Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2018, **28**, 623–625

Mendeleev Communications

Regioselective synthesis, structural diversification and cytotoxic activity of (thiazol-4-yl)furoxans

Alexander S. Kulikov,[a] Margarita A. Epishina,[a] Artem I. Churakov,[a,b] Lada V. Anikina,[c] Leonid L. Fershtat[a] and Nina N. Makhova*[a]

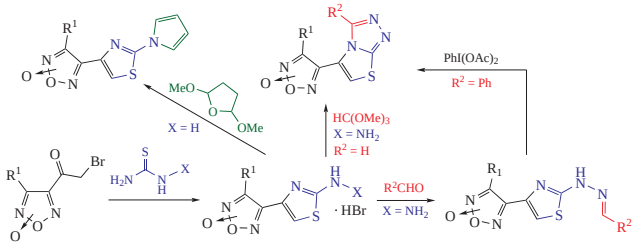
[a] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 5328; e-mail: mnn@ioc.ac.ru*

[b] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation*

[c] *Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation*

DOI: 10.1016/j.mencom.2018.11.020

The effective and regioselective synthesis of new (2-hydrazinylthiazol-4-yl)furoxan hydrobromides based on the condensation of (bromoacetyl)furoxans with thiosemicarbazide has been developed. The cytotoxic activity of their derivatives (with hydrazone, 4-thiazolo[2.3-*c*][1,2,4]triazole or pyrrole moieties) against two human cancer cell lines (A549, HCT116) was tested and several structures revealed moderate cytotoxic activity.

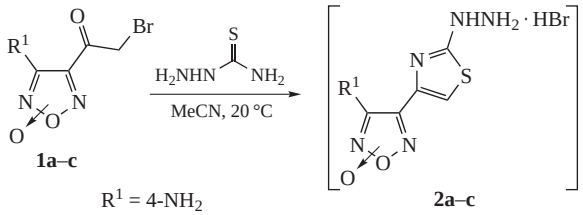


In modern drug design molecular hybridization approach became a powerful tool, leading to the creation of novel drug candidates with improved pharmacological profile. In recent decades, numerous efforts have been directed toward the construction of hybrid pharmaceuticals containing the NO-releasing fragments. Among the variety of nitrogen–oxygen organic motifs capable of releasing NO under physiological conditions, the furoxan (1,2,5-oxadiazole 2-oxide) scaffold has attracted considerable attention due to its high stability under ambient conditions and the absence of nitrate tolerance under continuous therapy.[1] Recently, we have developed effective one-pot syntheses of various hybrid heterocyclic systems incorporating furoxan ring as NO-donor linked to different pharmacophoric nitrogen-containing heterocycles (isomeric 1,2,3- and 1,2,4-triazoles, 1,2,4- and 1,3,4-oxadiazoles, tetrazole, pyridine, tetrahydroisoquinoline, indenopyridine, isoxazole, isoxazoline, *etc.*).[2] Among them, a series of compounds with cytotoxic activity was revealed.[3]

To extend the range of possible cytotoxic furoxan derivatives with potential NO-donor properties, the specific goal of this work was the synthesis of new hybrid structures comprising the furoxan and 2-(hydrazin-4-yl)thiazole fragments, and studying the cytotoxic activity of various (thiazol-4-yl)furoxan derivatives.

Thiazole derivatives have received a great interest due to their association with various kinds of biological properties, found in a number of biologically active molecules such as sulfathiazole (antimicrobial drug),[4] ritonavir (antiretroviral drug),[5] abafungin (antifungal drug),[6] tiazofurin (antineoplastic drug).[7] Thiazoles are well represented in biomolecules,[8] possess antibacterial activity against some gram-positive and gram-negative bacteria,[9] and a number of thiazole derivatives showed anticancer activity.[10] Therefore, assembling the furoxan NO-donor motif and the functionally (*e.g.*, hydrazinyl) substituted thiazole ring in one molecule may serve as a platform for search for new compounds with cytotoxic activity. One of the prospective methods for the preparation of 2-hydrazinylthiazoles is a condensation of thiosemicarbazide with α-halo carbonyl compounds. However, this reaction generally brings about three regioisomers including 2-amino-1,3,4-thiadiazine derivatives[11] (major regioisomer), 2-hydrazinylthiazoles, and 3-amino-2-thiazolinimines.[12]

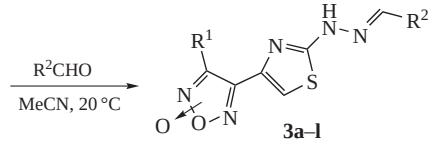
Available (bromoacetyl)furoxans[13] **1** were taken as initial α-halo carbonyl compounds. Reaction between 4-amino-3-(bromoacetyl)furoxan **1a** and thiosemicarbazide (in equimolar amounts)



1a–c

R^1 = 4-NH_2
R^1 = 3-Me
R^1 = 4-(1*H*-pyrrol-1-yl)

2a–c
84–95%



3a–l

3	R^1	R^2	Yield (%)
a	4-NH_2	4-$MeOC_6H_4$	83
b	3-Me	4-$MeOC_6H_4$	79
c	4-(1*H*-pyrrol-1-yl)	4-$MeOC_6H_4$	83
d	4-NH_2	3-MeO-4-$EtOC_6H_3$	64
e	4-NH_2	3,4,5-$(MeO)_3C_6H_2$	83
f	4-NH_2	PhCH=CH	82
g	3-Me	Ph	81
h	3-Me	4-ClC_6H_4	58
i	3-Me	PhCH=CH	85
j	4-(1*H*-pyrrol-1-yl)	PhCH=CH	61
k	4-(1*H*-pyrrol-1-yl)	3-MeO-4-$EtOC_6H_3$	70
l	4-(1*H*-pyrrol-1-yl)	3,4,5-$(MeO)_3C_6H_2$	58

Scheme 1

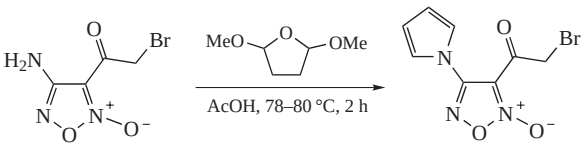
Table 1 Optimization of the reaction conditions for the synthesis of 4-amino-3-(2-hydrazinylthiazol-4-yl)furoxan hydrobromide **2a**.

Entry	Solvent	*T*/°C	*t*/h	Additive	Isolated yield of **2a** (%)
1	EtOH	20	4	–	63
2	EtOH	3→20	2	$NaHCO_3$	40[a]
3	MeCN	0	0.2	DBU	decomposition
4	MeCN	0	12	HBr	73
5	MeCN	20	4	–	95
6	DMF	20	1	–	90
7	DMSO	20	2	–	92

[a] HBr-free product decomposed quickly.

was selected as a model one for the optimization of the synthesis of 4-amino-3-(2-hydrazinylthiazol-4-yl)furoxan hydrobromide **2a** (Scheme 1). Solvents, temperature and reaction times were varied. The reaction in EtOH at room temperature afforded the target hydrobromide **2a** in moderate yield (Table 1, entry 1). Attempts to obtain HBr-free product by application of bases were unsuccessful. The use of $NaHCO_3$ led to the desired compound, but it decomposed quickly upon storage (entry 2), while employment of DBU in MeCN resulted in total decomposition (entry 3). Addition of HBr (MeCN) increased the yield slightly (entry 4). High yields of the compound **2a** were achieved when the reaction was carried out in MeCN, DMF or DMSO without any additives, although the reaction time differed (entries 5–7). Therefore, the optimal conditions for the synthesis of thiazole **2a** comprised an interaction of equimolar amounts of the reactants in MeCN for 4 h at 20 °C.

The found optimal conditions were applied to the synthesis of other (2-hydrazinylthiazol-4-yl)furoxans **2b**,**c**.† 3-Bromoacetyl-4-(1*H*-pyrrol-1-yl)furoxan **1c** was prepared by Clauson–Kaas condensation of compound **1a** with 2,5-dimethoxytetrahydrofuran (Scheme 2).



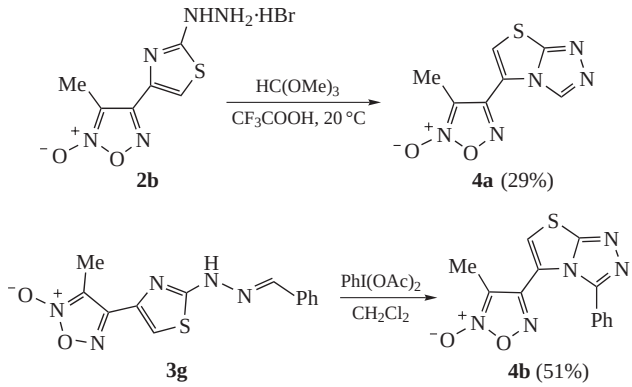
1a **1c** (77%)

Scheme 2

(2-Hydrazinylthiazol-4-yl)furoxan hydrobromides **2b**,**c** were successfully obtained in high yields. It is of note that the transformation occurs completely regioselectively in contrast to the similar reaction of α-bromoacetophenones resulting in 2-amino-1,3,4-thiadiazine derivatives.[11] The structure of the synthesized hydrobromides **2a**–**c** was estimated by spectral and analytical data. Since all signals of protons in ^{1}H NMR spectra of compounds **2a**,**b** were broadened, while compound **2c** decomposed in DMSO-d_6, the structures of **2a**–**c** were additionally confirmed by their conversion into 4-methoxybenzaldehyde hydrazones **3a**–**c** (see Scheme 1).

Hydrazones are of importance as intermediates for the development of new pharmacologically active compounds,[14] including a variety of nitrogen-containing heterocyclic structures. In particular, fused 1,2,4-triazoles can be prepared *via* oxidative condensation of hetarylhydrazones.[15] In addition, hydrazones themselves possess a wide range of pharmacological activities.[16] For example, furoxan-containing hydrazones revealed antitubercular,[17] antiplatelet and *in vivo* antithrombotic effects.[18] A series of furoxan-based chalcone hybrids can be considered as promising *in vivo* phase II enzyme inducers which is very useful in chemopreventive cancer therapy.[19] Therefore, we have synthesized a series of hydrazones **3a**–**l** based on (2-hydrazinylthiazol-4-yl)furoxans. Hydrazones **3a**,**b** were obtained by treatment of hydrazine hydrobromides **2a**,**b** with 4-methoxybenzaldehyde.‡ Hydrazones **3c**–**l** were obtained through a one-pot protocol *via* an interaction of the corresponding (bromoacetyl)furoxans **1** with thiosemicarbazide followed by addition of the corresponding aldehyde§ (see Scheme 1).

To broaden the set of the thiazolylfuroxans for the study of the cytotoxic activity, we performed a representative annulation of the 1,2,4-triazole ring into the thiazole one (Scheme 3). For this aim two approaches can be used, namely, an oxidative condensation of hydrazones[15] and condensation of hydrazinylthiazoles with ortho esters under acid catalysis.[20] Compound **4a** was prepared by the condensation of salt **2b** with trimethyl orthoformate in CF_3COOH. Thiazolo[2,3-*c*][1,2,4]triazole **4b** was synthesized *via* oxidative condensation of hydrazone **3g** under the action of $PhI(OAc)_2$ (see Scheme 3).



2b **4a** (29%)

3g **4b** (51%)

Scheme 3

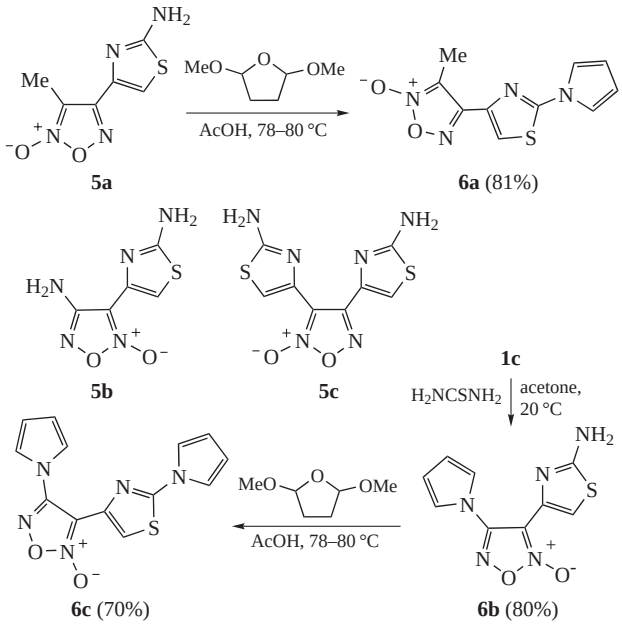
In addition, a set of compounds for the cytotoxic activity screening was extended to the previously synthesized[21] mono- and diaminothiazolylfuroxans **5a**–**c** along with the pyrrole **6a** obtained by the Clauson–Kaas condensation of compound **5a**, and pyrroles **6b**,**c** prepared by the consecutive transformation of bromoacetyl derivative **1c** (Scheme 4).¶

The cytotoxic activity of compounds **3**–**6** (18 compounds) was tested *in vitro* by MTT assay against two human cancer cell lines: A549 (lung adenocarcinoma) and HCT116 (colon cancer). Camptothecin was used as positive control. Cell viability was evaluated after 72 h of exposure to the compounds at 100–1.56 μM concentrations (Table S1, Online Supplementary Materials). The biological investigations have shown that the majority of com-

† *Synthesis of (2-hydrazinylthiazol-4-yl)furoxan hydrobromides* **2a**–**c**. An appropriate bromoacetylfuroxan **1a**–**c** (3 mmol) was added to a magnetically stirred suspension of thiosemicarbazide (0.27 g, 3 mmol) in MeCN (3 ml) at room temperature. The reaction mixture was stirred for 4 h at the same temperature and the resulting solid (**2a**–**c**) was filtered off, washed with MeCN (2 ml) and dried in air.

‡ *Synthesis of 3(4)-(2-hydrazinylthiazol-4-yl)-4(3)-R^1-furoxan 4-methoxybenzaldehyde hydrazones* **3a**,**b**. 4-Methoxybenzaldehyde (0.27 g, 2 mmol) was added to a suspension of compound **2a** or **2b** (2 mmol) in MeCN (6 ml). The mixture was stirred at room temperature for 24 h, then water (12 ml) was added. The resulting solid was filtered off, washed with water (6 ml) and cold EtOH (4 ml) and dried in air.

§ *One-pot synthesis of hydrazones* **3c**–**l**. An appropriate (bromoacetyl)-furoxan **1a**–**c** (3 mmol) was added to a stirred suspension of thiosemicarbazide (0.27 g, 3 mmol) in MeCN (3 ml) at room temperature. The mixture was stirred for 4 h, then a solution of the corresponding aldehyde (3 mmol) in MeCN (3 ml) was added. The stirring was continued for 24 h, then water (18 ml) was added, the resulting solid was filtered off, washed with water (6 ml) and cold EtOH (4 ml) and dried in air.



Scheme 4

pounds revealed rather low cytotoxic activity against both cell lines. The most active compounds against A549 cell line were hydrazones **3j** (IC_{50} 36.04 μM), **3c** (IC_{50} 90.11 μM) and **3i** (IC_{50} 96.29 μM). It was found that these compounds possessed selective activity against A549 cell line, while their cytotoxic activity against HCT116 cell line was lower (IC_{50} 70.74, 193.04 and 247.62 μM, respectively).

In summary, an effective and regioselective synthesis of new hybrid structures comprising furoxan and 2-hydrazinylthiazol-4-yl fragments based on the condensation between (bromoacetyl)-furoxans and thiosemicarbazide has been developed. The structural diversification of synthesized compounds brought about a series of functional derivatives containing, along with thiazolylfuroxan moiety, hydrazone, 4-thiazolo[2,3-*c*][1,2,4]triazole and pyrrole subunits. The cytotoxic activity of the synthesized compounds was tested *in vitro* by MTT assay against two human cancer cell lines (A549 and HCT116) and several structures revealed moderate cytotoxicity. To the best of our knowledge, this study represents a new, important step in the construction of hybrid pharmacologically oriented structures containing the NO-releasing fragments.

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

Online Supplementary Materials

Supplementary data associated with this article (synthetic procedures, characteristics of the synthesized compounds and cytotoxicity assay) can be found in the online version at doi: 10.1016/j.mencom.2018.11.020.

References

1 (*a*) A. Gasco and K. Schoenafinger, in *Nitric Oxide Donors: For Pharmaceutical and Biological Applications*, eds. P. G. Wang, T. B. Cai and N. Taniguchi, Wiley-VCH, Weinheim, 2005, pp. 131–175; (*b*) S. Guglielmo, D. Cortese, F. Vottero, B. Rolando, V. P. Kommer, D. L. Williams, R. Fruttero and A. Gasco, *Eur. J. Med. Chem.*, 2014, **84**, 135; (*c*) N. Zhao, K. T. Tian, K. G. Cheng, T. Han, X. Hu, D. H. Li, Z. L. Li and H. M. Hua, *Bioorg. Med. Chem.*, 2016, **24**, 2971; (*d*) Y. Fang, R. Wang, M. He, H. Huang, Q. Wang, Z. Yang, Y. Li, S. Yang and Y. Jin, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 98; (*e*) N. N. Makhova and O. A. Rakitin, *Chem. Heterocycl. Compd.*, 2017, **53**, 849 (*Khim. Geterotsikl. Soedin.*, 2017, **53**, 849); (*f*) L. L. Fershtat and N. N. Makhova, *ChemMedChem*, 2017, **12**, 622; (*g*) N. E. Ustyuzhanina, L. L. Fershtat, M. L. Gening, N. E. Nifantiev and N. N. Makhova, *Mendeleev Commun.*, 2016, **26**, 513; (*h*) N. E. Ustyuzhanina, L. L. Fershtat, M. L. Gening, N. E. Nifantiev and N. N. Makhova, *Mendeleev Commun.*, 2018, **28**, 49.

2 (*a*) L. L. Fershtat, M. A. Epishina, A. S. Kulikov, I. V. Ovchinnikov, I. V. Ananyev and N. N. Makhova, *Tetrahedron*, 2015, **71**, 6764; (*b*) L. L. Fershtat, I. V. Ananyev and N. N. Makhova, *RSC Adv.*, 2015, **5**, 47248; (*c*) L. L. Fershtat, A. A. Larin, M. A. Epishina, A. S. Kulikov, I. V. Ovchinnikov, I. V. Ananyev and N. N. Makhova, *Tetrahedron Lett.*, 2016, **57**, 4268; (*d*) L. L. Fershtat, M. A. Epishina, I. V. Ovchinnikov, M. I. Struchkova, A. A. Romanova, I. V. Ananyev and N. N. Makhova, *Tetrahedron Lett.*, 2016, **57**, 5685; (*e*) L. L. Fershtat and N. N. Makhova, *Russ. Chem. Rev.*, 2016, **85**, 1097; (*f*) A. A. Larin, L. L. Fershtat, I. V. Ananyev and N. N. Makhova, *Tetrahedron Lett.*, 2017, **58**, 3993; (*g*) S. G. Zlotin, A. M. Churakov, I. L. Dalinger, O. A. Luk'yanov, N. N. Makhova, A. Yu. Sukhorukov and V. A. Tartakovsky, *Mendeleev Commun.*, 2017, **27**, 535; (*h*) L. L. Fershtat, A. A. Larin, M. A. Epishina, I. V. Ovchinnikov, A. S. Kulikov, I. V. Ananyev and N. N. Makhova, *RSC Adv.*, 2016, **6**, 31526; (*i*) L. L. Fershtat, M. R. Radzhabov, A. A. Romanova, I. V. Ananyev and N. N. Makhova, *Arkivoc*, 2017, (**iii**), 140.

3 A. S. Kulikov, A. A. Larin, L. L. Fershtat, L. V. Anikina, S. A. Pukhov, S. G. Klochkov, M. I. Struchkova, A. A. Romanova, I. V. Ananyev and N. N. Makhova, *Arkivoc*, 2017, (**iii**), 250.

4 J. R. Anacona, J. L. Rodriguez and J. Camus, *Spectrochim. Acta, Part A*, 2014, **129**, 96.

5 B. S. Taylor, G. Hunt, E. J. Abrams, A. Coovadia, T. Meyers, G. Sherman, R. Strehlau, L. Morris and L. Kuhn, *AIDS Res. Hum. Retrovirus.*, 2011, **27**, 945.

6 A. Al-mamari, M. Al-buryhi, M. Al-heggami and S. Al-hag, *Der Pharma Chemica*, 2014, **6**, 336.

7 N. Siddiqui, M. F. Arshad, W. Ahsan and M. S. Alam, *Int. J. Pharm. Sci. Drug Res.*, 2009, **1**, 136.

8 N. Siddiqui, S. K. Arya, W. Ahsan and B. Azad, *Int. J. Drug Dev. Res.*, 2011, **3**, 55.

9 K. Kapadiya, K. Kavadia, P. Manvar, R. Kotadiya, R. Kothari and K. Ranjan, *Chem. Biol. Interface*, 2015, **5**, 258.

10 (*a*) A. Chawla, A. S. Sheelmani, P. Chawla and R. K. Dhawan, *Adv. J. Pharm. Life Sci. Res.*, 2014, **2**, 1; (*b*) S. M. Gomha, M. R. Abdel-Aziz, N. A. Kheder, H. M. Abdel-Aziz, S. Alterary and Y. N. Mabkhot, *Chem. Centr. J.*, 2017, **11**, 105.

11 L. B. Kulikova, G. I. Ezhova, N. E. Kravchenko, O. V. Dorofeeva, A. S. Kulikov and A. G. Zavozin, *Russ. Chem. Bull., Int. Ed.*, 2007, **56**, 1631 (*Izv. Akad. Nauk, Ser. Khim.*, 2007, 1570).

12 (*a*) H. Beyer, *Z. Chem.*, 1969, **9**, 361; (*b*) R. E. Busby and F. W. Dominay, *J. Chem. Soc.*, 1980, **6**, 890.

13 A. B. Sheremetev, A. S. Kulikov and L. I. Khmel'nitskii, *Russ. Chem. Bull.*, 1993, **42**, 708 (*Izv. Akad. Nauk, Ser. Khim.*, 1993, 744).

14 S. Rollas and S. G. Küçükgüzel, *Molecules*, 2007, **12**, 1910.

15 R. Kamal, V. Kumar and R. Kumar, *Chem. Asian J.*, 2016, **11**, 1988.

16 N. N. Makhova and L. L. Fershtat, *Tetrahedron Lett.*, 2018, **59**, 2317.

17 G. F. S. Fernandes, P. C. Souza, L. B. Marino, K. Chegaev, S. Guglielmo, L. Lazzarato, R. Fruttero, M. C. Chung, F. R. Pavan and J. L. Dos Santos, *Eur. J. Med. Chem.*, 2016, **123**, 523.

18 L. A. Dutra, J. F. O. Guanaes, N. Johmann, M. E. L. Pires, C. M. Chin, S. Marcondes and J. L. Dos Santos, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 2450.

19 M. Cabrera, I. Mastandrea, G. Otero, H. Cerecetto and M. González, *Bioorg. Med. Chem.*, 2016, **24**, 1665.

20 T. Nagamatsu and T. Fujita, *Heterocycles*, 2002, **57**, 631.

21 A. S. Kulikov and N. N. Makhova, *Russ. Chem. Bull.*, 1998, **47**, 139 (*Izv. Akad. Nauk, Ser. Khim.*, 1998, 137).

¶ *Synthesis of (thiazol-4-yl)furoxans* **6a–c**. 2,5-Dimethoxytetrahydrofuran (1.40 g, 10.6 mmol) was added to a solution of (2-aminothiazol-4-yl)-furoxan **5a** or **6b** (10 mmol) in AcOH (20 ml) at room temperature. The mixture was stirred at 78–80 °C for 2 h (TLC monitoring, eluent $CHCl_3$–EtOAc, 3:1), AcOH was evaporated under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$–CCl_4, 1:3).

Received: 30th May 2018; Com. 18/5593