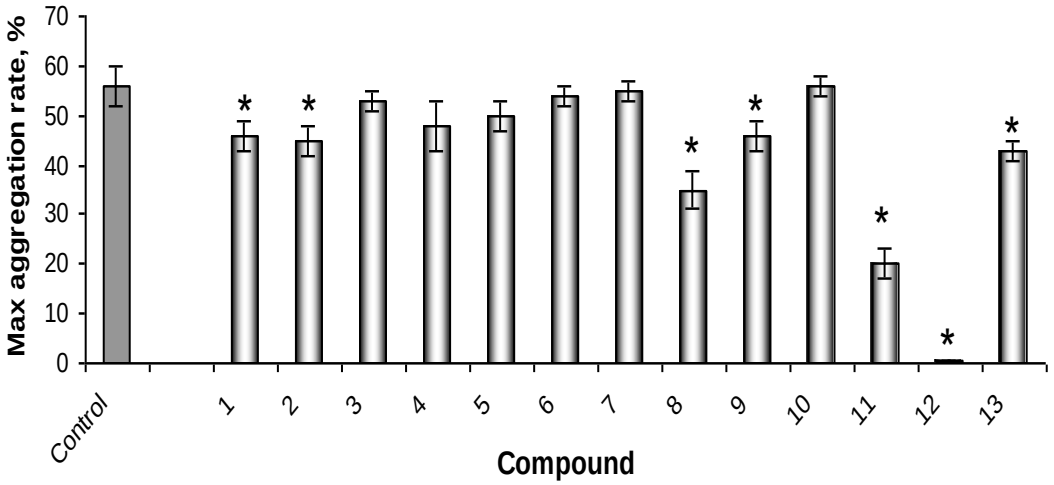


Fig. 2. The influence of the investigated dithiocarbamate derivatives of 9,10-anthracenedione (50μM) on the maximal degree of ADP-induced platelet aggregation in rabbit PRP

*Rabbit PRP was pre-incubated with 1% DMSO (control) at 37°C for 2 min, and then ADP (5 μM) was added. Data are presented as mean ± s. e. m. (n = 3). *P < 0.05 – compared with the control value.*

It was established that no investigated dithiocarbamates cause spontaneous platelet aggregation in the test concentration.

The correlation between the structure and activity of the tested compounds has been established based on obtained results. Dithiocarbamate derivative **12** had maximal inhibitory effect according to the results.

Derivatives **1**-**5** contained not only diethyldithiocarbamate fragment at the position 1 of the anthracene ring, but also substituents in positions 2 (chlorine or bromine atom) and 4 (*p*-tolylamide substituent) have less inhibitory effect. Compounds **1** and **2** inhibited process of ADP-dependent aggregation by 20 %. Dithiocarbamate **6**, which contains two diethyldithiocarbamate fragments, does not have anti-platelet activity. There is the following correlation among dithiocarbamates containing the morpholine ring. The presence of two morpholinodithiocarbamate fragments in the molecule (compound **7**) leads to the disappearance of the inhibitory effect. Migration of morpholinodithiocarbamate substituent from position 1 (compound **2**) into the position 2 of the anthracene ring (compound **8**) leads to increase of the inhibitory effect from 20 % to 40 %. It was determined that the introduction of chlorine atom in molecules of dithiocarbamate derivatives **11-13** causes an increase of inhibitory properties in comparison with compounds **8-10.** The replacement of diethylamine and pyrrolidine fragments on the piperidine fragment in the presence of chlorine atom in the molecule (compound **12**) leads to a pronounced anti-aggregative activity (full inhibition).

Thus, the results of screening test on the study of the anti-aggregation effect of non-protein low molecular weight compounds, namely dithiocarbamate derivatives of 9,10-anthracenediones, revealed two structures (**11** and **12**), which at a concentration of 50 μM inhibited ADP-dependent aggregation on 70 and 100%, respectively. In our opinion, these dithiocarbamates can be considered as promising anti-platelet agents that prompted us to further researches of their effects on platelet aggregation.

The study of the inhibitory effects of compounds **11** and **12** on ADP-induced platelet aggregation under conditions of their different concentrations (1-100 μM) in the incubation medium was the next step of our work. It was found that the studied derivatives influence on platelet aggregation in dose-dependent manner (Fig. 3).

ISSN: 0975-8585

The compound **11** completely inhibited ADP-induced platelet aggregation at a concentration of 100 µM. The decreasing of the concentration leds to a gradual lowering of the inhibitory effect. The IC_{50} value for this compound was ~30µM. The compound **12** was more effective. The complete inhibition of ADP-induced platelet aggregation was observed at the concentration of 50 µM. The effect was decreased in proportion with the lowering of the compound **12** concentration. The IC_{50} value for this compound was ~15 µM (Fig. 3).

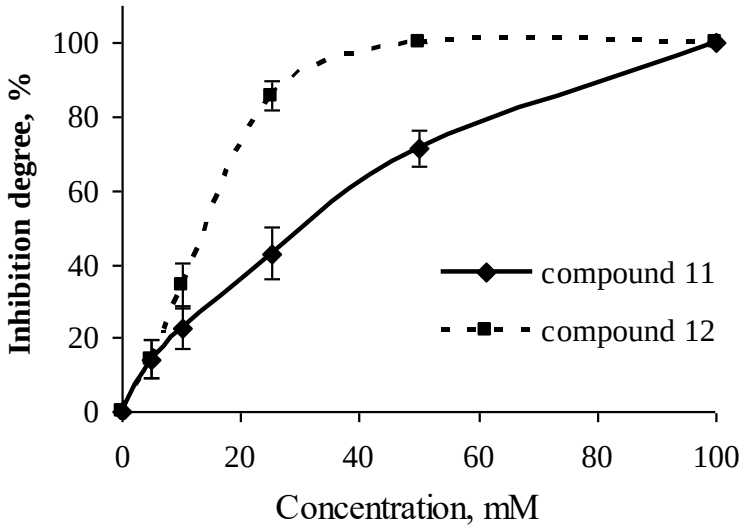


Fig. 3. Concentration-dependent inhibitory effect of compounds 11 and 12 on platelet aggregation induced by ADP in rabbit PRP

*Rabbir PRP was incubated with various concentrations of **11**, **12** (5-100 µM) or DMSO (1%) at 37 °C for 2 min, and then ADP (5 µM) was added to trigger aggregation. Data are presented as means ± s. e. m (n = 3).*

Platelets are activated by a number of physiological agonists. Another vital endogenous inducer of platelet aggregation is arachidonic acid. Arachidonic acid (AA) is released from the stores of the plasma membrane in response to numerous of platelet agonists. Released AA is then metabolized by cycloxegenase 1 (COX-1) to prostaglandin G2 which subsequently is converted to prostaglandin H2. Thromboxane synthase then produces thromboxane A2 (TxA2). After the TxA2 is synthesized, it can diffuse through the membrane of platelets, interact with the corresponding surface receptors on other cells, cause the changes in their shape, secretion of platelet granules, and activation of intracellular signaling and adapter molecules involved in the processes of cell activation and aggregation. The consequence of the TxA2 action is an activation of bigger amount of platelets that promotes a thrombus formation. It is proved that inhibition of TxA2 synthesis is effective approach in the antithrombotic therapy. The anti-aggregation drug, whose effectiveness is verified and confirmed by the results of numerous large-scale, placebo-controlled studies, is acetylsalicylic acid [25, 26]. Aspirin inhibits COX irreversibly by forming a covalent bond with the enzyme and further acetylation of an amino acid that extends into a narrow, hydrophobic channel within the enzyme molecule that leads to its active site. When this amino acid is acetylated, the channel is blocked and AA, the precursor to prostaglandin, can no longer reach the COX catalytic site. Therefore, aspirin prevents the TxA2 synthesis [27, 28]. Based on the fact that among the derivatives of 9,10-anthracenedione there are substances that are able to selectively inhibit COX, we also investigated the effect of compounds **11** and **12** on the aggregation induced by arachidonic acid.

The results showed that the platelet aggregation induced by arachidonic acid was also sensitive to the action of the tested compounds (Fig. 4).

ISSN: 0975-8585

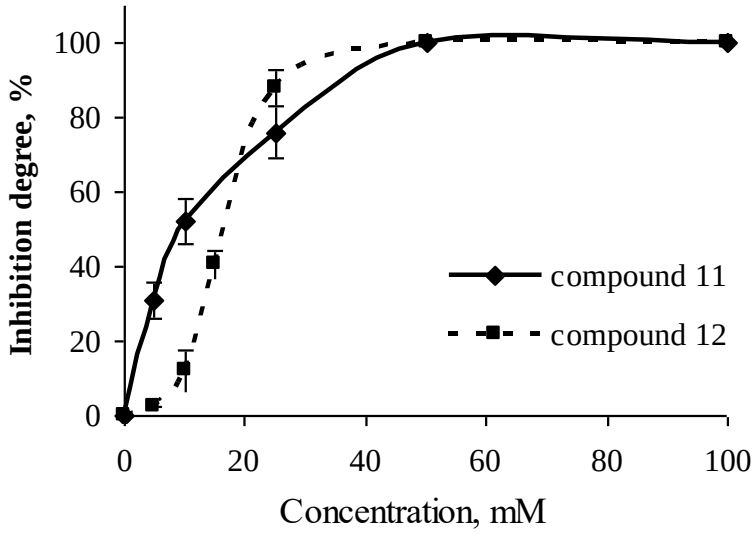


Fig. 4. Concentration-dependent inhibitory effect of compounds 11 and 12 on platelet aggregation induced by arachidonic acid in rabbit PRP

*Rabbir PRP was incubated with various concentrations of **11**, **12** (5-100 μM) or DMSO (1%) at 37 °C for 2 min, and then arachidonic acid (100 μM) was added to trigger aggregation. Data are presented as means ± s. e. m (n = 3).*

The inhibitory effect of compound **11** had already appeared in concentration of 5 μM, and gradually intensified with increasing of concentrations, reaching a maximum at 50 μM. The IC_{50} value for this compound was ~10 μM.

The complete inhibition of platelet aggregation induced by arachidonic acid under the influence of the compound **12** was noticed in its concentration of 50 μM. The IC_{50} value for this compound was ~18 μM.

CONCLUSIONS

Thus, the experimental data indicate that the search for potential anti-thrombotic agents among dithiocarbamate derivatives of the 9,10-anthraquinone is very perspective. The research of the structure-activity relationships of the dithiocarbamate derivatives of 9,10-anthracenediones allowed the establishment of certain interesting facts about the nature of pharmacophores in their structure that provide anti-platelet activity. Two dithiocarbamate derivatives of the 9,10-anthraquinone (compounds **11** and **12**) with high *in vitro* antiplatelet activity for rabbit PRP with IC_{50} in ranges at 15-30 μM for ADP-dependent aggregation and 10-18 μM for aggregation induced by arachidonic acid have been identified. Obtained data are insufficient to propose a mechanism of the inhibitory effect of the test compounds on the functioning of platelets, and indicating the feasibility of further research, but the results can form the basis for the direct synthesis of new compounds with the purpose of creating effective antiplatelet agents.

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