

N-(4-Methoxyphenyl)-substituted bicyclic isothioureas: effect on morphology of cancer cells

Anna V. Evdokimova,^a Alexander A. Alexeev,^a Evgeniya V. Nurieva,^{*,a}
Elena R. Milaeva,^{a,b} Sergei A. Kuznetsov^c and Olga N. Zefirova^{a,b}

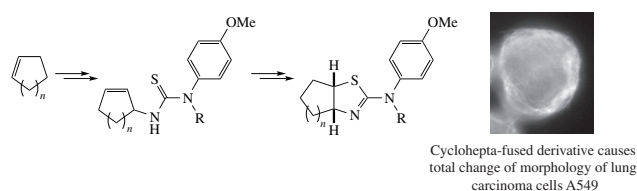
^a Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation.
E-mail: E.Selunina@org.chem.msu.ru

^b Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. E-mail: olgaz@med.chem.msu.ru

^c Institute of Biological Sciences, University of Rostock, D-18059 Rostock, Germany.
E-mail: sergei.kuznetsov@uni-rostock.de

DOI: 10.1016/j.mencom.2021.05.003

Bicyclic isothioureas of N-(4-methoxyphenyl)-2-aminocycloalkane[d]thiazole type were obtained using intramolecular electrophilic cyclization of N-(cycloalk-2-enyl)-N'-(4-methoxyphenyl)thioureas. Isothiourea fused with seven-membered ring caused noticeable changes of the morphology of human lung carcinoma cells A549, but without affecting their microtubule net.



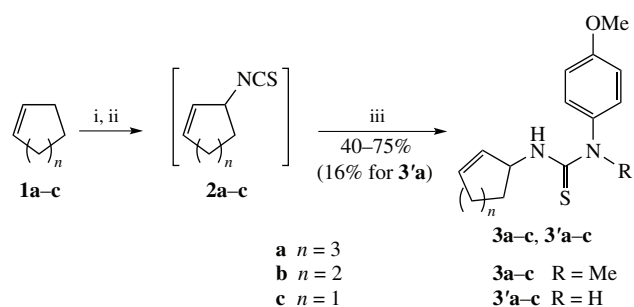
Keywords: thiazolin-2-amines, cycloalka[d]thiazoles, thioureas, isothioureas, heterocyclization, verubulin, lung carcinoma A549, cancer cell morphology.

In loving memory of our Great Teacher academician Nikolay S. Zefirov

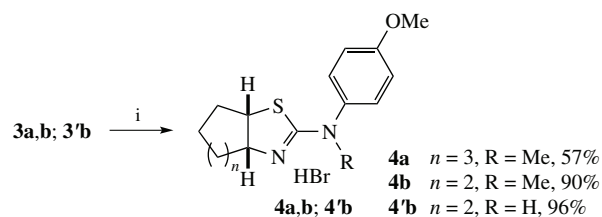
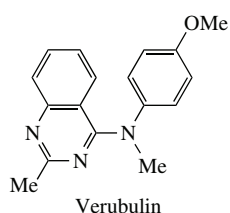
Interchange in aromatic residues is a classical approach used in drug design for optimization of characteristics of the initial molecule.^{1–4} The replacement of aromatic moiety by non-aromatic one is applied more rarely due to the pronounced differences in their geometric and electronic parameters and a consequent loss of activity. In some cases, however, such replacement allows one to enhance the activity (owing to acquired ability to fill more completely the available space in the target binding site) or to improve the pharmacological parameters of the initial molecule.⁵ Literature search reveals examples of successive replacement of aromatic group (thiazole, pyridine, etc.) by cyclic isothiourea moiety in the design of inhibitors of menin, mixed lineage leukemia fusion protein interaction,⁶ ligands of γ -aminobutyric acid receptors⁷ and inhibitors of enzyme nitric oxide synthase.⁸ In the present work, we carried out an analogous replacement in the structure of verubulin.⁹ The latter is a potent (but not clinically approved)¹⁰ ligand of colchicine binding site of cell protein tubulin, a validated molecular target of anticancer drugs.^{11–15} The proposed modification was aimed to study the potential of cyclic isothioureas as tubulin-targeted antimetabolic agents.

Schemes 1–3 demonstrate the synthesis of target compounds utilizing an intramolecular cyclization of cycloalkenyl-substituted ureas as the key step under the conditions elaborated

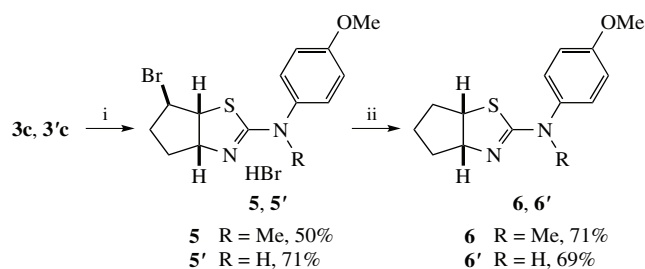
in our previous works.^{16–19} First, a convenient procedure involving sequential reactions of allylic bromination, nucleophilic substitution and addition to appropriate anisidine was fulfilled without evaporation and isolation of intermediate highly volatile bromides and isothiocyanates **2a–c**. In this way, substituted thioureas **3a–c**, **3'a–c** were obtained as racemic mixtures with acceptable yields. ¹H NMR spectra of these compounds contain the signal for CHN-cycloalkenyl proton at



Scheme 1 Reagents and conditions: i, NBS, AIBN, CCl₄, reflux, 1.5 h; ii, KSCN, MeOH, 0–22 °C, 2 h; iii, 4-MeOC₆H₄N(R)H, DIPEA, CH₂Cl₂, 22 °C, 12 h.



Scheme 2 Reagents and conditions: i, HBr, CH₂Cl₂, 22 °C, 24 h.



Scheme 3 Reagents and conditions: i, Br₂, CH₂Cl₂, 22 °C, 24 h; ii, Bu₃SnH, AIBN, toluene, 100 °C, 10 h.

4.9–5.5 ppm and the peaks for cycloalkenyl olefinic protons at 5.3–5.7 and 6.7–5.9 ppm (for the synthetic details and all characteristics of novel compounds, see Online Supplementary Materials).

Thioureas **3a,b** and **3'b** were converted into corresponding fused bicyclic isothioureas **4a,b** and **4'b** using electrophilic addition and simultaneous intramolecular cyclization under mild conditions by treatment with AcBr/MeOH (see Scheme 2). The cyclization occurred diastereoselectively with the formation of products with *cis*-configuration in accordance with our earlier study.¹⁶ In ¹H NMR spectra of **4a,b** and **4'b**, the two resonances in the range of 3.8–3.9 and 4.1–4.5 ppm correspond to the chemical shifts of protons at the ring junction of the fused system, while the signals at 60–67 and 48–52 ppm in ¹³C NMR spectra refer to the appropriate carbon atoms. The peak of thiazole C² atom is observed in the range of 169–174 ppm. Spectral data of compounds **4a,b** and **4'b** indicate the presence of one tautomer, however they are not diagnostic for exact elucidation of its structure. It should be mentioned, that we failed to isolate an NH analogue of **4a** with seven-membered ring, because the reaction with thiourea **3'a** led to a complex mixture of products.

To synthesize the target isothioureas fused with five-membered rings, the cyclization of thioureas **3c, 3'c** was carried out in the presence of bromine as more active electrophile (see Scheme 3). The chromatographic purification gave individual diastereoisomers **5, 5'** (one signal sets were observed in their ¹H NMR spectra). In ¹H NMR spectrum of compounds **5, 5'**, the peak for C⁶H–Br proton was observed at ~4.3 ppm and in ¹³C NMR spectrum, the corresponding carbon atom resonated in the range of 60–63 ppm. The configuration of bromine atom was assigned as *trans* towards thiazol ring on the basis of ¹H NOESY experiment (see Online Supplementary Materials). Bromo substituted derivatives **5, 5'** were subjected to reductive debromination by treatment with tri-*n*-butyltin hydride to give the target compounds **6, 6'** (mass spectral data: [M+H]⁺ = 263 for **6** and 249 for **6'**). The disappearance of the peaks at ~4.1–4.3 ppm in ¹H NMR and at ~70 ppm in ¹³C NMR confirms the removal of C⁶-bromine atom from initial compounds **5, 5'**. Analogously to **4**, compounds **5** and **6** were obtained as single tautomers.

Isothioureas **4a,b, 4'b** and **6, 6'** were studied for their ability to change the morphology and microtubule net of human lung carcinoma cells A549 using the standard procedures.^{20–22} The compounds with cyclohexane and cyclopentane rings **4b, 4'b** and **6, 6'** independently in the presence or absence of *N*-methyl substituent did not show any effect even at a high concentration of 100 μM. Only cyclohepta-fused isothioureas **4a** demonstrated noticeable changes in cell morphology (cell contraction and rounding) giving evidence to its cytotoxic action both at 10 and 100 μM (Figure 1). Though compound **4a** caused the termination of the cell growth, no changes in microtubules (MTs) were observed.

Therefore, one may suggest that the antimetabolic action of isothioureas **4a** relates to the interaction with molecular target

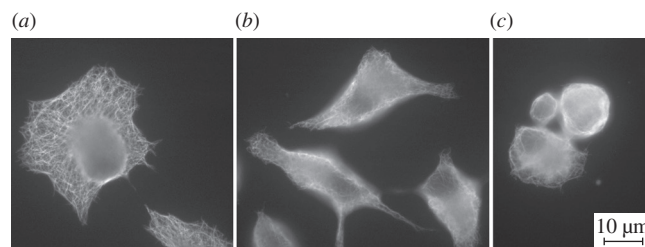


Figure 1 Immunofluorescence microscopy of the cells A549 treated for 24 h with (a): 0.5% DMSO (normal MTs); (b) 10 μM of **4a** (normal MTs, cell contraction); (c) 100 μM of **4a** (normal MTs, rounded cells).

other than tubulin. It is worth mentioning that the positive features of the lead molecule Verubulin include its ability to cause tumour vascular disruption and to inhibit the growth of multidrug resistant cells.¹⁰ Hence, theoretically its structural analogue **4a** may maintain these properties, which makes interesting further studies of novel cyclic isothioureas in the experiments *in vitro* and *in vivo*.

This work was supported by Russian Science Foundation (project no. 19-13-00084). Part of the work, comprising the data for compounds **3'b, 4'b, 5'**, was performed within the State assignment AAAA-A16-116032250004-2. The authors acknowledge B. Wobith (Rostock University) for technical assistance, Yu. K. Grishin (Moscow University) for performance of NMR experiments and the German organization DAAD for support of academic co-operation between Moscow (State) and Rostock Universities.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.05.003.

References

- S. R. Langdon, P. Ertl and N. Brown, *Mol. Inf.*, 2010, **29**, 366.
- G. A. Patani and E. J. LaVoie, *Chem. Rev.*, 1996, **96**, 3147.
- N. A. Meanwell, *J. Med. Chem.*, 2011, **54**, 2529.
- The Practice of Medicinal Chemistry*, 2nd edn., ed. C. G. Wermuth, Academic Press, Amsterdam, Boston, 2003.
- F. Lovering, J. Bikker and Ch. Humblet, *J. Med. Chem.*, 2009, **52**, 6752.
- J. Grembecka, S. He, A. Shi, T. Purohit, A. G. Muntean, R. J. Sorenson, H. D. Showalter, M. J. Murai, A. M. Belcher, T. Hartley, J. L. Hess and T. Cierpicki, *Nat. Chem. Biol.*, 2012, **8**, 277.
- A. Giraudo, J. Krall, F. Bavo, B. Nielsen, K. T. Kongstad, B. Rolando, R. De Blasio, D. E. Gloriam, R. Löffler, L. Thiesen, K. Harpsøe, K. Frydenvang, D. Boschi, P. Wellendorph, M. L. Lolli, A. A. Jensen and B. Frølund, *J. Med. Chem.*, 2019, **62**, 5797.
- S. Ueda, H. Terauchi, A. Yano, M. Matsumoto, T. Kubo, Y. Kyoya, K. Suzuki, M. Ido and M. Kawasaki, *Bioorg. Med. Chem.*, 2004, **12**, 4101.
- N. Sirisoma, A. Pervin, H. Zhang, S. Jiang, J. A. Willardsen, M. B. Anderson, G. Mather, C. M. Pleiman, S. Kasibhatla, B. Tseng, J. Drewe and S. X. Cai, *J. Med. Chem.*, 2009, **52**, 2341.
- Sh.-B. Wang, X.-F. Wang, B. Qin, E. Ohkoshi, K.-Y. Hsieh, E. Hamel, M.-T. Cui, D.-Q. Zhu, M. Goto, S. L. Morris-Natschke, K.-H. Lee and L. Xie, *Bioorg. Med. Chem.*, 2015, **23**, 5740.
- E. C. McLoughlin and N. M. O'Boyle, *Pharmaceuticals*, 2020, **13**, 8.
- I. A. Gracheva, E. S. Shchegryavina, H.-G. Schmalz, I. P. Beletskaya and A. Yu. Fedorov, *J. Med. Chem.*, 2020, **63**, 10618.
- L.-Y. Xia, Y.-L. Zhang, R. Yang, Zh.-Ch. Wang, Y.-D. Lu, B.-Zh. Wang and H.-L. Zhu, *Curr. Med. Chem.*, 2019, **26**, 1.
- K. Haider, S. Rahaman, M. Sh. Yar and A. Kamal, *Expert Opin. Ther. Pat.*, 2019, **29**, 623.
- G. La Regina, A. Coluccia, V. Naccarato and R. Silvestri, *Eur. J. Pharm. Sci.*, 2019, **131**, 58.
- E. V. Nurieva, T. P. Trofimova, A. A. Alexeev, A. N. Proshin, E. A. Chesnakova, Yu. K. Grishin, K. A. Lyssenko, M. V. Filimonova, S. O. Bachurin and O. N. Zefirova, *Mendeleev Commun.*, 2018, **28**, 390.
- A. A. Alexeev, E. V. Nurieva, T. P. Trofimova, E. A. Chesnakova, Yu. K. Grishin, K. A. Lyssenko, M. V. Filimonova and O. N. Zefirova, *Mendeleev Commun.*, 2019, **29**, 14.

- 18 A. A. Alexeev, E. V. Nurieva, K. A. Lyssenko, Yu. K. Grishin and O. N. Zefirova, *Struct. Chem.*, 2019, **30**, 473.
- 19 E. V. Nurieva, A. A. Alexeev, Yu. K. Grishin, V. A. Tafeenko and O. N. Zefirova, *Mendeleev Commun.*, 2020, **30**, 145.
- 20 A. Al-Haddad, M. A. Shonn, B. Redlich, A. Blocker, J. K. Burkhardt, H. Yu, J. A. Hammer, III, D. G. Weiss, W. Steffen, G. Griffiths and S. A. Kuznetsov, *Mol. Biol. Cell*, 2001, **12**, 2742.
- 21 N. A. Zefirov, Yu. A. Evteeva, A. I. Krasnoperova, A. V. Mamaeva, E. R. Milaeva, S. A. Kuznetsov and O. N. Zefirova, *Mendeleev Commun.*, 2020, **30**, 421.
- 22 E. V. Nurieva, N. A. Zefirov, N. Fritsch, E. R. Milaeva, S. A. Kuznetsov and O. N. Zefirova, *Mendeleev Commun.*, 2020, **30**, 706.

Received: 20th February 2021; Com. 21/6458