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Design of hetarylthiofuroxans by nucleophilic substitution of NO_2 group in nitrofuroxans

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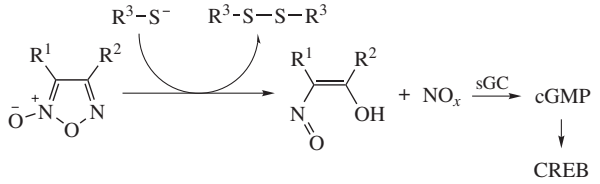
Hetarylthiofuroxans were synthesized by nucleophilic substitution of the nitro group in 4-nitrofuroxans under the action of hetarylthiols in the DBU–MeCN system at room temperature, reactivity of 4-nitrofuroxans being dependent on the C(3)-substituent.

Furoxan derivatives are of increased attention owing to a plethora of interesting biological activities.[1–6] This interest is focused on highly reactive furoxans that produce large fluxes of NO. The most commonly accepted mechanism underlying furoxan activity involves a thiol-dependent NO release.[7] NO stimulates the cyclic guanosine monophosphate (cGMP) production through the activation of NO-sensitive soluble guanylyl cyclase (sGC or NO-GC), which in turn leads to the activation of the memory-related transcription factor, a cyclic adenosine monophosphate (cAMP) response element binding the protein (CREB) (Scheme 1).[8–10]



Scheme 1

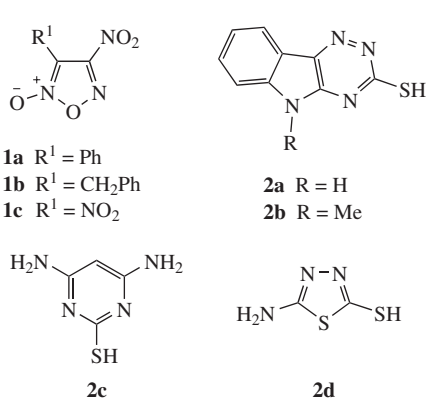
While NO exerts a cytotoxic effect at high concentrations, low levels of NO are potentially protective, particularly in the central nervous system.[11] An ability of furoxans to release NO in the presence of thiols depends on the nature of substituents at carbon atoms of the furoxan ring and therefore different substituents can assumingly control a propensity for the NO release.[12] Isomeric 3-aryl-4-nitro- and 4-aryl-3-nitrofuroxans proved to be the most effective NO donors upon the coincubation with 5 mM L-cystein.[12]

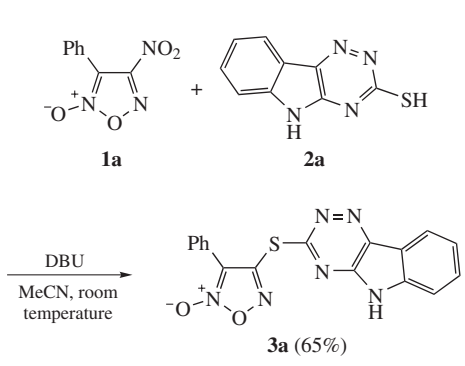
This research is to examine the dependence of 4-nitrofuroxan reactivity on the C(3)-substituent of the furoxan ring (Ph, Bn and NO_2, compounds **1a–c**, for their synthesis see refs. 13–15) under the action of pre-synthesized hetarylthiols **2a–c**[16,17] and commercial 5-amino-1,3,4-thiadiazole-2-thiol **2d**. Triazinethiols **2a**,**b** represent a promising family for the search of antihypoxic agents.[18] Diaminopyrimidinethiol **2c** supported on gold nanoparticles has a broad spectrum of antibacterial and bactericidal activities against superbugs.[19] Thiol **2d** proved to be a versatile carbonic anhydrase inhibitor against cytosolic human isozymes I and II and the transmembrane, tumor-associated human carbonic anhydrase IX.[20] A combination of the furoxan ring – a potential NO donor – and pharmacophoric heterocycles **2a–d** in one molecule is likely to bring about a wide range of pharmacological activities.

Although S_NAr substitution in nitro-activated substrates is well documented,[21] examples of the nitro group substitution in nitrofuroxans are scarce.[22] The range of S-nucleophiles is restricted and consists of several highly reactive mercaptanes[23] in the presence of Et_3N and thiophenol in the presence of aqueous NaOH.[24] Sulfur-bridged hetarylfuroxans had been so far unknown.

To find the proper reaction conditions, we used 4-nitro-3-phenylfuroxan **1a** and triazinethiol **2a** as initial compounds (Scheme 2). For the reaction to proceed it is necessary to generate the thiolate-anion from thiol **2a** under the action of basic reagents. Since furoxans fall into a base-sensitive type of heterocycles, we employed the base in an equimolar amount. However, the conditions described (Et_3N–MeCN, NaOH–acetone)[22,23] for the known nucleophilic substitutions in nitrofuroxans with S-nucleophiles appeared ineffective in this case. Therefore, we tested eight base–solvent systems such as KOH–DMF, K_2CO_3–DMF, K_2CO_3–acetone, Et_3N–MeCN, DBU–MeCN, DBU–[bmim]BF_4, [bmim]OH–DMF and [bmim]OH–[bmim]BF_4. Target hetarylthiofuroxan **3a** was only obtained in the DBU–MeCN system at room temperature.

This procedure was extended to other 4-nitrofuroxans **1b**,**c** and hetarylthiols **2b–d** (Scheme 3). As expected, the reaction afforded the corresponding hetarylthiofuroxans **3a–h** (Table 1).†





Scheme 2

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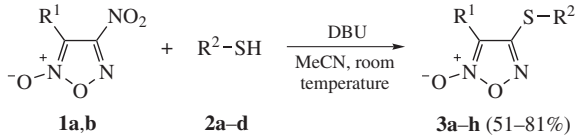
Table 1 Synthesis of hetarylthiofuroxans **3a–h**.

Reactants	*t*/h	Product	Isolated yield (%)
1a + 2a	8	**3a**	65
1a + 2b	8	**3b**	67
1a + 2c	8	**3c**	81
1a + 2d	8	**3d**	57
1b + 2a	10	**3e**	62
1b + 2b	10	**3f**	56
1b + 2c	10	**3g**	53
1b + 2d	10	**3h**	51



1a,**b** **2a–d** **3a–h** (51–81%)

Scheme 3

However, yields of products **3e–h** from 3-benzyl-4-nitrofuroxan **1b** were somewhat lower than those of compounds **3a–d** from 4-nitro-3-phenylfuroxan **1a** and required a longer reaction time (TLC monitoring).

† High resolution mass spectra (HRMS) were measured on a Bruker micrOTOF II instrument using electrospray ionization (ESI). The NMR spectra were recorded using a Bruker AM-300 spectrometer at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C spectra in DMSO-d_6 at 25 °C. TMS was used as the internal standard. TLC was conducted on silica gel plates (Silufol UV-254).

Caution! Dinitrofuroxan **1c** must be used only as a solution in appropriate solvent due to its propensity to explode.

General procedure for the preparation of hetarylthiofuroxans **3a–i**. DBU (0.5 mmol, 0.08 g) was added dropwise at room temperature to a magnetically stirred suspension of appropriate thiol **2** (0.5 mmol) in 1 ml of MeCN. The mixture was stirred for 10 min and then appropriate nitrofuroxan **1** (0.5 mmol) was added. The mixture was stirred for 0.5–10 h until initial compound **1** disappeared (TLC monitoring, eluent – $CHCl_3$). Next, H_2O (7 ml) was added and the reaction mixture was acidified with 1 N HCl until pH 1. The produced solid was filtered off, washed with water and the MeCN minimal volume (~1 ml), and air-dried. Compounds **3i** and **4** were prepared as a mixture. The mixture was treated with 2 ml of DMSO at 60 °C for 30 min; the undissolved solid was filtered off, water-washed and air-dried to afford disulfide **4**. The filtrate was diluted with water and the solid was filtered off, water-washed and air-dried to afford compound **3i**.

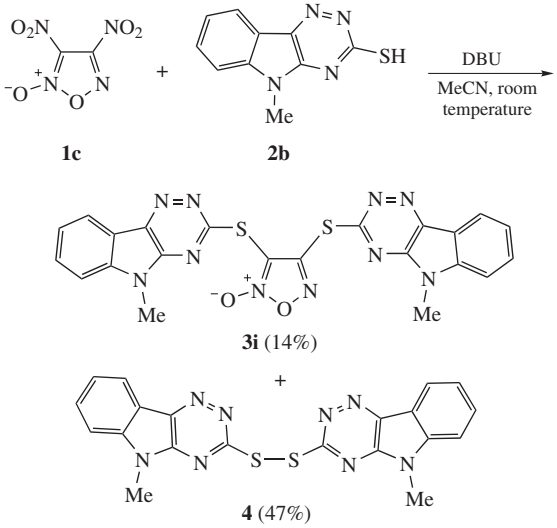
*4-[(5*H*-[1,2,4]Triazino[5,6-*b*]indol-3-yl)thio]-3-phenylfuroxan* **3a**. Yield 0.12 g (65%), mp 187–189 °C. ^{1}H NMR, δ: 7.43 (br. s, 3H, Ph), 7.45 (br. s, 2H, Ph), 7.54 (d, 1H, Ar, ^3J 8.6 Hz), 7.70 (dd, 1H, Ar, ^3J 7.5 Hz), 7.86 (d, 1H, Ar, ^3J 8.6 Hz), 8.30 (dd, 1H, Ar, ^3J 7.7 Hz), 12.87 (s, 1H, NH). ^{13}C NMR, δ: 112.83 ($C^3_{furoxan}$), 121.93, 127.54, 128.93, 130.97 (Ph), 122.77, 122.93, 126.11, 127.78, 129.04, 130.40, 151.43 and 161.70 ($C_{triazineindole}$), 152.54 ($C^4_{furoxan}$), 163.21 (CS). HRMS (ESI), *m*/*z*: 363.0657 $[M + H]^+$ (calc. for $C_{17}H_{10}N_6O_2S$, *m*/*z*: 363.0619).

*4-[(5-Methyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio]-3-phenylfuroxan* **3b**. Yield 0.125 g (67%), mp 216–218 °C. ^{1}H NMR, δ: 3.69 (s, 3H, Me), 7.44 (br. s, 5H, Ph), 7.76, 7.88, 8.27 (all br. s, 4H, Ar). ^{13}C NMR, δ: 27.61 (Me), 111.47 ($C^3_{furoxan}$), 122.08, 127.83, 128.97, 131.80 (Ph), 116.84, 121.88, 123.36, 126.93, 129.12, 130.97, 142.10 and 161.33 ($C_{triazineindole}$), 153.66 ($C^4_{furoxan}$), 166.44 (CS). HRMS (ESI), *m*/*z*: 377.0815 $[M + H]^+$ (calc. for $C_{18}H_{12}N_6O_2S$, *m*/*z*: 377.0776).

4-[(4,6-Diaminopyrimidin-2-yl)thio]-3-phenylfuroxan **3c**. Yield 0.12 g (81%), mp 139–141 °C. ^{1}H NMR, δ: 5.08 (s, 1H, $CH_{pyrimidine}$), 6.25 (s, 4H, $2NH_2$), 7.50 (s, 3H, Ph), 7.85 (s, 2H, Ph). ^{13}C NMR, δ: 79.73 ($CH_{pyrimidine}$), 112.28 ($C^3_{furoxan}$), 122.92, 128.08, 129.47, 130.67 (Ph), 155.82 ($C^4_{furoxan}$), 163.55 ($2CNH_2$), 165.15 ($SC_{pyrimidine}$). HRMS (ESI), *m*/*z*: 303.0665 $[M + H]^+$ (calc. for $C_{12}H_{10}N_6O_2S$, *m*/*z*: 303.0619).

4-[(5-Amino-1,3,4-thiadiazol-2-yl)thio]-3-phenylfuroxan **3d**. Yield 0.08 g (57%), mp 282–284 °C. ^{1}H NMR, δ: 7.63 (s, 5H, Ph), 7.91 (s, 2H, NH_2). ^{13}C NMR, δ: 108.23 ($C^3_{furoxan}$), 121.41, 128.45, 129.33, 131.42 (Ph), 152.44 ($C^4_{furoxan}$), 159.61 (CNH_2), 172.93 (SCS). HRMS (ESI), *m*/*z*: 294.0123 $[M + H]^+$ (calc. for $C_{10}H_7N_5O_2S_2$, *m*/*z*: 294.0075).

The behavior of dinitrofuroxan **1c** in the reaction with thiols **2a–d** differed from that of other nitrofuroxans. The interaction between compound **1c** and thiol **2b** in the 1 : 1 molar ratio unexpectedly resulted in a mixture of minor disubstituted adduct **3i** and major disulfide **4** (Scheme 4). Varying the reaction temperature (–10 °C, 0 °C, room temperature) and molar ratio of compounds **1c** : **2b** from 2 : 1 to 1 : 2 merely provided disulfide **4**, whereas the reaction of thiol **2b** with nitrofuroxans **1a**,**b** failed to yield any disulfide. Other thiols did not couple with dinitro-



1c **2b**

3i (14%)

+

4 (47%)

Scheme 4

*3-Benzyl-4-[(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio]furoxan* **3e**. Yield 0.11 g (62%), mp 142–144 °C. ^{1}H NMR, δ: 4.21 (s, 2H, CH_2), 7.31 (s, 5H, Ph), 7.63–7.84 (m, 4H, Ar), 12.24 (s, 1H, NH). ^{13}C NMR, δ: 28.86 (CH_2), 111.04 ($C^3_{furoxan}$), 121.66, 128.06, 128.98, 131.14 (Ph), 122.85, 122.99, 126.78, 128.05, 129.37, 130.86, 149.08 and 161.92 ($C_{triazineindole}$), 152.98 ($C^4_{furoxan}$), 163.67 (CS). HRMS (ESI), *m*/*z*: 377.0809 $[M + H]^+$ (calc. for $C_{18}H_{12}N_6O_2S$, *m*/*z*: 377.0776).

*3-Benzyl-4-[(5-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio]furoxan* **3f**. Yield 0.11 g (56%), mp 155–157 °C. ^{1}H NMR, δ: 3.74 (s, 3H, Me), 3.92 (s, 2H, CH_2), 7.31 (s, 5H, Ph), 7.69, 7.78, 8.14 (all br. s, 4H, Ar). ^{13}C NMR, δ: 27.34 (Me), 28.15 (CH_2), 111.32 ($C^3_{furoxan}$), 124.13, 128.35, 129.93, 131.03 (Ph), 117.81, 120.91, 122.31, 126.46, 129.37, 130.76, 142.01 and 162.48 ($C_{triazineindole}$), 154.87 ($C^4_{furoxan}$), 167.12 (CS). HRMS (ESI), *m*/*z*: 391.0979 $[M + H]^+$ (calc. for $C_{19}H_{14}N_6O_2S$, *m*/*z*: 391.0932).

3-Benzyl-4-[(4,6-diaminopyrimidin-2-yl)thio]furoxan **3g**. Yield 0.08 g (53%), mp 111–113 °C. ^{1}H NMR, δ: 4.39 (s, 2H, CH_2), 5.18 (s, 1H, $CH_{pyrimidine}$), 6.20 (s, 4H, $2NH_2$), 7.34 (s, 5H, Ph). ^{13}C NMR, δ: 29.52 (CH_2), 79.86 ($CH_{pyrimidine}$), 109.68 ($C^3_{furoxan}$), 123.32, 128.67, 129.47, 132.09 (Ph), 155.12 ($C^4_{furoxan}$), 163.79 ($2CNH_2$), 166.88 ($SC_{pyrimidine}$). HRMS (ESI), *m*/*z*: 317.0803 $[M + H]^+$ (calc. for $C_{13}H_{12}N_6O_2S$, *m*/*z*: 317.0776).

4-[(5-Amino-1,3,4-thiadiazol-2-yl)thio]-3-benzylfuroxan **3h**. Yield 0.08 g (51%), mp 203–205 °C. ^{1}H NMR, δ: 4.13 (s, 2H, CH_2), 7.35 (s, 5H, Ph), 7.98 (s, 2H, NH_2). ^{13}C NMR, δ: 29.03 (CH_2), 109.46 ($C^3_{furoxan}$), 121.88, 128.34, 129.97, 131.06 (Ph), 151.73 ($C^4_{furoxan}$), 159.89 (CNH_2), 172.04 (SCS). HRMS (ESI), *m*/*z*: 308.0282 $[M + H]^+$ (calc. for $C_{11}H_9N_5O_2S_2$, *m*/*z*: 308.0231).

*3,4-Bis[(5-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio]furoxan* **3i**. Yield 0.035 g (14%), mp 294–296 °C. ^{1}H NMR, δ: 3.82 and 3.88 (2s, 6H, 2Me), 7.43 (d, 2H, Ar, ^3J 7.5 Hz), 7.69 (m, 4H, Ar), 8.05 (d, 2H, Ar, ^3J 7.5 Hz). ^{13}C NMR, δ: 27.63 and 28.12 (2Me), 112.97 ($C^3_{furoxan}$), 116.92, 121.22, 122.49, 123.11, 126.57, 127.76, 128.80, 129.09, 130.44, 131.87, 142.03 and 161.13 ($C_{triazineindole}$), 155.83 ($C^4_{furoxan}$), 167.46 and 167.85 (2CS). HRMS (ESI), *m*/*z*: 515.0737 $[M + H]^+$ (calc. for $C_{22}H_{14}N_{10}O_2S_2$, *m*/*z*: 515.0776).

*1,2-Bis(5-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)disulfane* **4**. Yield 0.10 g (47%), mp 301–303 °C. ^{1}H NMR, δ: 3.56 (s, 6H, 2Me), 7.60, 7.87, 8.09, 8.41 (all br. s, 8H, Ar). ^{13}C NMR, δ: 28.94 (Me), 115.63, 121.86, 126.76, 128.16, 130.63, 133.16, 142.16, 165.28 ($C_{triazineindole}$), 172.14 (CS). HRMS (ESI), *m*/*z*: 431.0805 $[M + H]^+$ (calc. for $C_{20}H_{14}N_8S_2$, *m*/*z*: 431.0816).

furoxan **1c** and decomposed in 96 h. Previous investigations of the chemical behavior of dinitrofuroxan **1c** demonstrated a propensity for the 4-NO_2 group substitution, while the 3-NO_2 group substitution only occurred with a highly nucleophilic sodium ethoxide.[25] The formation of disulfides in known reactions of nitrofuroxans with sulfur nucleophiles was not observed before. The found reaction is the first example of the 3-NO_2 group nucleophilic substitution in nitrofuroxans under the action of sulfur nucleophiles. Formation of disulfide **4** can be explained by the oxidative nature of dinitrofuroxan **1c** and a higher reductive capacity of thiol **2b** compared to thiols **2a**,**c**,**d**.

Structures of synthesized compounds **3a–i**, **4** were established by ^{1}H and ^{13}C NMR spectroscopy and high resolution mass spectrometry (HRMS) with electrospray ionization (ESI).

In conclusion, we have reported a general approach to hetarylthiofuroxans by nucleophilic substitution in nitrofuroxan derivatives in good yields and under mild conditions. Some relationship between the type of C(3)-substituent in 4-nitrofuroxans and their reactivity was revealed. The target compounds are of interest as promising antibacterial and cytotoxic agents as well as prospective NO donors. Investigations on their pharmacological activity are now in progress.

References

1 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, *Brit. J. Pharmacol.*, 2000, **129**, 1163.

2 C. Cena, M. L. Lolli, L. Lazzarato, E. Guaita, G. Morini, G. Coruzzi, S. P. McElroy, I. L. Megson, R. Fruttero and A. Gasco, *J. Med. Chem.*, 2003, **46**, 747.

3 M. F. Buonsanti, M. Bertinaria, A. Di Stilo, C. Cena, R. Fruttero and A. Gasco, *J. Med. Chem*., 2007, **50**, 5003.

4 L. Boiani, G. Aguirre, M. Gonzalez, H. Cerecetto, A. Chidichimo, J. J. Cazzulo, M. Bertinaria and S. Guglielmo, *Bioorg. Med. Chem*., 2008, **16**, 7900.

5 K. Chegaev, C. Cena, M. Giorgis, B. Rolando, P. Tosco, M. Bertinaria, R. Fruttero, P.-A. Carrupt and A. Gasco, *J. Med. Chem.*, 2009, **52**, 574.

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

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

8 Y. F. Lu, E. R. Kandel and R. D. Hawkins, *J. Neurosci.*, 1999, **19**, 10250.

9 C. L. Bon and J. Garthwaite, *J. Neurosci*., 2003, **23**, 1941.

10 J. E. Haley, G. L. Wilcox and P. F. Chapman, *Neuron*, 1992, **8**, 211.

11 G. R. J. Thatcher, A. C. Nicolescu, B. M. Bennett and V. Toader, *Free Radical Biol. Med.*, 2004, **37**, 1122.

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

13 L. L. Fershtat, M. I. Struchkova, A. S. Goloveshkin, I. S. Bushmarinov and N. N. Makhova, *Heteroatom Chem*., 2014, **25**, 226.

14 I. V. Ovchinnikov, N. N. Makhova and L. I. Khmel'nitskii, *Mendeleev Commun*., 1993, 210.

15 I. V. Ovchinnikov, N. N. Makhova and L. I. Khmel'nitskii, *Russ. Chem. Bull*., 1995, **44**, 702 (*Izv. Akad. Nauk, Ser. Khim*., 1995, 722).

16 R. Sharma, A. K. Pandey, R. Shivahare, K. Srivastava, S. Gupta and P. Chauhan, *Bioorg. Med. Chem. Lett*., 2014, **24**, 298.

17 S. Mohebbi, J. M. Falcon-Perez, E. Gonzalez, O. Millet, J. M. Mato and F. Kobarfard, *Chem. Pharm. Bull*., 2012, **60**, 70.

18 A. B. Tomchin, O. Yu. Uryupov, T. I. Zhukova, T. A. Kuznetsova, M. V. Kostycheva and A. V. Smirnov, *Pharm. Chem. J*., 1997, **31**, 125.

19 Y. Zhao, Z. Chen, Y. Chen, J. Xu, J. Li and X. Jiang, *J. Am. Chem. Soc*., 2013, **135**, 12940.

20 G. L. Almajan, A. Innocenti, L. Puccetti, G. Manole, S. Barbuceanu, I. Saramet, A. Scozzafava and C. T. Supuran, *Bioorg. Med. Chem. Lett*., 2005, **15**, 2347.

21 F. Terrier, *Modern Nucleophilic Aromatic Substitution*, Wiley-VCH, Weinheim, 2013.

22 N. N. Makhova and A. S. Kulikov, *Russ. Chem. Rev*., 2013, **82**, 1007.

23 D. Boschi, G. Sorba, M. Bertinaria, R. Fruttero, R. Calvino and A. Gasco, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1751.

24 A. Gasco, V. Mortarini, G. Rua and A. Serafino, *J. Heterocycl. Chem.*, 1973, **10**, 587.

25 T. I. Godovikova, O. A. Rakitin, S. P. Golova, S. A. Vozchikova, M. V. Povorin and L. I. Khmel'nitskii, *Chem. Heterocycl. Compd.*, 1994, **30**, 465 (*Khim. Geterotsikl. Soedin.*, 1994, 529).

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