Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2016, **26**, 513–515
Mendeleev Communications

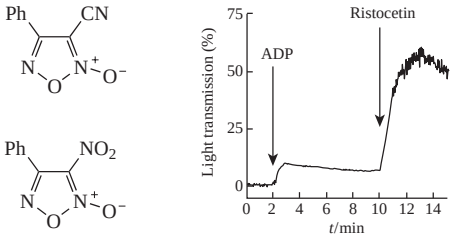
New insight into the antiaggregant activity of furoxans

Nadezhda E. Ustyuzhanina,* Leonid L. Fershtat, Marina L. Gening, Nikolay E. Nifantiev and Nina N. Makhova*

N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 8784; e-mail: ustnad@gmail.com, mnn@ioc.ac.ru

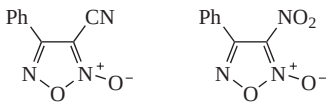
DOI: 10.1016/j.mencom.2016.11.018

The measurement of the antiaggregant properties of 3-cyano-4-phenylfuroxan and 3-nitro-4-phenylfuroxan has demonstrated that these compounds effectively inhibit platelet agglutination induced by adenosine diphosphate and adrenaline but not ristocetin. When collagen was used as an inducer, only a slight delay of aggregate formation was observed. Therefore, these furoxan derivatives could be considered as a basis for the development of next generation agents with improved antiaggregant activity.



A challenging task of modern organic chemistry is a search for new molecular systems possessing a wide spectrum of useful properties, in particular, biological activity.[1] Furoxans represent a distinct group of heterocyclic compounds exhibiting an interesting profile of biological activity,[2] including neuroprotective, precognitive,[3] cytotoxic,[4] antihelmintic,[5] antibacterial,[6] and fungicidal[7] activities. It is expected that the biological activity of furoxans is connected with their capacity to release NO.[8]

It was reported[9,10] that furoxans could be regarded as promising antiaggregant agents. In particular, 3-cyano-4-phenylfuroxan **1**[11] and 3-nitro-4-phenylfuroxan **2**[12] (Figure 1) inhibit platelet aggregation induced by collagen. Based on the observed levels of activity, compounds **1** and **2** can be considered as potential starting points for a further rational design of next generation agents with improved antiaggregant activity and water solubility. However, the reported data for **1** and **2** concerning their antiaggregant properties were studied always within 5 min after inducing platelet agglutination. Therefore, before starting the synthesis and biological study of novel furoxans, a thorough investigation into the antiaggregant properties of compounds **1** and **2** should be performed. Here, we report on the influence of **1** and **2** on platelet aggregation studied with the use of an expanded list of inducers and within longer periods of monitoring.



1 **2**

Figure 1 Structures of furoxan derivatives **1** and **2**.

The antiaggregant activity of **1** and **2** was studied *in vitro* using platelet rich plasma (PRP). Four platelet agglutination inducers, namely, collagen, ristocetin, adenosine diphosphate (ADP), and adrenaline, were applied in the experiments. The effects of the samples were measured according to an approved procedure.[†]

[†] http://www.renam.ru/katalog/reagenty-dlya-issledovaniya-funkcii-trombocitov/agrenam.

Table 1 Delay in platelet aggregation induced by collagen in the presence of different amounts of agents **1** or **2** compared to a control.

Agent	Concentration/nmol ml^{-1}		
	37.5	75	150
1	1 min	1.5 min	2.1 min
2	50 s	1.8 min	3.0 min

In the tests, an inducer was added after plasma heating at 37 °C for 2 min.[‡]

In the experiments with collagen, where three concentrations of **1** and **2** were tested, only a delay in aggregate formation was observed (Table 1). Thus, in the control group, platelet agglutination was started after 5 min, while the reaction started after about 6 min in the presence of **1** or **2** in a concentration of 37.5 nmol ml^{-1} [Figure 2(*a*)]. An increase in the concentration of the test compounds led to a bigger delay in the reaction, whereas the levels of agglutination were similar (about 70% of light transmission was detected).

Both of the furoxans were inactive in the experiments with ristocetin (data not shown). However, the application of ADP and adrenaline revealed very promising effects of both compounds

[‡] *Experimental procedure*. The test was performed using a Biola platelet aggregation analyzer (Biola Ltd, Russia) according to an established procedure. Agrenam (Renam, Russia) kit and adrenaline were used for the experiments. Blood with a citrate buffer (9:1) was centrifuged at 1000 rpm for 10 min, and platelets rich plasma (PRP) was collected. The 0.5 M stock solutions of compounds **1** and **2** in DMSO were prepared. The stock solutions were diluted with water to concentrations of 5, 2.5 and 1.25 mM. To 300 μl of heated at 37 °C PRP, 10 μl of a solution of a tested sample was added, and the mixture was incubated at 37 °C for 2 min with stirring. Then, a solution (30 μl) of collagen (2 mg ml^{-1}) was added, and light transmission was measured for 8 min at 37 °C. Aqueous solutions of DMSO with appropriate concentrations were used as controls. Inducers (30 μl) other than collagen, namely, ADP (0.2 mM), ristocetin (15 mg ml^{-1}) and adrenaline (110 μM), were used in the experiments with 1.25 mM solutions of **1** and **2**. If aggregation did not occur, another inducer of aggregation was added and light transmission was measured for 5 min at 37 °C.

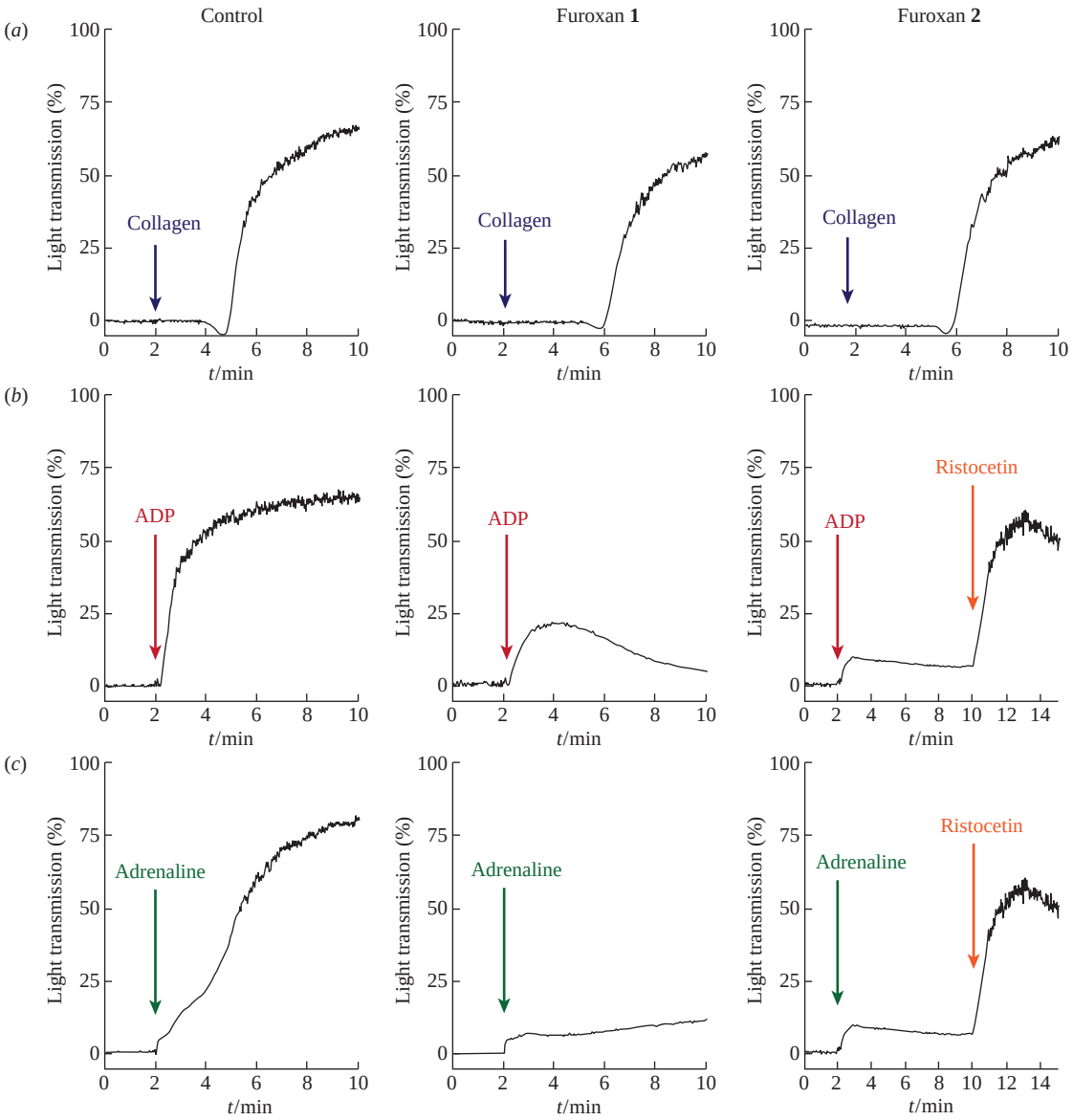


Figure 2 Effect of furoxans **1** and **2** on *in vitro* platelet aggregation.

1 and **2** even at the lowest test concentration of 0.0375 μmol ml^{-1} [Figure 2(*b*),(*c*)]. Thus, in the control group, the reaction was started immediately after ADP or adrenaline addition, while a low level of platelet aggregation was observed in the test groups (<25%). Note that the addition of ristocetin after 10th min of the experiments switched on agglutination immediately [Figure 2(*b*),(*c*)], which indicated that platelets were still capable to cell–cell interaction after the furoxan treatment. These results indicate that compounds **1** and **2** possess a selective mechanism of platelet aggregation inhibition. ADP and adrenaline are considered the main agents causing thrombus formation.[13,14] Therefore, it seems promising to study the effect of furoxans **1** and **2** in more detail.

In conclusion, furoxan derivatives **1** and **2** were found to inhibit platelet agglutination induced by adenosine diphosphate and adrenaline rather than ristocetin. In the case of collagen as an inducer, only a delay of aggregate formation was observed. Therefore, these results are promising in search of novel furoxan derivatives with improved antiaggregant activity.

This work was supported by the Russian Science Foundation (grant no. 14-50-00126).

References

1 (*a*) V. P. Ananikov, K. I. Galkin, M. P. Egorov, A. M. Sakharov, S. G. Zlotin, E. A. Redina, V. I. Isaeva, L. M. Kustov, M. L. Gening and N. E. Nifantiev, *Mendeleev Commun.*, 2016, **26**, 365; (*b*) S. G. Zlotin, A. M. Churakov, O. A. Luk'yanov, N. N. Makhova, A. Yu. Sukhorukov and V. A. Tartakovsky, *Mendeleev Commun.*, 2015, **25**, 399.

2 (*a*) C. Jovene, E. A. Chugunova and R. Goumont, *Mini-Rev. Med. Chem.*, 2013, **13**, 1089; (*b*) H. Cerecetto and W. Porcal, *Mini-Rev. Med. Chem.*, 2005, **5**, 57; (*c*) A. Gasco, R. Fruttero, G. Sorba, A. Di Stilo and R. Calvino, *Pure Appl. Chem.*, 2004, **76**, 973.

3 I. T. Schiefer, L. VandeVrede, M. Fa, O. Arancio and G. R. J. Thatcher, *J. Med. Chem.*, 2012, **55**, 3076.

4 (*a*) Y. Lai, L. Shen, Z. Zhang, W. Liu, Y. Zhang, H. Ji and J. Tian, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6416; (*b*) G. Aguirre, M. Boiani, H. Cerecetto, M. Fernández, M. González, E. León, C. Pintos, S. Raymondo, C. Arredondo, J. P. Pacheco and M. A. Basombrío, *Pharmazie*, 2006, **61**, 54; (*c*) J. Zhao, S. Gou, Y. Sun, L. Fang and Z. Wang, *Inorg. Chem.*, 2012, **51**, 10317.

5 R. S. Treger, A. Cook, G. Rai, D. J. Maloney, A. Simeonov, A. Jadhav, C. J. Thomas, D. L. Williams, M. Cappello and J. J. Vermeier, *Int. J. Parasitol. Drugs Drug Resist.*, 2012, **2**, 171.

6 J. L. Dos Santos, C. Lanaro, R. C. Chelucci, S. Gambero, P. L. Bosquesi, J. S. Reis, L. M. Lima, H. Cerecetto, M. González, F. F. Costa and M. C. Chung, *J. Med. Chem.*, 2012, **55**, 7583.

7 G. Rai, C. J. Thomas, W. Leister and D. J. Maloney, *Tetrahedron Lett.*, 2009, **50**, 1710.

8 (*a*) L. Lazzarato, C. Cena, B. Rolando, E. Marini, M. L. Lolli, S. Guglielmo, E. Guaita, G. Morini, G. Coruzzi, R. Fruttero and A. Gasco, *Bioorg. Med. Chem*., 2011, **19**, 5852; (*b*) X.-Q. Zou, S.-M. Peng, C.-P. Hu, L.-F. Tan, H.-W. Deng and Y.-J. Li, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1222; (*c*) K. Chegaev, B. Rolando, S. Guglielmo, R. Fruttero and A. Gasco, *J. Heterocycl. Chem*., 2009, **46**, 866; (*d*) S. Balbo, L. Lazzarato, A. Di Stilo, R. Fruttero, N. Lombaert and M. Kirsch-Volders, *Toxicol. Lett*., 2008, **178**, 44; (*e*) M. F. Buonsanti, M. Bertinaria, A. Di Stilo, C. Cena, R. Fruttero and A. Gasco, *J. Med. Chem.*, 2007, **50**, 5003; (*f*) C. M. Turnbull, C. Cena, R. Fruttero, A. Gasco, A. G. Rossi and I. L. Megson, *Br. J. Pharmacol.*, 2006, **148**, 517; (*g*) P. Krause, E. Wätzig, H. Acil, S. König, K. Unthan-Fechner, D. Tsikas and I. Probst, *Nitric Oxide*, 2010, **23**, 220; (*h*) C. Cena, M. Bertinaria, D. Boschi, M. Giorgis and A. Gasco, *ARKIVOC*, 2006, **vii**, 301; (*i*) A. Ya. Kots, M. A. Grafov, Yu. V. Khropov, V. L. Betin, N. N. Belushkina, O. G. Busygina, M. Yu. Yazykova, I. V. Ovchinnikov, A. S. Kulikov, N. N. Makhova, N. A. Medvedeva, T. V. Bulargina and I. S. Severina, *Br. J. Pharmacol.*, 2000, **129**, 1163.

9 R. Ferioli, A. Fazzini, G. C. Folco, R. Fruttero, R. Calvino, A. Gasco, S. Bongrani and M. Civelli, *Pharmacol. Res*., 1993, **28**, 203.

10 R. Ferioli, G. C. Folco, C. Ferretti, A. M. Gasco, C. Medana, R. Fruttero, M. Civelli and A. Gasco, *Br. J. Pharmacol*., 1995, **114**, 816.

11 L. L. Fershtat, M. A. Epishina, A. S. Kulikov, I. V. Ovchinnikov, I. V. Ananyev and N. N. Makhova, *Tetrahedron*, 2015, **71**, 6764.

12 I. V. Ovchinnikov, A. O. Finogenov, M. A. Epishina, A. S. Kulikov, Yu. A. Strelenko and N. N. Makhova, *Russ. Chem. Bull., Int. Ed*., 2009, **58**, 2137 (*Izv. Akad. Nauk, Ser. Khim.*, 2009, 2072).

13 R. N. Puri and R. W. Colman, *Crit. Rev. Biochem. Mol. Biol.*, 1997, **32**, 437.

14 M. Ueno, M. Kodali, A. Tello-Montoliu and D. J. Angiolillo, *J. Atheroscler. Thromb*., 2011, **18**, 431.

Received: 7th July 2016; Com. 16/4987