Send Orders for Reprints to reprints@benthamscience.ae

Medicinal Chemistry, **2015**, *11*, 629-635

Compounds Combining Aminoadamantane and Monoterpene Moieties: Cytotoxicity and Mutagenic Effects

Evgeniy V. Suslov[a,*], Konstantin Yu. Ponomarev[a], Artem D. Rogachev[a,b], Michail A. Pokrovsky[b], Andrey G. Pokrovsky[b], Maria B. Pykhtina[c], Anatoly B. Beklemishev[c], Dina V. Korchagina[a], Konstantin P. Volcho[a,b] and Nariman F. Salakhutdinov[a,b]

[a]*N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090, Russia;* [b]*Novosibirsk State University, Novosibirsk, 630090, Russia;* [c]*Research Institute for Biochemistry, Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk, Russia*

Evgeniy V. Suslov

Abstract: A series of secondary amines combining monoterpenoid and aminoadamantane moieties have been synthesized. Their cytotoxic activity against human cancer cells CEM-13, MT-4, and U-937 has been studied for the first time. Most of the obtained compounds exhibited a significant cytotoxic activity with the median cytotoxic dose (CTD_{50}) ranging from 6 to 84 μM. The most promising results were obtained for compound **2b** which was synthesized from 1-aminoadamantane and (−)-myrtenal and revealed a high activity against all tumor lines used (CTD_{50} = 12÷21 μM) along with low toxicity with respect to MDCK cells (CTD_{50} = 1500 μM). The synthesized amines do not exert the genotoxic effect on cells of the biosensor strain based on recombinant *E. coli* cells bearing the pRAC-gfp plasmid.

Keywords: Adamantane, amine, anticancer activity, cytotoxicity, genotoxicity, monoterpene.

INTRODUCTION

The presence of the adamantyl moiety in a molecule is known to promote its exhibiting diverse biological activity [1-3]. Thus, one of the first antiviral drugs acting on the influenza virus was 1-aminoadamantane hydrochloride **1** (amantadine) (Fig. **1**) [4]. Unfortunately, almost all isolated strains of the influenza H3N2 and H1N1 viruses are resistant to this drug [5]. Recently, we have demonstrated that the addition of a monoterpenoid moiety to the 1-aminoadamantane molecule reconstitutes the antiviral activity of the latter [6]. Some of the synthesized compounds were found to exhibit sufficiently high cytotoxicity against MDCK cells. For example, CTD_{50} (the concentration causing death of 50% of cells in the culture) for compound **2a** (a mixture of *cis*- and *trans*-isomers at the 1:1 ratio, Fig. **1**) is 20.5 μM.

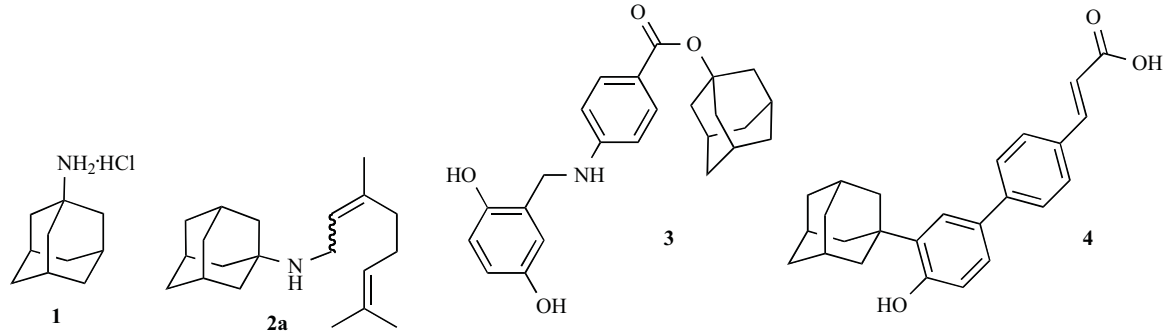
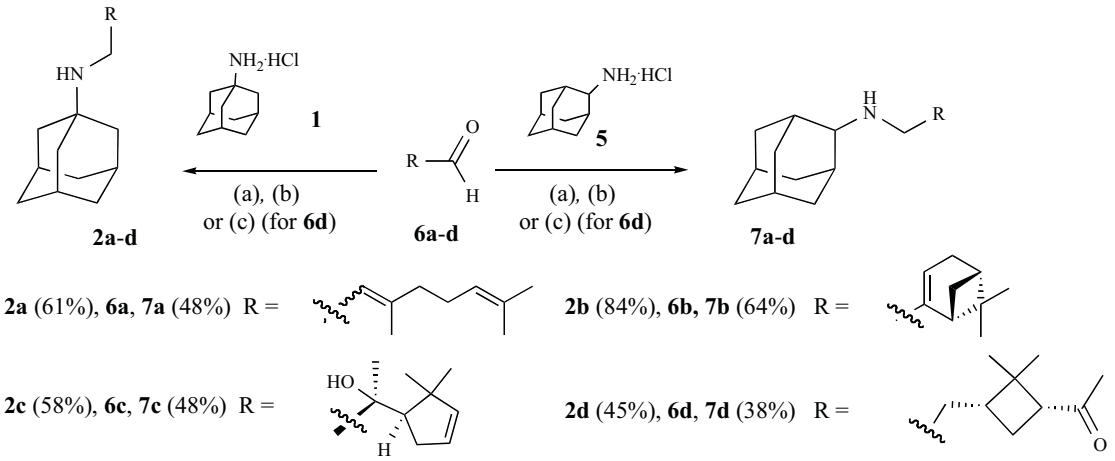


Fig. (1). Structures of biologically active adamantane-containing compounds **1**, **2a**, **3** and **4**.

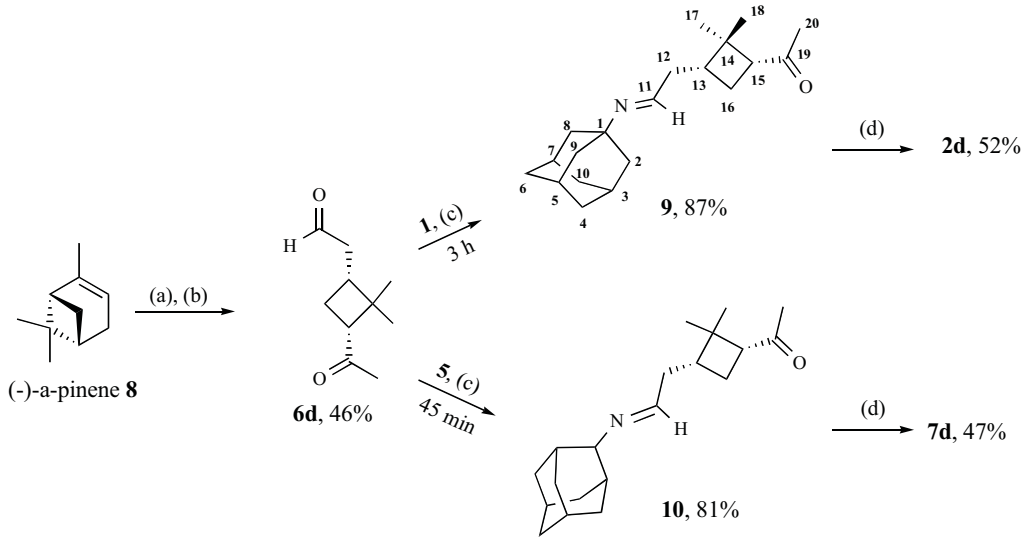
Some adamantane derivatives are known to have high cytotoxicity with respect to different cancer cell types [1, 7]. Thus, adaphostin **3** (NSC680410), which is currently undergoing preclinical trials, exhibits activity against leukemia, glioblastoma, and nonsmall cell lung cancer cell line [1, 8]. Adarotene **4** (ST1926) is active against ovarian carcinoma, neuroblastoma, and leukemia cells (Fig. **1**) [1, 9]. Moreover, monoterpenoids are known to possess the cytotoxic activity against some cancer cell lines [10]. For example, citral in-

*Address correspondence to this author at the Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, 630090, Novosibirsk, Russia; Tel: +73833308870; Fax: +73833309752; E-mail: redfox@nioch.nsc.ru

1875-6638/15 $58.00+.00



Scheme 1. Synthesis of compounds **2a-d** and **7a-d** ((a) MeOH, Et_3N; (b) $NaBH_4$, MeOH; (c) H_2, 10% Pd/C, H-Cube reactor).



Scheme 2. Synthesis of compounds **2d**, **6d**, **7d**, **9** and **10** ((a) O_3, -80°...-60°C, MeOH; (b) Me_2S, -60°C; (c) MeOH, Et_3N, 20°C; (d) H_2, 10% Pd/C, H-Cube reactor).

duced apoptosis in several hematopoietic cancer cell lines in concentration of 22.25 μM [11]. However, due to their metabolic instability, such monoterpenoids can not be considered as promising antitumor agents for practical use.

These findings prompted us to study the anticancer activity of the derivatives of 1- and 2-aminoadamantanes containing the monoterpenoid moiety which was the objective of this work.

RESULTS AND DISCUSSION

Chemistry

Previously [6, 12], we described the synthesis of compounds **2a-c** and **7a-c**. These compounds were obtained by a reaction of 1- and 2-aminoadamantane hydrochlorides **1** and **5** with citral **6a**, (−)-myrtenal **6b**, and (1*R*,6*R*)-2-(2,2-dimethylcyclopent-3-enyl)-2-hydroxypropanal **6c**, respectively, in the presence of triethylamine followed by the reduction of imines with $NaBH_4$ (Scheme **1**). Monoterpenoids **6a** and **6b** are the commercially available compounds; aldehyde **6c** was produced by isomerization of verbenol epoxide [13, 14]. In the present study, compounds **2a-c** and **7a-c** were synthesized in accordance with the procedures [6, 12]; the yields of the desired amines per starting aminoadamantanes ranged from 38% to 84%.

Moreover, expanding a set of adamantylamine derivatives possessing monoterpenoid moieties, we have synthesized compounds **2d** and **7d** for the first time. Ketoaldehyde **6d** required for the synthesis of these substances was obtained by ozonolysis of (−)-α-pinene **8** (Scheme **2**) in accordance with the procedure [15].

Imine **9** was produced by a reaction of 1-aminoadamantane **1** with aldehyde **6d** in methanol in the presence of Et_3N for 3 hours with the yield of 87% (Scheme **2**). If 2-aminoadamantane hydrochloride **5** was used, the reaction proceeded slightly faster, and complete conversion of compound **6d** was achieved in 45 min; the yield of imine **10** was 81%. Note that in terms of its reactivity with respect to hydrochlorides **1** and **5**, aldehyde **6d** is similar to (−)-myrtenal **6b**, which reacted with compound **5** faster than with adamantylamine **1** (20 min and 90 min, respectively). Interestingly, the inverse relationship was observed for citral **6a**. For this

Table 1. Cytotoxic activity of compounds 2a-d and 7a-d against lymphoblastoid cell lines and MDCK cells.

Compound	Cytotoxicity Against Cell Line (CTD_{50}, μM)[a]			
	CEM-13	U-937	MT-4	MDCK [6]
2a	5.9±2.1	27.9±3.0	>100	20.5
2b	11.9±0.6	21.1±1.6	11.9±1.1	1506.1
2c	>100	>100	62.6±9.4	145.6
2d	>100	>100	>100	n.d. [b]
7a	16.7±8.2	83.6±22.2	25.4±4.3	980.8
7b	42.1±6.6	>100	42.1±4.3	157.6
7c	>100	>100	>100	731.5
7d	69.2±29.5	49.5±6.8	>100	n.d.
Doxorubicin	3.4±2.1	0.17±0.06	2.8±1.8	n.d.

[a] CTD_{50} - concentration (μM) causing the death of 50% of the cells in culture
[b] n.d. – not determined

aldehyde, a complete conversion of starting materials was achieved one day after the start of the reaction with 2-aminoadamantane hydrochloride **5** and in 5 hours, when reacted with compound **1**.

The imine bond in compounds **9** and **10** was reduced using flow hydrogenation reactor "H-Cube Pro". The yield of the desired amines **2d** and **7d** after purification by column chromatography was 52% and 47%, respectively.

After producing the set of compounds **2a-d** and **7a-d**, we studied their cytotoxic activity.

Study of Biological Activity

Cytotoxicity

The cytotoxic activity of compounds **2a-d** and **7a-d** was studied on human cancer cells CEM-13, MT-4 (T-cellular human leucosis cells), and U-937 (human monocytes). The cytotoxic activity (Table **1**) was determined by measuring the concentration inhibiting tumor cell viability by 50% (CTD_{50}) using the conventional MTT assay, which allows estimating the number of survived cells [16]. Compounds with the CTD_{50} values less 100 μM were considered to be active. Doxorubicin was used as a positive control (see concentrations in Table **1**). For comparison, Table **1** presents the previously obtained data on cytotoxicity of the tested compounds with respect to non-cancer MDCK cells [6].

Compound **2a**, obtained from 1-aminoadamantane hydrochloride **1** and acyclic monoterpenoid citral **6a**, exhibited a high cytotoxic activity against the CEM-13 cell line, which was comparable to that of the reference drug, doxorubicin, and much lower activity against U-937 cells (Table **1**). The MT-4 cell line was resistant to the action of this amine. It should be noted that compound **2a** having high cytotoxicity with respect to both MDCK and cancer cells looks like unpromising for further studies.

The most promising results were obtained for compound **2b** synthesized from 1-aminoadamantane **1** and the bicyclic monoterpenoid myrtenal **6b**. Compound **2b** exhibited significant activity against all used tumor cell lines with CTD_{50} ranging from 12 μM to 21 μM, being two orders of magnitude less toxic to non-cancerous MDCK cells (Table **1**).

Compound **2c** demonstrated an appreciable activity against the MT-4 cell line only, and amine **2d** revealed no cytotoxic effect in all experiments.

The substitution of the 1-aminoadamantane moiety with the isomeric 2-aminoadamantane one when switching from type **2** compounds to type **7** compounds changed the observed patterns (Table **1**). Compound **7a** with an acyclic substituent appeared to be least toxic to non-cancerous MDCK cells, whereas a derivative of (−)-myrtenal, compound **7b**, exhibited appreciable toxicity with respect to this cell line. Both compounds, **7a** and **7b**, demonstrated significant cytotoxicity against cancer cells CEM-13 and MT-4, with amine **7a** being almost two times more active.

Compound **7c** was not active against all cancer cell lines studied, and compound **7d** revealed a moderate activity against U-937 and CEM-13. It is interesting that an isomer of amine **7d**, compound **2d**, was not active against all cancer cell lines used.

Thus, it was found that the compounds combining aminoadamantane and monoterpenoid moieties can possess significant cytotoxicity with respect to cancer cells. The most promising was compound **2b** obtained from 1-aminoadamantane **1** and (-)-myrtenal **6b**, which demonstrated a significant activity against all the tumor lines used along with low toxicity to MDCK cells.

Note that additional studies are needed to elucidate the mechanisms of cancer cell death caused by the action of compound **2b**.

Evaluation of Mutagenic Effects of the Compounds on Bacterial Biosensor Cells

Mutagenicity is an important feature of potential drugs (including anticancer ones) that largely determines their prospects. In the present work, the potential genotoxicity of compounds **2a-d** and **7a-d** was investigated using a whole-cell biosensor based on recombinant *E. coli* cells.

Chromosomal DNA repair in bacteria is induced by activation of different systems, depending on the nature of DNA damage. One of the main ways to DNA repair involves the *recA*-mediated SOS response. At the first stage, expression of the *recA* gene is activated, which leads to synthesis of RecA protein. This protein initiates self-cleavage of the LexA repressor that inhibits the SOS response genes under normal conditions. RecA plays a key role in several DNA repair pathways in bacteria. The creation of artificial genetic constructs, in which the genes of reporter proteins (chromogenic, fluorescent, chemiluminescent) are linked to the regulatory regions of the *recA* genes of various bacteria, underlies the development of most of sensitive whole-cell biosensor systems designated to determine genotoxicity of compounds under study. A large number of similar whole-cell bacterial biosensors for rapid and sensitive detection of the genotoxic effect of mutagens of chemical and physical nature on cells have been developed to date [17-20].

In this study, the possible genotoxicity was investigated using a whole-cell biosensor based on *E. coli* recombinant cells bearing the pRAC-gfp plasmid derived from the previously constructed expression vector pREB-gfp [19]. The pREB-gfp and pRAC-gfp plasmids provided expression of the gene encoding the reporter green fluorescent protein (GFP) in biosensor cells under the control of the regulatory region of the *recA* gene from *Proteus mirabilis*. It was previously shown that after the biosensor cell strains are treated with chemical mutagens [16, 19], the cells responded by an appropriate level of GFP synthesis. There was a direct correlation between the effect of different doses and the exposure time of the biosensor cells to mutagens of physical (UV irradiation) and chemical nature (nalidixic acid, mitomycin C, 5-bromo-2-deoxyuridine, actinomycin D, and 1,1-dimethylhydrazine) and the intensity of fluorescence emitted by the indicator strain culture [19]. In preliminary experiments, it was found that nalidixic acid had a dose-dependent genotoxic effect on *E. coli* cells, bearing the pRAC-gfp plasmid, at concentrations ranging from 4.3 to 215 μM.

Concentrations of compounds **2a-d** and **7a-d**, at which studies of their genotoxicity were carried out, were chosen individually for each substance, based on the data obtained in a study of cytotoxicity of these compounds on CEM-13, MT-4, and U-937 cell lines, and ranged from 56 to 200 μM. A compound can be considered to be genotoxic if the induction factor of GFP synthesis is 2 or higher [21]. The positive control samples contained nalidixic acid as a mutagen; the negative control samples contained water.

None of the tested compounds **2a-d** and **7a-d** at the studied doses induced GFP synthesis in cells of the BL-21(DE3) biosensor *E. coli* strain bearing the pRAC-gfp plasmid. The induction factor of GFP synthesis was less than 2.0 in all the analyzed samples, while that in positive control samples containing 86 μM nalidixic acid was 3.4. These results indicate that none of the studied compounds activates the SOS response system in cells of the biosensor strain (i.e. provides the genotoxic effect at the tested concentrations). The data obtained during the experiments and used to calculate the induction factors of GFP synthesis are shown in the supplementary materials.

CONCLUSION

A number of compounds combining monoterpenoid and aminoadamantane moieties were synthesized; their cytotoxic activity against human cancer cells CEM-13, MT-4 (T-cellular human leucosis cells), and U-937 (human monocytes) was studied for the first time. Most of the obtained compounds exhibited a significant cytotoxic activity with CTD_{50} ranging from 6 to 84 μM. The most promising results were obtained for compound **2b** synthesized from 1-aminoadamantane hydrochloride **1** and (−)-myrtenal **6b**, which demonstrated a significant activity against all cancer cell lines used with CTD_{50} ranging from 12 μM to 21 μM, being two orders of magnitude less toxic to non-cancer MDCK cells. None of the tested compounds was found to activate the SOS response in cells of the biosensor strain based on recombinant *E. coli* cells bearing the pRAC-gfp plasmid, i.e. they did not have the genotoxic effect at the studied concentrations.

EXPERIMENTAL METHODS

Chemistry

Reagents were purchased from commercial suppliers and used as received. Compounds **2a-c**, **7a-c** were synthesized in accordance with [6, 12], the experimental procedures and spectral data for these compounds are provided in the supporting materials. Solvents were purified by distillation before use. Column chromatography (CC) was performed on silica gel (60-230 μ, Macherey-Nagel). Optical rotation: *polAAr 3005* spectrometer; MeOH soln. concentration g/100 mL, specific rotation is expressed as (deg mL)/(g dm). GC: 7820A gas chromatograph (Agilent Tech., USA); flame-ionization detector; HP-5 capillary column (⌀ 0.25 mm × 30 m × 0.25 μm), He as a carrier gas (flow rate 2 mL/min, flow division 99:1). ^{1}H- and ^{13}C-NMR spectra: *Bruker DRX-500* spectrometer (500.13 MHz (^{1}H) and 125.76 MHz (^{13}C) in $CDCl_3$); chemical shifts δ in ppm rel. to residual chloroform [δ(H) 7.24, δ(C) 76.90 ppm], *J* in Hz. The structures of the products were determined by analyzing their ^{1}H NMR spectra, ^{1}H,^{1}H double- resonance spectra, *J*-modulated ^{13}C NMR spectra (JMOD) and ^{13}C,^{1}H-type 2D heteronuclear correlation with one bond and long-range spin-spin coupling constants (COSY, ^{1}J(C,H) = 135 Hz, COLOC, $^{2,3}J$(C,H) = 10 Hz). Numeration of atoms in the compounds is given for assigning the signals in the NMR spectra and does not coincide with that for the names according to the nomenclature of the compounds. HR-MS: *DFS Thermo Scientific* spectrometer in a full scan mode (0-500 *m/z*, 70 eV electron impact ionization, direct sample injection).

Hydrogenation of imines **9** and **10** to corresponding amines **2d** and **7d** was performed in flow reactor "H-Cube Pro" (ThalesNano, Inc) using 10% Pd/C as catalyst (Cat-Cart^{TM} catalyst cartridge system, 30 mm). Hydrogenation conditions: solvent – MeOH; concentration of imine 0.05 M; temperature 40^{o}C, the reactor pressure 1 bar, flow rate 0.5 mL/min.

The purity of the target compounds was determined by gas chromatography methods. All of the target compounds reported in this paper have a purity of more than 95%.

Spectral and analytical investigations were carried out at Collective Chemical Service center of Siberian Branch of the Russian Academy of Sciences.

Synthesis and Characterization

1-((1*R*,3*R*)-3-(2-adamantan-1-yl)amino)ethyl)-2,2-dimethylcyclobutyl)ethan-1-one (2d)

Triethylamine (0.100 g, 0.99 mmol) and aldehyde **6d** (0.098 g, 0.58 mmol) were added to a solution of 1-adamantylamine hydrochloride **1** (0.100 g, 0.53 mmol) in methanol (2 mL). The mixture was stirred for 3 h, then the solvent was distilled off and the residue was suspended in hexane (15 mL). The suspension was filtered, precipitation was discarded and hexane was evaporated from the filtrate. This gave 1-((1*R*,3*R*)-3-(2-((adamantan-1-yl)imino)ethyl)-2,2-dimethylcyclobutyl)ethan-1-one **9** (0.139 g, 87%).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.78 (s; Me-17), 1.18 (s; 3H, Me-18), 1.75 (ddd; 1H, ^{2}J = 11.0, $J_{16,13}$ = $J_{16,15}$ = 7.4 Hz, H-16), 1.92 (s; 3H, Me-20), 1.98-2.02 (m; 3H, H-3, H-5, H-7), 2.05-2.21 (m; 3H, 2H-12, H-13), 2.72 (dd; 1H, $J_{15,16'}$ = 10.2, $J_{15,16}$ = 7.4 Hz, H-15), 7.42 (dd; 1H, $J_{11,12}$ = 5.3, $J_{11,12'}$ = 4.3 Hz, H-11). The signals of the other protons appeared in 1.44-1.65 and 1.85-1.96 ppm in the form of overlapping multiplets. ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 56.56 (s, C-1), 42.84 (t, C-2, C-8, C-9), 29.24 (d, C-3, C-5, C-7), 36.26 (t, C-4, C-6, C-10), 156.60 (d, C-11), 37.31 (t, C-12), 39.25 (d, C-13), 43.33 (s, C-14), 53.98 (d, C-15), 22.64 (t, C-16), 17.23 (q, C-17), 30.27 (q, C-18), 207.35 (s, C-19), 29.48 (q, C-20). HR-MS: found *M* 301.2397, calculated *M* 301.2400, $C_{20}H_{31}NO$.

Hydrogenation of 0.125 g (0.42 mmol) of imine **9** in the flow reactor "H-Cube Pro" and further separation using column chromatography led to amine **2d** (0.065 g, 52%). $[\alpha]_D^{31}$ = -12.5 (*C* 1.75, MeOH).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.78 (s; 3H, Me-17), 1.21 (s; 3H, Me-18), 1.20-1.31 (m; 1H, H-12), 1.39 (dddd; 1H, ^{2}J = 13.2, $J_{12',11'}$ = 9.3, $J_{12',11}$ = 6.0, $J_{12',13}$ = 5.5 Hz, H-12'), 1.53 and 1.59 (br.d; 6H, *J* = 12 Hz, 2H-4, 2H-6, 2H-10), 1.52-1.56 (m; 6H, 2H-2, 2H-8, 2H-9), 1.73-1.91 (m; 3H, H-13, 2H-16), 1.95 (s; 3H, Me-20), 1.96-2.01 (m; 3H, H-3, H-5, H-7), 2.38 (ddd; 1H, ^{2}J = 10.6, $J_{11,12}$ = 9.3, $J_{11,12'}$ = 6.0 Hz, H-11), 2.44 (ddd; 1H, ^{2}J = 10.6, $J_{11',12'}$ = 9.3, $J_{11',12}$ = 5.7 Hz, H-11'), 2.73 (dd; 1H, $J_{15,16}$ = 10.0, $J_{15,16'}$ = 7.4 Hz, H-15). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 50.04 (s, C-1), 42.68 (t, C-2, C-8, C-9), 29.41 (d, C-3, C-5, C-7), 36.59 (t, C-4, C-6, C-10), 38.20 (t, C-11), 31.57 (t, C-12), 40.10 (d, C-13), 43.15 (s, C-14), 54.12 (d, C-15), 23.03 (t, C-16), 17.03 (q, C-17), 30.34 (q, C-18), 207.68 (s, C-19), 29.85 (q, C-20). HR-MS: found *M* 303.2549, calculated *M* 303.2557, $C_{20}H_{33}NO$.

1-((1*R*,3*R*)-3-(2-adamantan-2-yl)amino)ethyl)-2,2-dimethylcyclobutyl)ethan-1-one (7d)

Triethylamine (0.100 g, 0.99 mmol) and aldehyde **6d** (0.1 g, 0.59 mmol) were added to a solution of 2-adamantylamine hydrochloride **5** (0.100 g, 0.53 mmol) in methanol (2 mL). The mixture was stirred for 45 min, then the solvent was distilled off and the residue was suspended in hexane (15 mL). The suspension was filtered, precipitation was discarded and hexane was evaporated from the filtrate. This gave 1-((1*R*,3*R*)-3-(2-((adamantan-2-yl)imino)ethyl)-2,2-dimethylcyclobutyl)ethan-1-one **10** (0.130 g (81%)).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.78 (s; 3H, Me-17), 1.19 (s; 3H, Me-18), 1.38 (dm; 2H, ^{2}J ~ 12.5 Hz, H-4, H-9), 1.48-1.54 (m; 2H, H-1, H-3), 1.91 (s; 3H, Me-20), 2.04-2.22 (m; 5H, H-4', H-9', 2H-12, H-13), 2.71 (dd; 1H, $J_{15,16}$ = 10.2, $J_{15,16'}$ = 7.4 Hz, H-15), 3.02 (br.t, 1H, $J_{2,1(3)}$ ~ 2.5 Hz, H-2), 7.53 (dd, 1H, $J_{11,12}$ = 5.1, $J_{11,12'}$ = 4.0 Hz, H-11). The signals of the other protons appeared in 1.60-1.95 ppm in the form of overlapping multiplets. ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 34.96 and 35.09 (d, C-1, C-3), 74.44 (d, C-2), 31.65 and 31.68 (t, C-4, C-9), 27.90 (d, C-5), 37.93 (t, C-6), 27.19 (d, C-7), 37.18 and 37.23 (t, C-8, C-10), 159.25 (d, C-11), 36.76 (t, C-12), 38.91 (d, C-13), 43.19 (s, C-14), 54.08 (d, C-15), 22.86 (t, C-16), 17.33 (q, C-17), 30.51 (q, C-18), 206.33 (s, C-19), 29.76 (q, C-20). HR-MS: found *M* 301.2395, calculated *M* 301.2400, $C_{20}H_{31}NO$.

Hydrogenation of imine **10** (0.13 g, 0.43 mmol) in the flow reactor "H-Cube Pro" and further separation using column chromatography led to amine **7d** (0.062 g, 47%). $[\alpha]_D^{31}$ = 1.3 (*C* 0.47, MeOH).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.83 (s; 3H, Me-17), 1.25 (s; 3H, Me-18), 1.45 (br.d; 2H, ^{2}J = 12.3 Hz, H-4, H-9), 1.45-1.53 (m; 1H, H-12'), 1.35 (dtd; 1H, ^{2}J = 13.0, $J_{12,11}$ = 8.7, $J_{12,13}$ = 6.0 Hz, H-12), 1.63-1.69 (m; 4H, 2H-6, H-8, H-10), 1.70-1.75 (m; 1H, H-5), 1.77-1.96 (m; 10H, H-1, H-3, H-4', H-7, H-8', H-9', H-10', H-13, 2H-16), 1.99 (s; 3H, Me-20), 2.41-2.51 (m; 2H, 2H-11), 2.64 (br.s; 1H, H-2), 2.77 (dd; 1H, $J_{15,16}$ = 10.0, $J_{15,16'}$ = 7.6 Hz, H-15). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 31.97 and 32.00 (d, C-1, C-3), 61.78 (d, C-2), 31.25 and 31.27 (t, C-4, C-9), 27.55 (d, C-5), 37.87 (t, C-6), 27.75 (d, C-7), 37.51 (t, C-8, C-10), 45.08 (t, C-11), 31.03 (t, C-12), 40.27 (d, C-13), 43.25 (s, C-14), 54.27 (d, C-15), 23.20 (t, C-16), 17.11 (q, C-17), 30.42 (q, C-18), 207.84 (s, C-19), 29.94 (q, C-20). HR-MS: found [*M*-H] 302.2479, calculated [*M*-H] 302.2478, $C_{20}H_{32}NO$.

Biological Study

Cell Viability Assays

The human cancer cells MT-4, CEM-13 (the cells of T-cellular human leucosis), and U-937 (human monocytes) were used in this study. The cells were cultured in the RPMI-1640 medium that contained 10% embryonic calf serum, *L*-

glutamine (2 mmol/L), gentamicin (80 μg/mL), and lincomycin (30 mg/mL) in a CO_2 incubator at 37°C. The compounds were dissolved in DMSO and added to the cellular culture at the required concentrations. Cells (0.5×10^6 cells per mL) were placed on 96-well microliter plates and cultivated at 37 °C in 5% CO_2/95% air for 72 h. Three wells were used for each concentration. The cells which were incubated with the same final concentration of DMSO without compounds were used as a control. Cell viability was assessed through an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2*H*-tetrazolium bromide] conversion assay. 1% MTT was added to each well. Four hours later DMSO was added and mixed for 15 min. Finally, the optical density values were monitored at 570 nm.

Genotoxicity Assays

Evaluation of possible genotoxicity of the compounds under study was carried out using a whole-cell biosensor based on the recombinant BL-21(DE3) *E. coli* strain bearing the pRAC-gfp plasmid. Experiments were performed according to the following scheme. The sample (3mL) of the overnight culture of the biosensor *E. coli* strain was inoculated into a flask containing 100 mL of fresh Luria-Bertani (LB) medium and was cultured in a shaker at 37 °C until the optical density OD_{600} reached 0.5–0.6. Then, 1 mL aliquots of the biosensor strain culture were added into wells of sterile 12-well plates containing 110 μL of one of the analyzed compounds dissolved in aqueous DMSO at different concentrations. Nalidixic acid (to the final concentration of 86 μM) or water was added to the wells serving as positive or negative controls, respectively, instead of the analyzed compounds.

The plates were then placed in a shaker and incubated at 37°C and 140 rpm for 2 hours. After completing the incubation, the plates were placed to a refrigerator (4°C) for 15 hours for maturation of the chromophore (GFP). At the final stage, the relative number of cells in each well was determined by measuring the optical density of the culture at 600 nm. The fluorescence of a cell suspension was measured using an RF-5301PC spectrofluorophotometer (Shimadzu, Japan) (excitation wavelength λ_{ex} = 491 nm, emission wavelength λ_{em} = 511 nm). Based on the absorbance and fluorescence (**F**) data obtained for a biosensor cell suspension, the factor of induction (**Fi**) was calculated using the following equation [21, 22]:

$Fi = (Ft_{m510nm} \times ODc_{m\ 600\ nm}) / (FC_{m\ 510\ nm} \times ODt_{m\ 600\ nm})$, where

Fi is the factor of induction;

$\mathbf{Ft_{m\ 510\ nm}}$ is the mean fluorescence of samples with equal concentrations of an analyzed compound and DMSO;

$\mathbf{Fc_{m\ 510\ nm}}$ is the mean fluorescence of negative control samples with equal concentrations of DMSO;

$\mathbf{ODc_{m\ 600\ nm}}$ is the mean optical density of the control samples (negative control) with equal concentrations of DMSO;

$\mathbf{ODt_{m\ 600\ nm}}$ is the mean optical density of the samples with equal concentrations of an analyzed compound and DMSO.

The results of genotoxicity evaluation for the analyzed compounds and different concentrations of DMSO are provided in the supplementary materials.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The work was partly supported by the Ministry of Education and Science of the Russian Federation and Ministry of Education, Science and Innovation Policy of the Novosibirsk Region, as well as by RFBR, research project No. 15-33-20198.

SUPPLEMENTARY MATERIAL

Additional supporting information may be found in the online version of this article at the publisher's web-site

REFERENCES

[1] Wanka, L.; Iqbal, K.; Schreiner, P. R. The lipophilic bullet hits the targets: medicinal chemistry of adamantane derivatives. *Chem. Rev.,* **2013**, *113*, 3516-3604.

[2] Lamoureux, G.; Artavia, G. Use of the adamantane structure in medicinal chemistry. *Curr. Med. Chem.,* **2010**, *17*, 2967-2978.

[3] Levandovskiy, I. A.; Sharapa, D. I.; Shamota, T. V.; Rodionov, V. N.; Shubina, T. E. Conformationally restricted GABA analogs: from rigid carbocycles to cage hydrocarbons. *Future Med. Chem.,* **2011**, *3*, 223-241.

[4] Davies, W. L.; Grunert, R. R.; Haff, R. F.; McGahen, J. W.; Neumayer, E. M.; Paulshock, M.; Watts, J. C.; Wood, T. R.; Hermann, E. C.; Hoffmann, C. E. Antiviral activity of 1-adamantanamine (amantadine). *Science,* **1964**, *144*, 862-863.

[5] Fiore, A. E.; Fry, A.; Shay, D.; Gubareva, L.; Bresee, J. S.; Uyeki, T. M. Antiviral agents for the treatment and chemoprophylaxis of influenza. Recommendations of the Advisory Committee on immunization practices (ACIP). *CDC MMWR* **2011**, *60*(RR-1), pp. 1-24.

[6] Teplov, G. V.; Suslov, E. V.; Zarubaev, V. V.; Shtro, A. A.; Karpinskaya, L. A.; Rogachev, A. D.; Korchagina, D. V.; Volcho, K. P.; Salakhutdinov, N. F.; Kiselev, O. I. Synthesis of new compounds combining adamantanamine and monoterpene fragments and their antiviral activity against influenza virus A(H1N1)pdm09. *Lett. Drug Des. Discov.,* **2013**, *10*, 477-485.

[7] Gopalan, B.; Ponpandian, T.; Kachhadia, V.; Bharathimohan, K.; Vignesh, R.; Sivasudar, V.; Narayanan, S.; Mandar, B.; Praveen, R.; Saranya, N.; Rajagopal, S.; Rajagopal, S. Discovery of adamantane based highly potent HDAC inhibitors. *Bioorg. Med. Chem. Lett.,* **2013**, *23*, 2532-2537.

[8] Aguiló, J. I.; Iturralde, M.; Monleón, I.; Iñarrea, P.; Pardo, J.; Martínez-Lorenzo, M. J.; Anel, A.; Alava, M. A. Cytotoxicity of quinone drugs on highly proliferative human leukemia T cells: reactive oxygen species generation and inactive shortened SOD1 isoform implications. *Chem. Biol. Interact.,* **2012**, *198*, 18-28.

[9] Giannini, G.; Brunetti, T.; Battistuzzi, G.; Alloatti, D.; Quattrocicchi, G.; Cima, M. G.; Merlini, L.; Dallavalle, S.; Cincinelli, R.; Nannei, R.; Vesci, L.; Bucci, F.; Foderà, R.; Guglielmi, M. B.; Pisano, C.; Cabri, W. New retinoid derivatives as back-ups of Adarotene. *Bioorg. Med. Chem.,* **2012**, *20*, 2405-2415.

[10] Sobral, M. V; Xavier, A. L; Lima, T. C; de Sousa, D. P. Antitumor activity of monoterpenes found in essential oils. *Scientific World J.*, **2014**, *2014*, 1-35.

[11] Dudai, N.; Weinstein, Y.; Krup, M.; Rabinski, T.; Ofir, R. Citral is a new inducer of caspase-3 in tumor cell lines. *Planta Med.*, **2005**, *71*, 484-488.

[12] Kapitsa, I. G.; Suslov, E. V.; Teplov, G. V.; Korchagina, D. V.; Komarova, N. I.; Volcho, K. P.; Voronina, T. A.; Shevela, A. I.; Salakhutdinov, N. F. Synthesis and anxiolytic activity of 2-

aminoadamantane derivatives containing monoterpene fragments. *Pharm. Chem. J.,* **2012**, *46*, 263-265.

[13] Ardashov, O. V.; Il'ina, I. V.; Korchagina, D. V.; Volcho, K. P.; Salakhutdinov, N. F. Unusual α-hydroxyaldehyde with a cyclopentane framework from verbenol epoxide. *Mendeleev Commun*., **2007**, *17*, 303-305.

[14] Il'ina, I. V.; Koneva, E. A.; Korchagina, D. V.; Sal'nikov, G. E.; Genaev, A. M.; Volcho, K. P.; Salakhutdinov, N. F. Chiral Schiff bases synthesized from terpenes of pinane series in asymmetric metall complex oxidation of sulfides. *Russ. J. Org. Chem*., **2012**, *48*(2), 214-220.

[15] Barton, D. H. R.; Fontana, G. Radical chemistry based on (+)-cis-pinononic and (+)-cis-pinonic acids. *Synth. Commun.,* **1996**, *26*, 1953-1968.

[16] Sokolova, A. S.; Yarovaya, O. I.; Shernyukov, A. V.; Pokrovsky, M. A.; Pokrovsky, A. G.; Lavrinenko, V. A.; Zarubaev, V. V.; Tretiak, T. S.; Anfimov, P. M.; Kiselev, O. I.; Beklemishev, A. B.; Salakhutdinov, N. F. New quaternary ammonium camphor derivatives and their antiviral activity, genotoxic effects and cytotoxicity. *Bioorg. Med. Chem.,* **2013**, *21*, 6690-6698.

[17] Chen, Z.; Lu, M.; Zhuang, G.; Wang, H. Enhanced bacterial biosensor for fast and sensitive detection of oxidatively DNA damaging agents. *Anal. Chem.,* **2011**, *83*, 3248-3251.

[18] Biran, A.; Ben Yoav, H.; Yagur-Kroll, S.; Pedahzur, R.; Buchinger, S.; Shacham-Diamand, Y.; Reifferscheid, G.; Belkin, S. Microbial genotoxicity bioreporters based on sulA activation. *Anal. Bioanal. Chem*., **2011**, *400*, 3013-3024.

[19] Lavrinenko, I. A.; Lavrinenko, V. A.; Ryabchenko, A. V.; Beklemishev, A. B. Development of a biosensor test system with GFP reporter protein for detection of DNA damages. *Bull. Exp. Biol. Med.,* **2006**, *141*, 33-35.

[20] Mitchell, R. J.; Gu, M. B. An *Escherichia coli* biosensor capable of detecting both genotoxic and oxidative damage. *Appl. Microbiol. Biotechnol*., **2004**, *64*, 46-52.

[21] Ptitsyn, L. R.; Horneck, G.; Komova, O.; Kozubek, S.; Krasavin, E. A.; Bonev, M.; Rettberg, P. A biosensor for environmental genotoxin screening based on an SOS lux assay in recombinant Escherichia coli cells. *Appl. Environ. Microbial.,* **1997**, *63*, 4377-4384.

[22] Kostrzynska, M.; Leung, K. T.; Lee, H.; Trevors, J. T. Green fluorescent protein-based biosensor for detecting SOS-induced activity of genotoxic compounds. *J. Microbiol. Methods,* **2002**, *48*, 43-51.

Received: September 04, 2014 Revised: May 14, 2015 Accepted: May 14, 2015