Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2017, **27**, 240–242

Mendeleev
Communications

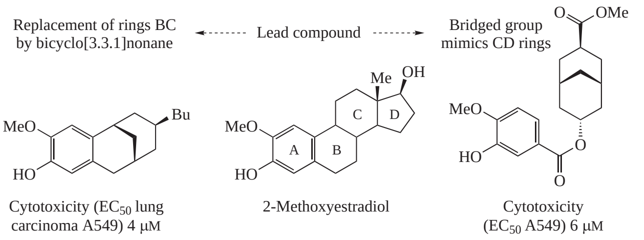
Synthesis of non-steroidal 2-methoxyestradiol mimetics based on the bicyclo[3.3.1]nonane structural motif

**Evgeniya V. Nurieva,[a] Nikolay A. Zefirov,[a] Alexandra V. Mamaeva,[a]
Yuri K. Grishin,[a] Sergei A. Kuznetsov[b] and Olga N. Zefirova*[a,c]**

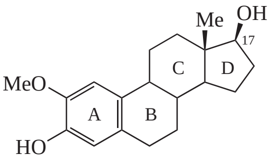
[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: olgaz@org.chem.msu.ru*
[b] *Institute of Biological Sciences, Cell Biology and Biosystems Technology, University of Rostock, D-18059 Rostock, Germany. E-mail: sergei.kuznetsov@uni-rostock.de*
[c] *Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation*

DOI: 10.1016/j.mencom.2017.05.007

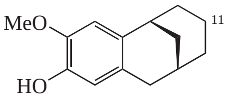
Two pairs of bicyclo[3.3.1]nonane derivatives with 2-methoxyphenol moiety were synthesized as non-steroidal 2-methoxyestradiol mimetics demonstrating noticeable cytotoxicity to human lung carcinoma cell line A549. Compounds with 2-methoxyphenol annulated with bicyclo[3.3.1]nonane fragment were obtained using BF_3-assisted oxirane opening in 5-(4-benzyloxy-3-methoxyphenyl)-1-oxaspiro[2.5]octanes with simultaneous intramolecular cyclization and reduction as the key step. *endo*-7-Hydroxybicyclo[3.3.1]nonane-*exo*-3-carboxylic acid was converted into two esters containing 2-methoxyphenol fragment in either acid or alcohol part of the molecule.



Antitumor activity of 2-methoxyestradiol (2-ME, **1**) has been attributed to its antitubulin, antiangiogenic, pro-apoptotic activities and reactive oxygen species (ROS) induction.[1] Being an endogenous metabolite in human organism, 2-ME is relatively low toxic to the normal cells at the concentrations effective for the anticancer therapy. Nevertheless, neither 2-ME itself nor its derivatives are used in clinics mainly because of the rapid *in vivo* metabolism of steroids.[2] This makes important the creation of non-steroidal analogues of compound **1**, however, little data on such a problem is still documented (see, *e.g.* ref. 3).



1



2

Moieties of bridged polycyclic compounds are not uncommon in drug design as structural templates replacing fused polycyclic (including steroid) cores of the lead compounds.[4–6] Therefore, we initiated the synthesis of bicyclo[3.3.1]nonane derivatives annulated with non-aromatic[7] or aromatic cycle[8] as non-steroidal 2-ME analogues and novel colchicine domain ligands. In the frame of these works, we elaborated a convenient method of synthesis of structural template **2**.[8]

Computer overlay of structures **1** and **2** in the most stable conformations demonstrates their good mapping and the benefit of replacement of C^{11}-positioned H-atom in **2** either with hydroxyalkyl group, mimicking the part of the D ring with C^{17} hydroxyl (Figure 1), or a flexible alkyl chain. The latter simulates the hydrophobic region, corresponding to the ring D of the initial steroid without C^{17} hydroxyl group, which is not strictly necessary for the antitumor activity of 2-ME analogues.[2]

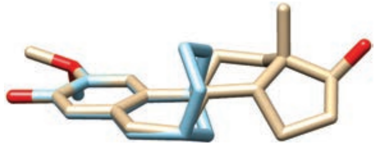
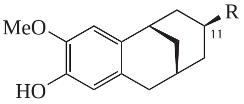
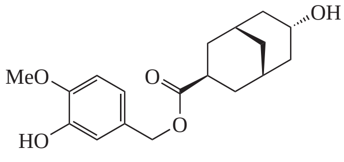


Figure 1 Computer superimposition of 2-ME (shown in ivory) and structure **2** (shown in blue) in the most stable conformations (made by visualization system USCF Chimera[9]).

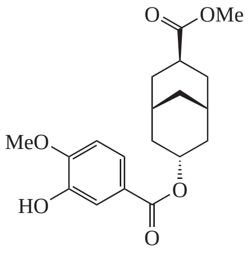
In this work we aimed to synthesize compounds **3a** and **3b** with *n*-butyl and 2-hydroxyethyl groups, respectively. It is seen from Figure 1 that *exo*-configuration of the C^{11} substituent is preferable (the configuration is relative as the compounds are racemates). Taking into account that during ligand–protein interaction the ligand can adopt not the most stable conformation, we



3a R = Bu
3b R = CH_2CH_2OH



4

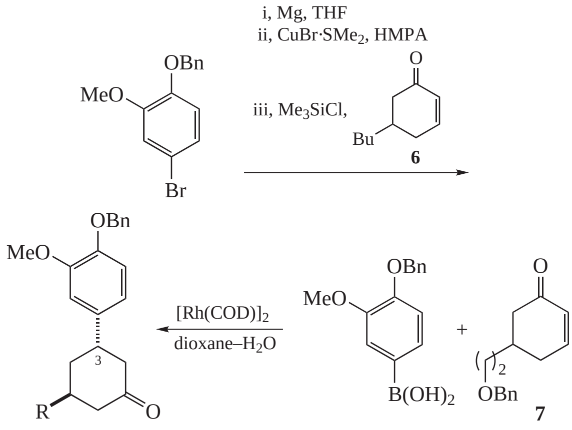


5

also imposed the structure **1** on a molecule **2** in a distorted chair–boat conformation. In this case, the *exo*-C^{11} substitution also fits the initial molecule better than the *endo*-substitution (see Online Supplementary Materials).

In parallel we tried to apply bicyclo[3.3.1]nonane core as the substituent for 2-ME fragment differing from BC ring system. In this work we obtained easily accessible esters **4** and **5** (conformationally more flexible than compounds **3a**,**b**) to enable the bridged system to adopt an optimal position in target proteins (close to that occupied by CD rings of 2-ME).

Compounds **3a** and **3b** were obtained *via* earlier elaborated protocol[8] involving the Friedel–Crafts intramolecular cyclization as a key step. Conjugate addition of arylcuprate obtained from 1-benzyloxy-4-bromo-2-methoxybenzene to *rac*-5-butylcyclohex-2-en-1-one **6**[10] afforded ketone **8a** in a moderate yield (Scheme 1). A similar attempted access to ketone **8b** gave a complex mixture of products. Ketone **8b** was synthesized by Rh-catalyzed 1,4-addition of 4-benzyloxy-3-methoxyphenylboronic acid to *rac*-5-(2-benzyloxyethyl)cyclohex-2-enone **7** (see Scheme 1).[†] In the ^1H NMR spectra of cyclohexenones **8a** and **8b**, the C^3-positioned axial proton is displayed as multiplet at 3.28 and 3.25 ppm, respectively.



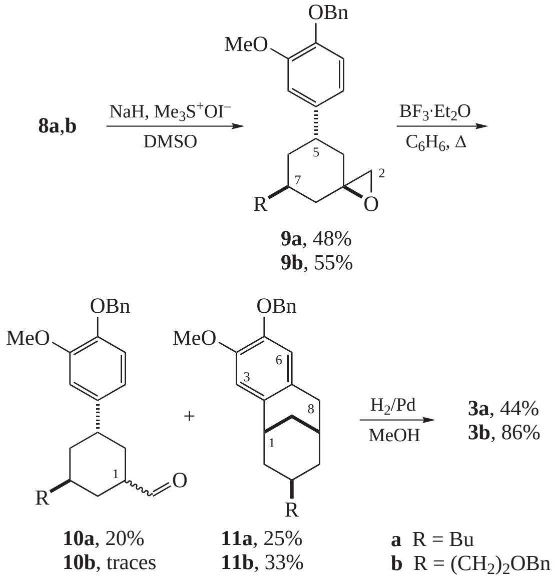
8a R = Bu (35% from **6**)
8b R = $(CH_2)_2OBn$ (59% from **7**)

Scheme 1

Compounds **8a** and **8b** were formed as individual *trans*-diastereomers. The NOE experiment for both products showed that irradiation at the HC^3 proton resonance did not cause response of HC^5 proton, but led to an increase in the intensity of the peaks of methylene group protons of the substituent at C^5 (see Online Supplementary Materials).

The Corey–Chaykovsky epoxidation of ketones **8a** and **8b** resulted in the oxirane diastereomers **9a** and **9b**, respectively (Scheme 2, the configuration was assigned in compliance with the literature data[11] and NMR spectroscopy).

The epoxide formation was proved both by the presence of two doublets (J ~ 5 Hz) of oxirane protons in the region 2.50–2.56 ppm in ^1H NMR spectra of **9a**,**b** and the appearance of additional resonance of carbon atom at 51.91 (**9a**) or 51.79 ppm (**9b**) in the ^{13}C NMR spectra. The *trans*-arrangement of the aryl substituent and epoxide oxygen in **9a** was confirmed by the NOE experiment revealing correlations between resonances of oxirane protons with the signals of H^{4eq} and H^{8eq}. *trans*-Configuration of the substituents at C^5 and C^7 in **9a** was also confirmed in the course of NOE experiment (see Online Supplementary Materials).



Scheme 2

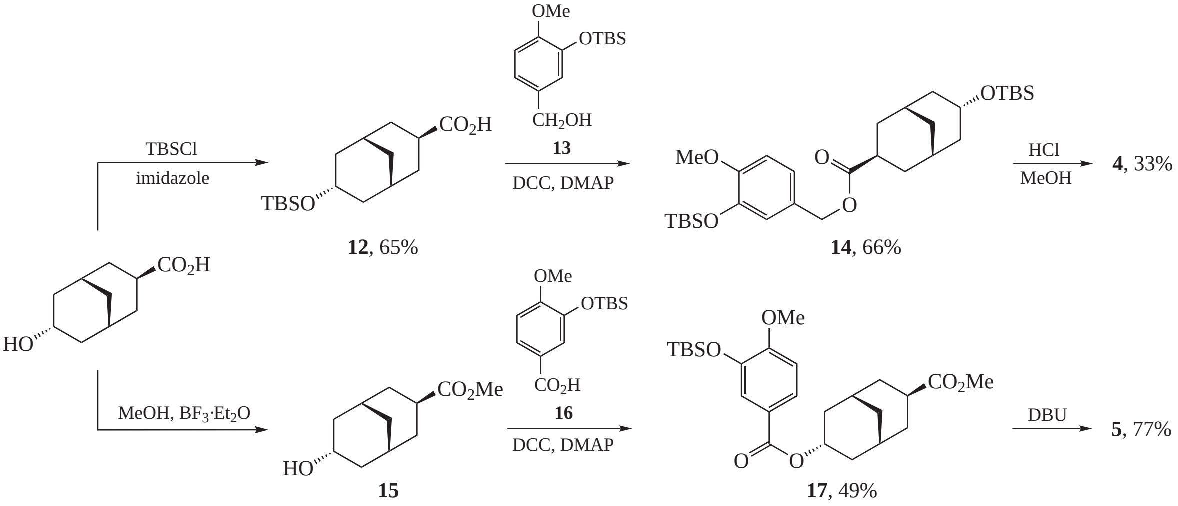
Analogously to compound **2**,[8] the BF_3-assisted epoxide opening in **9a** gave aldehyde **10a** and a product of intramolecular cyclization **11a**. Aldehyde **10a** was formed as a mixture of C^1 isomers in a ratio 2 : 1 according to the integral intensities of the signals in the ^1H NMR spectrum (the aldehyde proton singlets are displayed at 9.68 ppm for the major isomer and 9.81 ppm for the minor one). In the ^1H NMR spectrum of the cyclization product **11a** two protons resonate at 6.60 and 6.67 ppm. The presence of a doublet at 2.54 ppm (J 17.4 Hz) and a doublet of doublets at 3.01 ppm (J 17.4, 7.2 Hz) corresponding to aliphatic diastereotopic C^8 protons proves the formation of additional cycle. The ^{13}C NMR spectrum of **11a** contains eleven signals of aliphatic carbon atoms, four of butyl group and seven of bridged fragment.

For the analogous epoxide opening in compound **9b**, we used TLC to monitor the relationship between the aldehyde–cyclization product ratio and the reaction time (to clearly prove the sequence of the process). Prolongation of the reaction to 2 h allowed us to obtain mostly cyclization product **11b** (aldehyde **10b** was detected in a trace amount based on the aldehyde proton peak at 9.66 ppm). In the ^1H NMR spectrum of compound **11b** two singlets for aryl protons are observed at 6.59 and 6.66 ppm, the signals of diastereotopic C^8 protons are displayed as a doublet at 2.54 ppm (J 17.4 Hz) and a doublet of doublets at 3.01 ppm (J 17.4, 7.0 Hz).

Literature search revealed several examples of the BF_3-assisted epoxide opening followed by intramolecular cyclization[12] and an example of this process followed by elimination.[13] Obviously, the treatment of epoxides **9a**,**b** with $BF_3 \cdot Et_2O$ as the Lewis acid leads to the corresponding aldehydes *via* carbocationic species. In turn, attack of the benzene ring (activated by RO-substituents) by carbocation $R–CH^+–OBF_3$ results in aromatic substitution and formation of bicyclo[3.3.1]nonane framework with the OBF_3 group at C^8. In the reported processes the final products of such reactions are alcohols[12] or the corresponding alkenes.[13] However, in case of oxiranes **9a**,**b** the intermediate alcohols were not detected whereas $C^7=C^8$ or $C^8=C^9$ double bonds could not be created (*cf.*, *e.g.* the Bredt's rule[14]). Apparently, compounds **11a**,**b** were formed in the course of OBF_3 elimination and a hydride transfer to the corresponding carbocation giving C–H bond. This susceptibity towards reduction has been described for the bridged compounds and related adamantane derivatives, for example, adamantan-1-ol gave adamantane on treatment with $BF_3 \cdot Et_2O$ in THF at 160 °C.[15] The intriguing point in these cases is the source of hydride ion. In many papers the 'solvent' or 'media' is mentioned as a source of H^- without details, and only rarely the conclusions are supported by experiments with deuterated solvents.[15] In our

---

[†] For the synthesis and characteristics of compound **7** and all new compounds, see Online Supplementary Materials.



Scheme 3

case some amount of any starting compound may serve as hydride ion source. Obviously, special experiments using isotopic labeling are required to clear up the details of the mechanism in question.

Evidently, the intramolecular cyclization of **10a**,**b** is possible when the aryl and aldehyde substituents on cyclohexane ring are *cis* to one another leading to cyclization products **11a**,**b** as individual diastereomers, which is proved by their spectral data. Taking into account, that the configuration of *n*-butyl and 2-benzyloxyethyl substituents in ketones **8a**,**b** remains unchanged in the following reactions, these substituents were assigned to have *exo*-configuration in products **11a**,**b**. Deprotection of compounds **11a**,**b** resulted in the target 2-methoxyestradiol analogues **3a**,**b**, respectively.

The target compounds **4** and **5** were synthesized from *endo*-7-hydroxybicyclo[3.3.1]nonane-*exo*-3-carboxylic acid[16] (Scheme 3) *via* esterification of acid **12** with alcohol **13** or alcohol **15** with acid **16**.

Deprotection of compounds **14** and **17** led to the target esters **4** and **5**, respectively. The structures of these compounds were proved by NMR and IR spectral data, mass spectrometry [*m*/*z* 343 (M^+ + Na), 359 (M^+ + K) for **4**; 348 (M^+), 371 (M^+ + Na), 387 (M^+ + K) for **5**] and elemental analysis.

The primary biotesting of novel compounds **3a**,**b**, **4** and **5** was carried out in a standard MTT colorimetric assay on epithelial lung carcinoma cancer cells A549 (Table 1) using the described procedure.[17,18] 2-Methoxyestradiol **1** was used as a positive control; the cytotoxicity of compound **2** was also measured for comparison.

Table 1 The results of MTT test on the human lung carcinoma cell line A549.

Compound	Cytotoxicity, EC_{50}/μM	Compound	Cytotoxicity, EC_{50}/μM
3a	4 ± 2	**5**	6 ± 1
3b	> 50	**2**	> 50
4	47 ± 3	**1**	0.20 ± 0.05

According to the data given in Table 1, two compounds, **3a** and **5**, revealed a noticeable cytotoxicity (in submicromolar concentrations) towards A549 cells. Therefore, these non-steroidal analogues of 2-methoxyestradiol can be of interest for the study of mechanism of their cytotoxic action and further structural optimization.

This work was supported by the Russian Foundation for Basic Research (project no. 15-03-04894), Russian Academy of Sciences (OHNM-9) and the German Academic Exchange Service (DAAD) under the auspices of a collaborative agreement between Moscow and Rostock Universities. Authors acknowledge partial support from M. V. Lomonosov Moscow State University Program of Development and are grateful to Dr. Dmitrii V. Shishov for his kind help with the experiment.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2017.05.007.

References

1 B. S. Kumar, D. S. Raghuvanshi, M. Hasanain, S. Alam, J. Sarkar, K. Mitra, F. Khan and A. S. Negi, *Steroids*, 2016, **110**, 9.
2 N. A. Zefirov and O. N. Zefirova, *Russ. J. Org. Chem.*, 2015, **51**, 1207 (*Zh. Org. Khim.*, 2015, **51**, 1231).
3 M. P. Leese, F. L. Jourdan, M. R. Major, W. Dohle, E. Hamel, E. Ferrandis, A. Fiore, P. G. Kasprzyk and B. V. L. Potter, *ChemMedChem*, 2014, **9**, 85.
4 R. S. Muthyala, S. Sheng, K. E. Carlson, B. S. Katzenellenbogen and J. A. Katzenellenbogen, *J. Med. Chem.*, 2003, **46**, 1589.
5 O. N. Zefirova, E. V. Nurieva, H. Lemcke, A. A. Ivanov, N. V. Zyk, D. G. Weiss, S. A. Kuznetsov and N. S. Zefirov, *Mendeleev Commun.*, 2008, **18**, 183.
6 O. N. Zefirova and N. S. Zefirov, *Russ. Chem. Bull., Int. Ed.*, 2013, **62**, 325 (*Izv. Akad. Nauk, Ser. Khim.*, 2013, 329).
7 E. V. Nurieva, I. S. Semenova, V. N. Nuriev, D. V. Shishov, I. I. Baskin, O. N. Zefirova and N. S. Zefirov, *Russ. J. Org. Chem.*, 2010, **46**, 1892 (*Zh. Org. Khim.*, 2010, **46**, 1877).
8 D. V. Shishov, E. V. Nurieva, N. A. Zefirov, A. V. Mamaeva and O. N. Zefirova, *Mendeleev Commun.*, 2014, **24**, 370.
9 E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin, *J. Comput. Chem.*, 2004, **25**, 1605.
10 B.-D. Chong, Y.-I. Ji, S.-S. Oh, J.-D. Yang, W. Baik and S. Koo, *J. Org. Chem.*, 1997, **62**, 9323.
11 F. Toda and N. Imai, *J. Chem. Soc., Perkin Trans. 1*, 1994, 2673.
12 S. K. Taylor, M. Ivanovic, L. J. Simons and M. M. Davis, *Tetrahedron: Asymmetry*, 2003, **14**, 743 and references cited therein.
13 M. Julia and R. Labia, *Bull. Soc. Chim. Fr.*, 1972, 4151.
14 J. Bredt, *Justus Liebigs Ann. Chem.*, 1924, **437**, 1.
15 J. Li, Q. Liu, H. Shen, R. Huang, X. Zhang, Y. Xiong and C. Chen, *RSC Adv.*, 2015, **5**, 85291.
16 G. E. Renzoni and W. T. Borden, *J. Org. Chem.*, 1983, **48**, 5231.
17 O. N. Zefirova, H. Lemcke, M. Lantow, E. V. Nurieva, B. Wobith, G. E. Onishchenko, A. Hoenen, G. Griffiths, N. S. Zefirov and S. A. Kuznetsov, *ChemBioChem*, 2013, **14**, 1444.
18 A. A. Beloglazkina, B. Wobith, E. S. Barskaia, N. A. Zefirov, A. G. Majouga, E. K. Beloglazkina, N. V. Zyk, S. A. Kuznetsov and O. N. Zefirova, *Med. Chem. Res.*, 2016, **25**, 1239.

Received: 21st October 2016; Com. 16/5080