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New hybrid furoxan structures with antiaggregant activity

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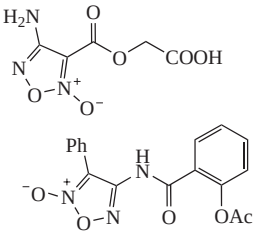
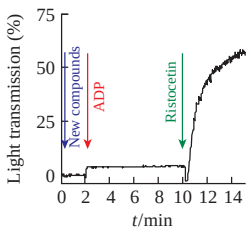
A series of functionalized furoxans was synthesized, and their effect on the platelet aggregation was estimated using a set of inducers. New hybrid structures comprising the furoxan ring and glycolic or acetylsalicylic acid motifs effectively inhibit aggregate formation induced by adenosine diphosphate and adrenaline. Studies of their NO-donor ability showed that their antiplatelet effects were mainly independent of the NO action.





Until recently, only few antiplatelet agents (aspirin, ticlopidine, dipyridamole) and anticoagulants (vitamin K antagonists, unfractionated heparin, low molecular weight heparins and heparinoids) were in clinical use.[1,2] A search for novel antiaggregant compounds with improved pharmacokinetic profiles has resulted in new effective agents (*e.g.*, clopidogrel,[3] a chemical analogue of ticlopidine[4] with minimal bone-marrow suppressing effects, thromboxane synthase inhibitors and receptor blockers, antagonists of platelet receptor glycoproteins Ib and IIb/IIIa[5]). However, these antiaggregants have serious disadvantages such as bleeding, gastrotoxicity, possible cardiotoxicity, or ischaemic stroke. Therefore, a search for new selective antiaggregant and anticoagulant agents remains relevant.

In recent decade, a number of new antiaggregant and cardiovascular drug candidates have been found in a series of 1,2,5-oxadiazole 2-oxides (furoxans).[6] Furoxans are valuable scaffolds for the design of complex molecular systems in the context of the development of practical technologies.[7] In addition, furoxans are capable of releasing nitric oxide (NO) in the presence of thiol-cofactors.[8] Recently, we have found that 3-cyano-4-phenylfuroxan and 3-nitro-4-phenylfuroxan inhibit platelet agglutination induced by adenosin diphosphate and adrenaline.[9] In addition, these compounds demonstrated high cardiovascular activity.[6(*a*)] Later, we have studied the influence of the water soluble furoxan derivatives on platelet aggregation using a set of inducers. Known vasodilator 3-carbamoyl-4-(hydroxymethyl)-furoxan (CAS-1609)[10–13] was shown to be most active. Other furoxans containing amide groups also revealed good cardiovascular activity.[14] These results served as a good basis for the search of new structures with antiaggregant activity in a series of furoxancarboxamides as well as among hybrid structures incorporating the furoxan ring.

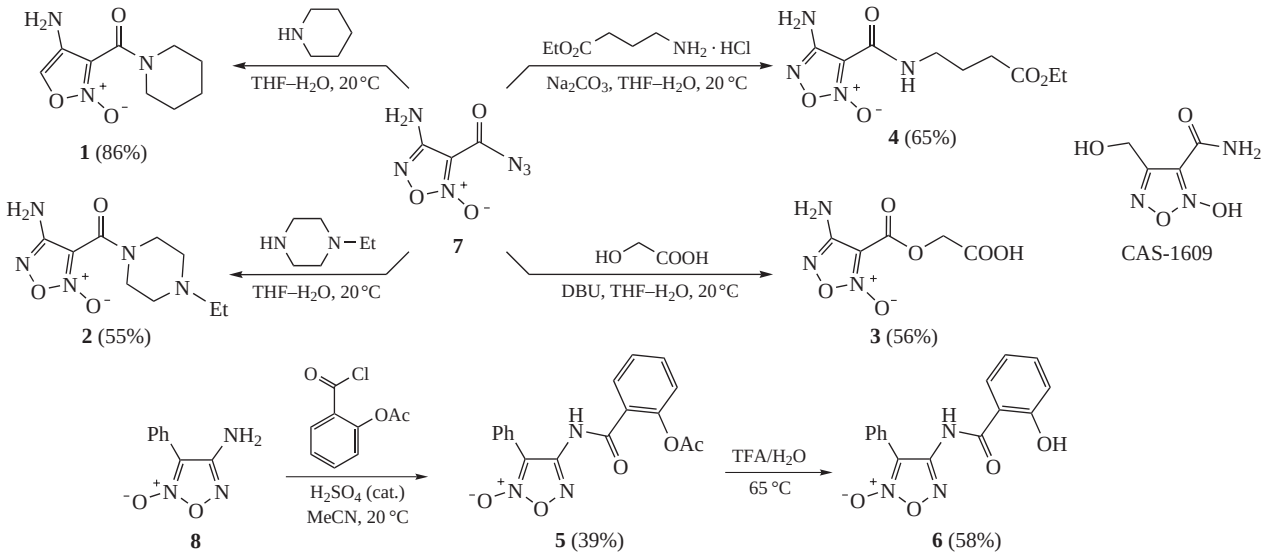
Herein, we report the antiaggregant activity of new water-soluble functionally substituted furoxans **1**–**6** using an expanded list of inducers. Along with furoxancarboxamides **1**, **2**, **4**, we synthesized a series of hybrid structures comprising the furoxan moiety linked to the fragments of glycolic acid (compound **3**), acetylsalicylic acid (aspirin, compound **5**) and salicylic acid (compound **6**). Glycolic acid motif is a structural component of lamifiban, nonpeptide glycoprotein (GP) IIb/IIIa receptor antagonist,[15] and aspirin is a well-known anti-platelet and cardiovascular agent.[1,2] Compound CAS-1609 was taken as the reference to compare the obtained results. In addition, the NO-donor ability of the synthesized furoxan derivatives was measured.

Furoxancarboxamides **1**, **2** and **4** were synthesized by a nucleophilic substitution of azido group in 4-amino-3-(azidocarbonyl)furoxan **7**[16] under the action of the corresponding amines (Scheme 1). Compound **3** was prepared analogously from furoxan **7** and glycolic acid in the presence of DBU. Amide **5** was synthesized by acylation of 4-amino-3-phenylfuroxan **8**[17] with *O*-acetylsalicylic acid chloride. Subsequent deacetylation of compound **5** with CF_3COOH afforded product **6** (see Scheme 1).†

Furoxans are known to possess cytotoxic activity,[18] therefore the ability of compounds **1**–**6** to influence cell proliferation was studied first. Human mononuclear cells (MNC) and cancer cells LnCap were used in the standard MTT test. None of the

† *4-Amino-3-furoxancarboxylic acid amides* **1**, **2**, **4**. For the preparation of amides **1** and **2**, piperidine or 4-ethylpiperazine (5.9 mmol) was added dropwise to a stirred solution of 4-amino-3-(azidocarbonyl)furoxan **7** (0.50 g, 2.8 mmol) in a mixture of THF (1 ml) and water (5 ml) at 20 °C. For the synthesis of amide **4**, ethyl-γ-aminobutyrate (0.90 g, 5.9 mmol) and Na_2CO_3 (0.62 g, 5.9 mmol) were used. The reaction mixture was stirred at 20 °C for 1 h, then diluted with water (50 ml). Amides **1** and **4** were filtered off, washed with cold water (2 × 25 ml), dried in air and recrystallized from EtOH. Amide **2** was extracted with EtOAc (3 × 20 ml), the extract was washed with cold water (2 × 25 ml) and dried ($MgSO_4$). Evaporation of the solvent afforded the crude product which was recrystallized from EtOH.

4-Amino-3-(carboxymethoxy)carbonyl-1,2,5-oxadiazole 2-oxide **3**. DBU (1.01 ml, 6.8 mmol) was added to a stirred solution of glycolic acid (0.30 g, 3.4 mmol) in a mixture of THF (1 ml) and water (5 ml) at room temperature. In 5 min, 4-amino-3-(azidocarbonyl)furoxan **7** (0.30 g, 1.7 mmol) was added in one portion. The mixture was stirred at room temperature for 5 h until the consumption of the initial compound (TLC monitoring, eluent $CHCl_3$–EtOAc, 10 : 1). The mixture was treated with 10% HCl (30 ml), extracted with EtOAc (3 × 20 ml), the combined extracts were washed with cold water (2 × 25 ml) and dried ($MgSO_4$). Evaporation of the solvent afforded the crude product which was recrystallized from EtOH.



Scheme 1

compounds induced cell death in the experiments. The lack of the toxic effect could be explained by the absence of the nitro groups in the structures **1**–**6** in contrast to previously studied nitrobifuroxans that caused cell death probably due to release of great amount NO.[13]

The *in vitro* antiaggregant activity of compounds **1**–**6** was studied using platelet rich plasma (PRP). Five inducers of platelets aggregation, namely adrenaline, adenosine diphosphate (ADP), collagen, ristocetin, and arachidonic acid, were employed in the experiments. The effects of the samples were measured according to the previously described procedure.[12] PRP with a sample was heated at 37 °C for 2 min, then an inducer was added and light transmission was recorded.

Similarly to the previously studied CAS-1609, none of the compounds inhibited platelet aggregation induced by ristocetin and arachidonic acid. In the experiments with adrenaline, furoxans **3** and **5** demonstrated significant effect comparable with that of CAS-1609, while other compounds (**1**, **2** and **6**) were inactive or showed a weak activity (**4**) (Figure S1, Online Supplementary Materials). Thus, in the control group and in the presence of furoxans **1**, **2** or **6**, the reaction was started immediately after adrenaline addition, while in the presence of compounds **3**, **5** or CAS-1609 only slight platelets aggregation was observed. It is remarkable that addition of ristocetin at the 10th minute of the experiments switched on agglutination immediately, which indicated the remaining ability of platelets to cell-cell interaction after the treatment with furoxans **3** and **5**.

In the experiments with ADP, furoxans **3** and **5** also showed activity similarly to CAS-1609, while the others demonstrated no effect. Application of the collagen as inducer caused the delay in aggregates formation in the presence of **3** and **5** (Figure S2). Thus, in the control group and in the presence of compounds **1**, **2**, **4** or **6**, the platelets agglutination was started at the 6th minute of the experiment, while the addition of **3** or **5** similarly to CAS-1609 triggered the reaction after the 8th minute. These results indicate that compounds **3** and **5** possess a selective mechanism of inhibition of platelets aggregation mediated by ADP and adrenaline, but not ristocetin, collagen and arachidonic acid. It is noteworthy that ADP and adrenaline are considered to be the main agents causing thrombus formation.[19,20]

As it was mentioned above, furoxans behave as NO-donors in the presence of thiol cofactors.[8] At the same time, the formation of nitrite-anion as a result of NO oxidation may be quantified according to Griess assay and thus may serve as a reliable tool for measuring the amount of NO release. The amounts of NO_2^- produced from the selected furoxans **1**–**6** and CAS-1609 (as a reference compound) under physiological conditions (pH 7.4, 37 °C) after 1 h incubation were measured *via* the Griess reaction using a spectrophotometric technique. Furoxan CAS-1609 was found to be the most powerful NO-donor (26.2% NO_2^-). Among studied compounds **1**–**6**, furoxan **4** incorporating γ-aminobutyrate subunit showed the highest level of NO release (14.1%). Other investigated furoxan derivatives were found to be weak NO-donors (3.2–5.2% NO_2^-) (Figure 1). Higher NO-donor ability of CAS-1609 evidently connects with its cardiovascular properties. Apparently, these results suggest that the antiplatelet effects of furoxans **3** and **5** are mainly independent of the NO action. Therefore, we extended the scope of furoxans with antiaggregant activity and synthesized new compounds with high activity and low toxicity.

In conclusion, a series of the furoxancarboxamides and hybrid structures, comprising furoxan and glycolic or acetylsalicylic (aspirin) acids motifs, have been synthesized and investigated as potential agents with antiaggregant properties using an expanded list of inducers. The hybrid compounds whose structures incorporate

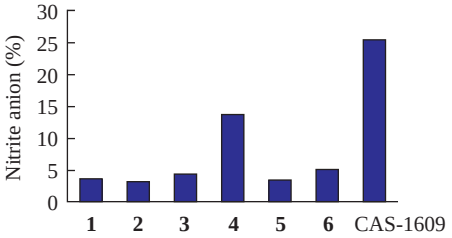


Figure 1 Nitrite anion release from furoxans **1**–**6** and CAS-1609 according to Griess test results.

4-(2-Acetoxybenzamido)-3-phenyl-1,2,5-oxadiazole 2-oxide **5**. 4-Amino-3-phenylfuroxan **8** (0.80 g, 4 mmol) was added to a stirred solution of acetylsalicylic acid chloride (0.90 g, 4.5 mmol) in MeCN (10 ml). Then a catalytic amount of conc. H_2SO_4 (0.1 ml) was added. The mixture was stirred at room temperature for 3 h until the consumption of the initial compound (TLC monitoring, eluent $CHCl_3$–EtOAc, 10 : 1). The solvent was evaporated and the title compound was purified by column chromatography on SiO_2 ($CHCl_3$–EtOAc, 10 : 1).

4-(2-Hydroxybenzamido)-3-phenyl-1,2,5-oxadiazole 2-oxide **6**. Amide **5** (0.30 g, 0.8 mmol) was added to a stirred mixture of TFA (5 ml) and water (0.25 ml). The mixture was stirred at 65 °C for 2 h, then cooled, diluted with water (50 ml) and extracted with EtOAc (3×20 ml). The combined extracts were washed with cold water (2×50 ml) and dried ($MgSO_4$).

furoxan ring linked to the glycolic or acetylsalicylic acid subunits revealed a high antiplatelet activity which was comparable to that of the previously investigated compound CAS-1609. In addition, these compounds possess a selective mechanism of inhibition of platelets aggregation mediated by ADP and adrenaline but not ristocetin, collagen and arachidonic acid, and their antiplatelet effects are mainly independent of the NO action. To the best of our knowledge, we have discovered a new hybrid furoxan structure with high antiaggregant activity and low toxicity and this strategy should be continued.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.11.010.

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