

New functionalized aminofurazans as potential antimitotic agents in the sea urchin embryo assay

Aleksei B. Sheremetev,^{*a} Dmitrii E. Dmitriev,^a Nataliya K. Lagutina,^a Mikhail M. Raihstat,^a
Alex S. Kiselyov,^b Marina N. Semenova,^c Natalie N. Ikizalp^d and Victor V. Semenov^{*a,d}

^a N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 5328; e-mail: sab@ioc.ac.ru, vs@zelinsky.ru

^b deCODE Chemistry, Chicago, IL 60517, USA

^c N. K. Kol'tsov Institute of Developmental Biology, Russian Academy of Sciences, 119334 Moscow, Russian Federation

^d Chemical Block Ltd., 3723, Limassol, Cyprus

DOI: 10.1016/j.mencom.2010.05.002

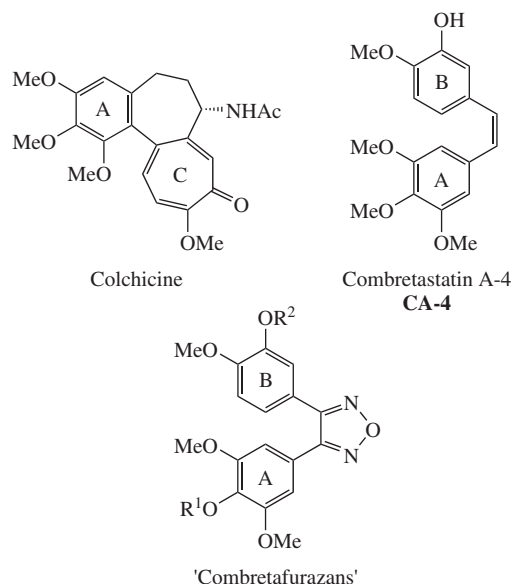
A series of new furazan (1,2,5-oxadiazole) derivatives based on structural overlap with combretastatin have been synthesized. Targeted molecules were evaluated using the sea urchin embryo assay; several agents demonstrated 1–4 $\mu\text{mol dm}^{-3}$ antiproliferative activity in this *in vivo* model.

Targeting tubulin in rapidly dividing tumor cells has been a well validated strategy for cancer therapy.^{1,2} Screening of natural products for cytotoxic activity yielded structurally diverse classes of mitotic spindle poisons. Most of the known anti-mitotic agents, including colchicine and *Vinca* alkaloids, inhibit tubulin polymerization. In contrast, TaxolTM has an opposite action: it stimulates polymerization *in vitro* and stabilizes spindle MT's.³ High toxicity found for the mitotic poisons^{4–9} prompted scientists to expand their search for the synthetic modulators of tubulin that (a) mimic natural products, (b) result in the same anti-mitotic effect and (c) display better efficacy/safety window. In general, colchicine derivatives are structurally simpler than *Vinca* alkaloids or TaxolTM derivatives, as exemplified by combretastatin A-4 (CA-4). This agent features a tilted 'biaryl' structural motif (A and B rings) connected by a hydrocarbon bridge of variable length. The linker provides for *cis*-configuration of the biaryl template necessary for the efficient interaction of a molecule with the colchicine binding site of tubulin.¹⁰

Note that five-membered heterocycle templates were reported to provide both non-isomerizable and metabolically stable isosteric replacement for the *cis*-styrene featured in combretastatins.¹¹ For example, combretafurazans were reported to be more potent in anti-mitotic agents compared to CA-4 in neuroblastoma cells.¹²

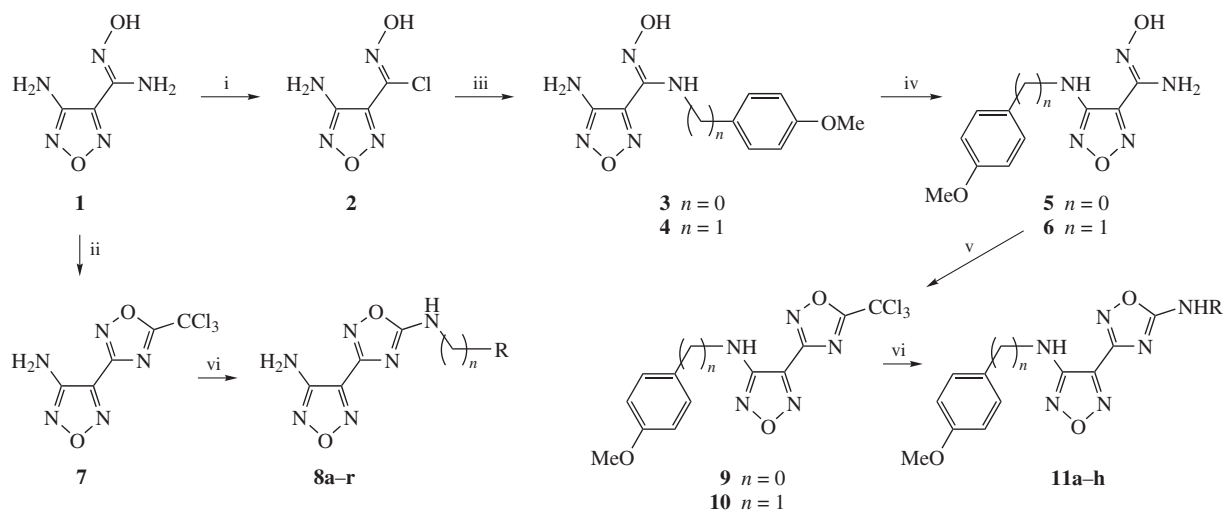
In our search for new synthetic anti-mitotic agents, we focused on heterocyclic molecules with pharmacophore arrangement that mimics combretastatin and related compounds. Initially, we studied derivatives of both 1,3,4-oxadiazoles^{13,14} and 1,3,4-triazoles.¹⁵ The biaryl (1,2,4-oxadiazol-3-yl)furazan template was expected to provide for the (i) isosteric replacement of the *cis*-styrene bond while maintaining proper alignment of the aryl substituents for the tubulin activity and (ii) yield better cell permeability compared to the substituted phenyl group.^{13–15} Considering complexity of both *in vitro* and *ex vivo* assays measuring tubulin activity of an agent, we decided to profile newly synthesized compounds in the sea urchin embryo model instead.¹⁶ This phenotypic *in vivo* assay includes (i) a fertilized egg test for antimitotic activity displayed by the cleavage alteration/arrest, and (ii) the behavioral monitoring of a free-swimming blastulae treated immediately after hatching.^{16,17}

Our chemistry efforts were centered around structural modifications of a 4-amino-3-(1,2,4-oxadiazol-3-yl)furazan template. An overview of this approach is summarized in Scheme 1.



Commercially available furazan derivative **1** provided for a good starting point in the synthesis. Key intermediate **2**^{18,19} was prepared by diazotization of amidoxime **1** as described previously. Treatment of **2** with substituted amines in the presence of Et₃N yielded corresponding amidoximes **3** and **4**. The subsequent ring-to-ring interconversion reaction^{20–22} took place with KOH in ethylene glycol at reflux to furnish **5** and **6**. Notably, this is the first example of the rearrangement applied to the synthesis of a bicyclic (1,2,4-oxadiazol-3-yl)furazan ring system. This step is both robust and high-yielding (~80% for both **5** and **6**).

In order to introduce a 1,2,4-oxadiazolyl-*N*-substituent into the targeted molecules, we first prepared trichloromethyl derivative **7**²³ from furazan derivative **1**^{24,25} using a modification of a published procedure. Specifically, the nucleophilic displacement of the CCl₃ group^{26,27} in compound **7** was achieved with amines in THF to afford desired molecules **8a–r**.[†] A wide variety of amine reagents were tolerated under the reaction conditions (Table 1). The yields of **8a–r** were 76–96%. Unfortunately, our attempts to further modify the unsubstituted amino group in these compounds *via* the reductive amination were unsuccessful



Scheme 1 Reagents and conditions: i, see refs. 18, 19; ii, see ref. 23; iii, *p*-MeOC₆H₄(CH₂)_nNH₂/NEt₃, EtOH or PrⁱOH; iv, KOH, ethylene glycol, reflux; v, CCl₃COCl, BuOAc, reflux; vi, RNH₂, THF.

under a variety of experimental conditions. Easy access to intermediates **5** and **6** (*vide supra*) allowed us to address this issue. Namely, ring-closing reactions of **5** and **6** to form targeted 1,2,4-oxadiazole derivatives **9** and **10** were accomplished with CCl₃COCl in butyl acetate at reflux. A final step towards desired molecules **11a–h** was accomplished *via* the nucleophilic displacement of a CCl₃ moiety with the respective amines in THF at room temperature. The yields ranged from 72 to 86% (Table 1).

We further elaborated the stepwise nucleophilic displacement of both NO₂ groups in 3,4-dinitrofurazan **12**²⁸ with N- and O-nucleophiles to access compound **14** as suggested by the earlier experimental data on the tubulin activity within the furazan series (Scheme 2).^{12,27,29} Synthetic protocols describing similar transformations in the furazan series are scarce, primarily due to the hazard of starting compound **12**. In our hands, this safety concern was successfully addressed by working with dilute (< 0.35 mol dm⁻³) solutions of **12** in CH₂Cl₂ at room temperature. While studying this conversion, we found that the initial introduction of the N-substituent was critical to the nucleophilic displacement of the remaining NO₂ group with the O-nucleophile. Following this protocol, we isolated compound **14** in 70.5% overall yield. Reversing the order of reactions, namely reacting **12** with the O-nucleophile followed by the N-nucleophile led to a complex mixture of products, none of them major (Scheme 2).

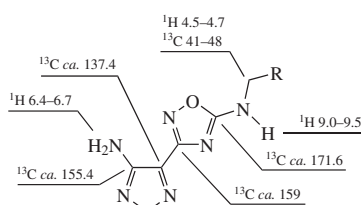
We further tested the molecules of **8a–r**, **11a–h** and **12** in the phenotypic sea urchin embryo assay in order to identify compounds targeting tubulin.^{16,17} Combretastatin A-4 disodium phosphate (CA-4P, OxiGene) served as a benchmark reference compound.¹⁶ We monitored the effect of furazans **8**, **11** and **14** on two specific developmental stages of the sea urchin embryo, namely: (i) fertilized egg to assess antimetabolic activity and (ii) behavioral monitoring of a free-swimming blastulae to detect changes in the embryo swimming pattern. In this assay,

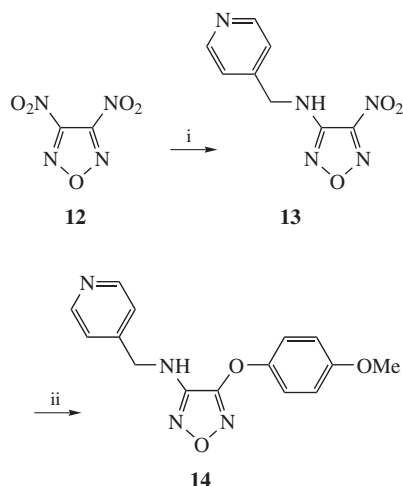
Table 1 Synthesis of 4-amino-3-(1,2,4-oxadiazol-3-yl)furazans **8a–r** and **11a–h**.

Product	<i>n</i>	R	Yield (%) ^a	Product	<i>n</i>	R	Yield (%) ^a
8a	1		94	8n	1		79
8b	1		89	8o	1		93
8c	1		90	8p	1		92
8d	2		96	8q	1		79
8e	1		88	8r	1		78
8f	1		93	11a	1	H	73
8g	1		85	11b	1		76
8h	1		76	11c	0		84
8i	1		91	11d	0		86
8j	1		87	11e	0		80
8k	1		92	11f	0		81
8l	1		85	11g	0		83
8m	1		79	11h	0		76

^aYields refer to the isolated analytically pure materials.[†]

[†] Both ¹H and ¹³C NMR data confirmed structures of the targeted furazans. For example, ¹³C NMR spectra of compounds **8a–r** featured four signals specific to quaternary carbons of the furazan-1,2,4-oxadiazole core. The IR spectrum of these molecules displayed intense aminofurazan-NH₂ vibrational bands at *ca.* 3440 and *ca.* 3320 cm⁻¹. Typical ranges for the ¹H and ¹³C NMR shifts of compounds **8a–r** are shown.





Scheme 2 Reagents and conditions: i, *p*-PyCH₂NH₂, CH₂Cl₂, Et₃N, room temperature; ii, *p*-MeOC₆H₄OH, K₂CO₃, DMSO, 80–100 °C.

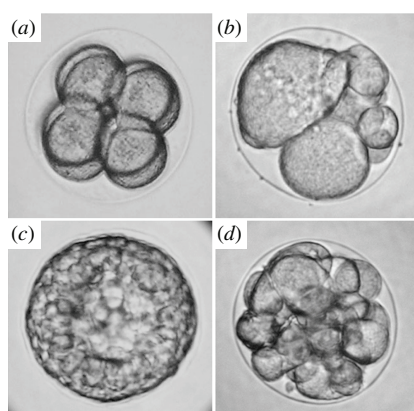


Figure 1 Effect of aminofurazans on the sea urchin embryo development. (a), (c) Intact embryos: (a) 8-cell stage; (c) early blastula. (b), (d) Typical cleavage abnormalities caused by aminofurazans. Fertilized eggs were exposed continuously to (b) 4 $\mu\text{mol dm}^{-3}$ of **8c** or (d) 2 $\mu\text{mol dm}^{-3}$ of **11g**. Time after fertilization: (a), (b) 3 h; (c), (d) 6 h. Average embryo diameter is 115 μm .

the molecules of **8b,c** and **11d,g** displayed EC₅₀ values of about 1–4 $\mu\text{mol dm}^{-3}$ suggesting an antimitotic tubulin destabilizing effect (Figure 1).

In summary, a series of furazan derivatives based on structural overlap with combretastatin have been designed, synthesized and tested in the phenotypic sea urchin embryo *in vivo* assay. The targeted molecules were prepared *via* a synthetic sequence involving the formation of key chloroamidoxime **2**. Compound **2** was converted to trichloromethyl 1,2,4-oxadiazoles **7**, **9** and **10** *via* a two-step protocol, namely, the ring-to-ring interconversion reaction of amidoximes **3** and **4** to yield compounds **5** and **6** with their subsequent cyclization. A nucleophilic displacement of the CCl₃ group in **7**, **9** and **10** with a series of amines furnished targeted furazans **8a–r** and **11a–h** in high yields. A furazan analogue of combretastatin **14** was prepared from 3,4-dinitrofurazan **12** *via* stepwise nucleophilic displacement of nitro groups with N- and O-nucleophiles. The order of reactions for **12** was discovered to be critical to the outcome of this conversion. All targeted furazan derivatives were conveniently purified *via* a straightforward recrystallization to furnish analytically pure materials immediately suitable for biological assays.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2010.05.002.

References

- 1 M. A. Jordan and L. Wilson, *Nature Rev. Cancer*, 2004, **4**, 253.
- 2 A. S. Kiselyov, K. Balakin, S. E. Tkachenko and A. V. Ivachtchenko, *Anti-Cancer Agents in Medicinal Chemistry*, 2007, **7**, 189.
- 3 L. Wordenmam and T. J. Mitchison, in *Microtubules*, eds. J. S. Hyams and C. W. Lloyd, Wiley-Liss, New York, 1994, p. 287.
- 4 E. K. Rowinsky, V. Chaudhry, D. R. Cornblath and R. C. Donehower, *J. Natl. Cancer Inst.*, 1993, **15**, 107.
- 5 H. C. Pitot, E. A. McElroy, Jr., J. M. Reid, A. J. Windebank, J. A. Sloan, C. Erlichman, P. G. Bagniewski, D. L. Walker, J. Rubin, R. M. Goldberg, A. A. Adjei and M. M. Ames, *Clin. Cancer Res.*, 1999, **5**, 525.
- 6 S. P. Cole, K. E. Sparks, K. Fraser, D. W. Loe, C. E. Grant, G. M. Wilson and R. G. Deeley, *Cancer Res.*, 1994, **54**, 5902.
- 7 A. Loricco, G. Rappa, R. A. Flavell and A. C. Sartorelli, *Cancer Res.*, 1996, **56**, 5351.
- 8 M. Kavallaris, D. Y. S. Kuo, C. A. Burkhart, D. L. Regl, M. D. Norris, M. Haber and B. Horwitz, *J. Clin. Invest.*, 1997, **100**, 1282.
- 9 P. Giannakakou, D. L. Sackett, Y. K. Kang, Z. Zhan, J. T. Buters, T. Fojo and M. S. Poruchynsky, *J. Biol. Chem.*, 1997, **272**, 17118.
- 10 E. Nogales, M. Whittaker, R. A. Milligan and K. H. Downing, *Cell*, 1999, **96**, 79.
- 11 T. Brown, H. Holt and M. Lee, *Top. Heterocycl. Chem.*, 2006, 7081.
- 12 G. C. Tron, F. Pagliai, E. D. Grosso, A. A. Genazzani and G. Sorba, *J. Med. Chem.*, 2005, **48**, 3260.
- 13 X. Ouyang, E. L. Piatnitski, V. Pattaropong, X. Chen, H.-Y. He, A. S. Kiselyov, A. Velankar, J. Kawakami, M. Labelle, L. Smith II, J. Lohman, S. P. Lee, A. Malikzay, J. Fleming, J. Gerlak, Y. Wang, R. L. Rosler, K. Zhou, S. Mitelman, M. Camara, D. Surguladze, J. F. Doody and M. C. Tuma, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1191.
- 14 X. Ouyang, A. Kiselyov, X. Chen, H.-Y. J. He, J. Kawakami, V. Pattaropong, E. Piatnitski, M. C. Tuma and J. Kincaid, *US Patent WO 2005/004818 A2*, 2005.
- 15 X. Ouyang, X. Chen, E. L. Piatnitskii, A. S. Kiselyov, H.-Y. He, Y. Mao, V. Pattaropong, Y. Yu, K. H. Kim, J. Kincaid, L. Smith, II, W. C. Wong, S. P. Lee, D. L. Milligan, A. Malikzay, J. Fleming, J. Gerlak, D. Dhanvanthri, J. F. Doody, H.-H. Chiang, S. N. Patel, Y. Wang, R. L. Rosler, P. Kussie, M. Labelle and M. C. Tuma, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 5154.
- 16 M. N. Semenova, A. S. Kiselyov and V. V. Semenov, *Biotechniques*, 2006, **40**, 765.
- 17 M. N. Semenova, A. S. Kiselyov, I. Y. Titov, M. M. Raihstat, M. Molodtsov, E. Grishchuk, I. Spiridonov and V. V. Semenov, *Chem. Biol. Drug Design*, 2007, **70**, 485.
- 18 V. G. Andrianov and A. V. Ereemeev, *Khim. Geterotsikl. Soedin.*, 1994, 470 [*Chem. Heterocycl. Compd. (Engl. Transl.)*, 1994, **30**, 370].
- 19 V. N. Yarovenko, M. M. Krayushkin, O. V. Lysenko, L. M. Kustov and I. V. Zavarzin, *Izv. Akad. Nauk, Ser. Khim.*, 1994, 444 (*Russ. Chem. Bull.*, 1994, **43**, 402).
- 20 V. G. Andrianov, V. G. Semenikhina, A. V. Ereemeev and A. P. Gaukhman, *Khim. Geterotsikl. Soedin.*, 1988, 1701 [*Chem. Heterocycl. Compd. (Engl. Transl.)*, 1988, **24**, 1410].
- 21 V. G. Andrianov and A. V. Ereemeev, *Khim. Geterotsikl. Soedin.*, 1990, 1443 [*Chem. Heterocycl. Compd. (Engl. Transl.)*, 1990, **26**, 1199].
- 22 V. G. Andrianov, V. G. Semenikhina and A. V. Ereemeev, *Khim. Geterotsikl. Soedin.*, 1991, 122 [*Chem. Heterocycl. Compd. (Engl. Transl.)*, 1991, **27**, 102].
- 23 V. G. Andrianov, E. N. Rozhkov and A. V. Ereemeev, *Khim. Geterotsikl. Soedin.*, 1994, 534 [*Chem. Heterocycl. Compd. (Engl. Transl.)*, 1994, **30**, 470].
- 24 T. Ichikawa, T. Kato and T. Takenishi, *J. Heterocycl. Chem.*, 1965, **2**, 253.
- 25 V. G. Andrianov, A. V. Ereemeev and Yu. B. Sheremet, *Khim. Geterotsikl. Soedin.*, 1988, 856 [*Chem. Heterocycl. Compd. (Engl. Transl.)*, 1988, **24**, 770].
- 26 R. Beaudegnies and S. Wendeborn, *Heterocycles*, 2003, **60**, 2417.
- 27 A. B. Sheremetev, V. G. Andrianov, E. V. Mantseva, E. V. Shatunova, N. S. Aleksandrova, I. L. Yudin, D. E. Dmitriev, B. B. Averkiev and M. Yu. Antipin, *Izv. Akad. Nauk, Ser. Khim.*, 2004, 569 (*Russ. Chem. Bull., Int. Ed.*, 2004, **53**, 596).
- 28 T. S. Novikova, T. M. Mel'nikova, O. V. Kharitonova, V. O. Kulagina, N. S. Aleksandrova, A. B. Sheremetev, T. S. Pivina, L. I. Khmel'nitskii and S. S. Novikov, *Mendeleev Commun.*, 1994, 138.
- 29 A. B. Sheremetev, O. V. Kharitonova, E. V. Mantseva, V. O. Kulagina, E. V. Shatunova, N. S. Aleksandrova, T. M. Mel'nikova, E. A. Ivanova, D. E. Dmitriev, V. A. Eman, I. L. Yudin, V. S. Kuzmin, Yu. A. Strelenko, T. S. Novikova, O. V. Lebedev and L. I. Khmel'nitskii, *Zh. Org. Khim.*, 1999, **35**, 1555 (*Russ. J. Org. Chem.*, 1999, **35**, 1525).

Received: 1st December 2009; Com. 09/3428