3-Nitro-2-trifluoromethyl-2*H*-chromenes and products of their reduction. Synthesis and cytotoxicity evaluation*

M. A. Baryshnikova,[a] A. Yu. Volkonskii,[b] D. V. Gusev,[b] N. O. Labodneva,[c] A. L. Sigan,[b] N. G. Yakunina,[a] and N. D. Chkanikov[b]*

[a]*Research Institute of Experimental Diagnostics and Tumor Therapy, N. N. Blokhin Russian Cancer Research Center, Russian Academy of Sciences, 24 Kashirskoe shosse, 115478 Moscow, Russian Federation. Fax: +7 (495) 324 6037. E-mail: ma_ba@mail.ru*

[b]*A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 ul. Vavilova, 119991 Moscow, Russian Federation. Fax: +7 (495) 135 5085. E-mail: dgusev@ineos.ac.ru*

[c]*D. I. Mendeleev Russian University of Chemistry and Technology, 9 Miusskaya pl., 125047 Moscow, Russian Federation. Fax: +7 (495) 135 5085. E-mail: n.labodneva@gmail.com*

A series of 3-nitro-2-trifluoromethyl-2*H*-chromenes was synthesized by the reactions of salicylaldehyde derivatives with 3,3,3-trifluoro-1-nitroprop-1-ene. Further transformations of the synthesized chromenes gave hitherto unknown 3-amino-2-trifluoromethylchromanes promising as precursors for the synthesis of fused heterocyclic systems. Cytotoxicity assay first revealed the pronounced activity of several 2-nitro-2-trifluoromethyl-2*H*-chromenes against human tumor cell lines.

Key words: salicylaldehyde, 3,3,3-trifluoro-1-nitroprop-1-ene, condensation, 3-nitro-2-trifluoromethyl-2*H*-chromenes, 3-amino-2-trifluoromethylchromanes, cytotoxicity.

Earlier, we by the reaction of a naturally occurring phenolic aldehyde gossypol (2,2′-di(1,6,7-trihydroxy-5-isopropyl-3-methyl-8-naphthaldehyde) with 3,3,3-trifluoro-1-nitroprop-1-ene have synthesized 6,6′-diisopropyl-8,8′-dimethyl-2,2′-dinitro-3,3′-bis(trifluoromethyl)-3*H*,3′*H*-[9,9′]di[benzo(f)chromenyl]-5,10,5′,10′-tetraol. This compound is of interest since it comprises two fused tricyclic systems with the chromene skeletons substituted with two trifluoromethyl and two nitro groups. Biomedical evaluation of this compound shows extension of the biological activity spectrum of the parent gossypol owing to the appearance of pronounced antitumor and fungicidal activities.[1] In this case, new valuable properties are apparently due to the presence of the trifluoromethyl-substituted chromene cores.

The aim of the present work is the synthesis of a library of 2-trifluoromethyl-substituted 3-nitro-2*H*-chromenes and 3-aminochromanes and *in vitro* evaluation of their biological activity.

* Dedicated to Academician of the Russian Academy of Sciences Yu. N. Bubnov on the occasion of his 80th birthday and to the 60th anniversary of A. N. Nesmeyanov Institute of Organoelement Compounds of the Russian Academy of Sciences.

Results and Discussion

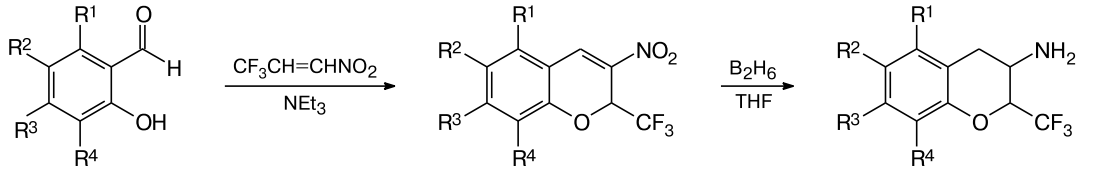
2*H*-Benzopyranes (chromenes) are a class of naturally occurring compounds found in a wide variety of plants. Chromene core is the essential parts and the structural determinant responsible for high biological activity of complex natural products (flavonoids, anthocyanins, *etc.*).[2] Chromene-based synthetic compounds bearing various substituents and functional groups have contribute substantially to the development of highly efficient therapeutics possessing anticoagulant, anti-oxidant, antitumor, antiviral, and HIV integrase inhibitory activities.[3—6] The high reactivity and structural diversity of chromenes promote the development of novel synthetic approaches towards these compounds and identifying the areas of their practical applications.

Over the past several decades, the introduction of the fluorine atoms into organic molecules has been used as an efficient strategy to extend therapeutic efficacy and pharmacological activity of parent compounds.[7] Until recently, only few methods to access fluorinated or polyfluoroalkylated chromenes have been developed. These multistep syntheses require poorly available reagents and proceed with unsatisfactory yields.[8—10] An convenient ap-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 11, pp. 2551—2555, November, 2014.

1066-5285/14/6311-2551

Scheme 1



1a—i **2a—i** **3a—f, h, i**

Compound **1—3**	R^1	R^2	R^3	R^4	Compound **1—3**	R^1	R^2	R^3	R^4
a	H	H	H	OMe	**f**	H	Cl	H	H
b	H	H	H	H	**g**	H	NO_2	H	H
c	H	Br	H	Br	**h**	H	Me	H	H
d	H	H	H	Me	**i**	H	OMe	H	H

proach for the synthesis of 3-nitro-2-trifluoromethyl-2*H*-chromenes was first suggested by Korotaev and co-workers.[11] This approach involves catalytic condensation of salicylic aldehydes with 3,3,3-trifluoro-1-nitroprop-1-ene *via* nucleophilic addition of the hydroxy groups at the activated C=C bond followed by the ring closure to give the corresponding 2*H*-chromene derivatives. Later, the general character of the suggested approach was demonstrated by the reaction of various nitroalkenes with salicylaldehyde derivatives and ketimines of *o*-hydroxyacetophenones.[12,13]

We also employed this synthetic pathway to obtain a wide variety of novel trifluoromethylated chromenes for the detailed study (Scheme 1). The reactions were carried out either in dichloromethane (method *A*) or in propane-2-ol (method *B*). To a mixture of salicylaldehyde derivatives **1a—i** and 3,3,3-trifuoro-1-nitroprop-1-ene, a solution of triethylamine used as a catalyst was added dropwise over a period of 1 h at 5—10 °C. When propan-2-ol was used as the solvent (5—10 °C, then 20 °C, reaction time of 24 h), precipitation of the crystalline reaction products, 3-nitro-2-trifuoromethyl-2*H*-chromenes bearing the corresponding substituents in the aromatic ring, was observed in the most cases. Only in the cases of 5-methoxy- (**1a**) and 5-methylsalicylaldehydes (**1d**), the reactions carried out by method ***A*** require 40 h to be completed (TLC data), which is due apparently to the electron-withdrawing effect of the substituents decreasing the electrophilicity of the carbonyl group of the starting compounds. It should be noted that in these cases, no precipitation occurred and the products were isolated after certain work-up of the reaction mixtures. The elemental analyses data and ^1H and ^{19}F NMR spectral characteristics for compounds **2a—i** are given in Tables 1 and 2, respectively. Mass spectrometry of compounds **2a—i** revealed good agreement between measured and calculated *m/z* values.

Table 1. Physicochemical characteristics and microanalysis data for compounds **2a—i**

Compound	M.p/°C	Yield (%)	Found / Calculated (%)			Molecular formula
			C	H	N	
2a	138—142	89	47.80	2.85	5.01	$C_{11}H_8F_3NO_4$
			48.01	2.93	5.09	
2b	88—91	67	48.51	2.52	5.70	$C_{10}H_6F_3NO_3$
			48.99	2.47	5.71	
2c	94—97	56	29.67	1.10	3.49	$C_{10}H_4Br_2F_3NO_3$
			29.81	1.00	3.48	
2d	97—99	33	50.67	3.21	5.32	$C_{11}H_8F_3NO_3$
			50.98	3.11	5.40	
2e	105—107	76	37.00	1.59	4.32	$C_{10}H_5BrF_3NO_3$
			37.07	1.56	4.32	
2f	101—104	84	42.54	1.92	4.87	$C_{10}H_5ClF_3NO_3$
			42.96	1.80	5.01	
2g	129—131	80	41.38	1.76	9.53	$C_{10}H_5F_3N_2O_5$
			41.40	1.74	9.65	
2h	79—81	26	50.45	3.24	5.42	$C_{11}H_8F_3NO_3$
			50.98	3.11	5.40	
2i	84—85	60	47.89	2.95	5.04	$C_{11}H_8F_3NO_4$
			48.01	2.93	5.09	

Table 2. ^{1}H and ^{19}F NMR spectra ($CDCl_3$) of compounds **2a—i**

Compound	NMR, δ (*J*/Hz)	
	^{1}H	^{19}F
2a	8.11 (s, 1 H, CH=); 7.02 (m, 3 H, R^1, R^2, R^3); 6.15 (m, 1 H, C<u>H</u>CF_3); 3.92 (s, 1 H, OMe)	−0.15
2b	8.12 (s, 1 H, CH=); 7.45 (m, 1 H, R^1); 7.37 (m, 1 H, R^4); 7.08 (m, 2 H, R^2, R^3); 6.06 (m, 1 H, C<u>H</u>CF_3)	−0.16
2c	8.02 (s, 1 H, CH=); 7.79 (s, 1 H, R^1); 7.46 (s, 1 H, R^3); 6.19 (m, 1 H, C<u>H</u>CF_3)	−0.09
2d	8.17 (s, 1 H, CH=); 7.31 (m, 2 H, R^1, R^3); 7.05 (m, 1 H, R^2); 6.19 (m, C<u>H</u>CF_3); 2.34 (s, 3 H, Me)	−0.37
2e	8.55 (s, 1 H, CH=); 7.91 (m, 1 H, R^1); 7.68 (m, 1 H, R^3); 7.14 (m, 1 H, R^4); 6.63 (m, 1 H, C<u>H</u>CF_3)	1.53
2f	8.05 (s, 1 H, CH=); 7.38 (m, 2 H, R^3, R^4); 7.04 (m, 1 H, R^1); 6.08 (m, 1 H, C<u>H</u>CF_3)	−1.14
2g	8.37 (s, 2 H, R^1, R^3); 8.20 (s, 1 H, CH=); 7.30 (s, 2 H, R^4); 6.25 (m, 1 H, C<u>H</u>CF_3)	−1.18
2h	8.08 (s, 1 H, CH=); 7.24 (m, 1 H, R^1); 7.15 (m, 1 H, R^3); 6.96 (m, 1 H, R^4); 6.05 (m, 1 H, C<u>H</u>CF_3); 2.33 (s, 1 H, Me)	−0.09
2i	8.08 (s, 1 H, CH=); 7.00 (m, 2 H, R^3, R^4); 6.85 (m, 1 H, R^1); 6.04 (m, 1 H, C<u>H</u>CF_3); 3.81 (s, 3 H, OMe)	0.04

To date, several examples of the involvement of 3-nitro-2-trihalomethyl-2*H*-chromenes in the synthesis of the chromane-based compounds with potential biological activity for possible application as agrochemicals have been described.[14,15] However, no data on the synthesis of 3-amino-2-trifluoromethylchromanes and their applications as valuable syntones have been published. To achieve this goal, we performed exhaustive reduction of the double bond and nitro group in compounds **2a—f**,**h**,**i** with borane in THF (see Scheme 1). The corresponding 3-amino-2-trifluoromethylchromanes **3a—f**,**h**,**i** were isolated after certain work-up. The elemental analysis data and NMR spectral data for compounds **3a—f**,**h**,**i** are summarized in Tables 3 and 4, respectively.

Note that in all described reactions, the product yields do not exceed 50% regardless of the substrate nature. It was found that all reaction mixtures obtained after isolation of target 3-amino-2-trifluoromethylchromanes contain 3-hydroxylamino-2-trifluoromethylchromanes as the main side products. In the case of compound **3c**, readily crystallized side product was isolated, to which a structure of *N*-[6,8-dibromo-2-(trifluoromethyl)-3,4-dihydro-2*H*-chromen-3-yl]hydroxylamine (**4**) was ascribed. Compound **4** is stable under reducing conditions (B_2H_6, THF) even at prolong reflux. ^{1}H and ^{19}F NMR spectroscopy revealed that formation of 3-hydroxylamino-substituted chromanes is a general feature of these reactions and causes the decrease in the target product yields.

Cytotoxicity assay of several selected compounds (**2a**,**b**,**d**,**e** and **3e**) was performed *in vitro* in Research Institute of Experimental Diagnostics and Tumor Therapy of N. N. Blokhin Russian Cancer Research Center of RAS. Cytotoxicity of compounds **2a**,**b**,**d**,**e** and **3e** was examined on human cell lines, namely, Jurkat (T-lymphoblastic leucosis), SKOV-3 (ovarian carcinoma cells), HCT-116 (colon cancer cells), A549 (lung adenocarcinoma), using

Table 3. Physicochemical characteristics and microanalysis data for compounds **3a—f**,**h**,**i**

Compound	Yield (%)	Found / Calculated (%)			Molecular formula	Note
		C	H	N		
3a	43	<u>50.78</u> 51.55	<u>5.01</u> 4.81	<u>3.15</u> 3.34	$C_{11}H_{12}F_3NO_2 \cdot C_7H_8O_3S$	Tosylate
3b	44	<u>43.45</u> 44.32	<u>4.56</u> 4.34	<u>4.13</u> 4.31	$C_{10}H_{10}F_3NO \cdot C_2H_2O_4 \cdot H_2O$	Oxalate
3c	46	<u>29.12</u> 29.19	<u>2.20</u> 2.20	<u>3.35</u> 3.40	$C_{10}H_8Br_2F_3NO \cdot HCl$	Hydrochloride
3d	49	<u>49.23</u> 49.36	<u>4.98</u> 4.90	<u>5.25</u> 5.23	$C_{11}H_{12}F_3NO \cdot HCl$	Hydrochloride
3e	44	<u>36.01</u> 36.12	<u>3.11</u> 3.03	<u>4.30</u> 4.21	$C_{10}H_9BrF_3NO \cdot HCl$	Hydrochloride
3f	45	<u>40.98</u> 41.69	<u>3.67</u> 3.50	<u>4.65</u> 4.86	$C1_0H_9ClF_3NO \cdot HCl$	Hydrochloride
3h	48	<u>49.03</u> 49.36	<u>4.78</u> 4.90	<u>5.12</u> 5.23	$C_{11}H_{12}F_3NO \cdot HCl$	Hydrochloride
3i	50	<u>46.57</u> 46.57	<u>4.87</u> 4.62	<u>4.67</u> 4.94	$C_{11}H_{12}F_3NO_2 \cdot HCl$	Hydrochloride

Table 4. ^{1}H and ^{19}F NMR spectra ($CDCl_3$) of compounds **3a—f,h,i**

Compound	NMR, δ (*J*/Hz)	
	^{1}H	^{19}F
3a	8.34 (s, 3 H, NH_3^+); 7.35 (m, 4 H, Tos); 7.01—6.75 (m, 3 H, R^1, R^2, R^3); 5.12 (m, 1 H, $CHCF_3$); 4.19 (br.s, 1 H, $C\underline{H}_2CH$—); 3.78 (s, 3 H, OMe); 3.2 (m, 1 H, $CH_2C\underline{H}$—); 2.99 (m, 1 H, $C\underline{H}_2CH$—); 2.28 (s, 3 H, Me, Tos)	4.70
3b	7.20—6.93 (m, 4 H, R^1, R^2, R^3, R^4); 4.97 (m, 1 H, $CHCF_3$); 3.98 (m, 4 H, $C\underline{H}_2CH$—, NH_3^+); 3.25 (m, 1 H, CH_2CH—); 2.86 (m, 1 H, $CH_2C\underline{H}$—)	5.16
3c*	7.55 (s, 1 H, R^3); 7.16 (s, 1 H, R^1); 4.36 (m, 1 H, $CHCF_3$); 3.70 (br.s, 1 H, $C\underline{H}_2CH$—); 3.16 (m, 1 H, $CH_2C\underline{H}$—); 2.75 (m, 1 H, $C\underline{H}_2CH$—); 1.32 (s, 2 H, NH_2)	3.66
3d	8.68 (s, 3 H, NH_3^+); 7.03—6.86 (m, 3 H, R^1, R^2, R^3); 5.12 (m, 1 H, $CHCF_3$); 4.16 (br.s, 1 H, $C\underline{H}_2CH$—); 3.48 (br.s, 1 H, $C\underline{H}_2CH$—); 3.27 (m, 1 H, $CH_2C\underline{H}$—); 2.18 (s, 3 H, Me)	4.43
3e	8.78 (s, 3 H, NH_3^+); 7.46—7.37 (m, 2 H, R^2, R^3); 6.98 (m, 1 H, R^1); 5.21 (m, 1 H, $CHCF_3$); 4.19 (br.s, 1 H, $C\underline{H}_2CH$—); 3.53—3.23 (m, 2 H, $C\underline{H}_2CH$—, $CH_2C\underline{H}$—)	4.77
3f	8.78 (s, 3 H, NH_3^+); 7.29 (m, 2 H, R^3, R^4); 7.03 (m, 1 H, R^1); 5.22 (m, 1 H, $CHCF_3$); 4.19 (br.s, 1 H, $C\underline{H}_2CH$—); 3.29 (m, 1H, $C\underline{H}_2CH$—); 3.29 (m, 1 H, $CH_2C\underline{H}$—)	4.77
3h	8.76 (s, 3 H, NH_3^+); 7.03—6.86 (m, 3 H, R^1, R^3, R^4); 5.12 (m, 1 H, $C\underline{H}CF_3$); 4.11 (br.s, 1 H, $C\underline{H}_2$—CH); 3.28 (m, 2 H, $C\underline{H}_2$—CH, CH_2—$C\underline{H}$); 2.23 (s, 3 H, Me)	4.78
3i	8.73 (s, 3 H, NH_3^+); 6.94—6.78 (m, 3 H, R^1, R^3, R^4); 5.1 (s, 1 H, $CHCF_3$); 4.15 (br.s, 1 H, $C\underline{H}_2CH$—); 3.70 (s, 3 H, OMe); 3.25 (m, 2 H, $C\underline{H}_2CH$—, $CH_2C\underline{H}$—)	4.79

* Data for a free base obtained from hydrochloride.

MTT colorimetric assay. Cancer cell cultures were obtained from the culture collection of N. N. Blokhin Russian Cancer Research.

The MMT assay is the best known method for the cytotoxicity evaluation. MTT assay involves the reduction of yellow water-soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial and cytoplasmic dehydrogenases of metabolically active cells to give water-insoluble purple formazan crystals. The amount of produced formazan directly correlates to the number of metabolically active cells and their viability and can be quantified using spectrophotometry.

The results of cytotoxicity evaluation are given in Table 5. Compounds **2a**,**b**,**d**,**e** exhibit the most pronounced cytotoxic effect against the Jurkat cell line.

Table 5. *In vitro* cytotoxicity of compounds **2a**,**b**,**d**,**e** and **3e**

Compound	Concentration /mol L^{-1}	Cell survival rate (%)			
		Jurkat	SKOV-3	HCT-116	A549
2a	$1 \cdot 10^{-7}$	87	94	98	91
	$1 \cdot 10^{-6}$	79	101	102	95
	$1 \cdot 10^{-5}$	33	76	73	90
	$1 \cdot 10^{-4}$	10	17	9	9
2b	$1 \cdot 10^{-7}$	88	100	93	98
	$1 \cdot 10^{-6}$	84	104	88	91
	$1 \cdot 10^{-5}$	54	101	86	93
	$1 \cdot 10^{-4}$	11	42	11	15
2d	$1 \cdot 10^{-7}$	73	83	85	86
	$1 \cdot 10^{-6}$	71	116	94	81
	$1 \cdot 10^{-5}$	57	88	94	76
	$1 \cdot 10^{-4}$	19	31	12	17
2e	$1 \cdot 10^{-7}$	95	95	87	85
	$1 \cdot 10^{-6}$	84	86	82	86
	$1 \cdot 10^{-5}$	50	98	86	80
	$1 \cdot 10^{-4}$	9	23	10	14
3e	$1 \cdot 10^{-7}$	89	93	96	101
	$1 \cdot 10^{-6}$	87	95	95	100
	$1 \cdot 10^{-5}$	87	93	105	102
	$1 \cdot 10^{-4}$	72	82	80	83

In summary, the obtained results show a good promise for further studies of physiological activities of this class of compounds.

Experimental

^{1}H and ^{19}F NMR spectra were run on Bruker AMX-400 and Bruker AMX-300 instruments with working frequencies of 400.13 and 376.50 MHz, respectively, at 20 °C. The chemical shifts are given in the δ scale relative to the residual solvent signal (^{1}H) and CF_3COOH (^{19}F, an external standard). Mass spectra (EI, 70 eV) were obtained with a Kratos MS-890 instrument. The starting compounds **1a—i** are commercially available. 3,3,3-Trifluoro-1-nitroprop-1-ene was synthesized by the known procedure.[16]

8-Methoxy-3-nitro-2-trifluoromethyl-2*H*-chromene (2a). ***A.*** To a solution of 3-methoxysalicylaldehyde **1a** (0.25 g, 1.61 mmol) and 3,3,3-trifluoro-1-nitroprop-1-ene (0.23 g, 1.63 mmol) in dichloromethane (1.5 mL), a solution of triethylamine (16 mg, 0.16 mmol) in dichloromethane (1.5 mL) was added dropwise over a period of 1 h at 5 °C. The obtained solution was stirred for 40 h, washed with 1% aqueous HCl and water (3×15 mL). The aqueous layer was extracted with dichloromethane (2×10 mL), the organic layer was separated, dried with sodium sulfate, and the solvent was removed *in vacuo*. The dark brown residue was recrystallized from hexane, the crystals were washed with hexane and dried. Yield 87%. Compounds **2b—i** were synthesized similarly. Physicochemical characteristics and spectral data for compounds **2a—i** are given in Tables 1 and 2.

B. To a solution of 3-methoxysalicylaldehyde **1a** (4.56 g, 30 mmol) and 3,3,3-trifluoro-1-nitropropene (4.2 g, 30 mmol) in propan-2-ol (15 mL), a solution of triethylamine (0.3 g, 3 mmol) in propan-2-ol (15 mL) was added dropwise over a period

of 1 h at 5 °C. The reaction mixture was stirred for 24 h at ~20 °C and then cooled to 5 °C. The precipitate formed was collected by filtration, washed with cold propan-2-ol, and dried *in vacuo*. No further purification of the product was required. Yield 89%. Compounds **2b—i** were synthesized similarly.

3-Amino-8-methoxy-2-trifluoromethylchromane (3a). A three-necked flask (100 mL) equipped with a thermometer, a pressure equalizing dropping funnel, and a condenser was charged with 1 *M* solution of B_2H_6 in THF (10 mL, 10 mmol) followed by cooling to 5 °C. Then, a solution of compound **2a** (0.6 g, 2.5 mmol) in THF (10 mL) was added over a period of 2 h with stirring. After 1 h, $NaBH_4$ (50 mg) was added, the cooling was removed, and the reaction mixture was refluxed for 8 h. After cooling down, the mixture was poured onto ice (200 g) with stirring. The obtained mixture was acidified with 1 *M* HCl to pH 2 and then heated at 70 °C for 2 h with stirring. After cooling to 20 °C, the mixture was extracted with diethyl ether (3×25 mL). The aqueous layer was separated, basified with 10% aqueous NaOH, and extracted with diethyl ether. Combined organics were dried with sodium sulfate and the solvent was removed *in vacuo*. The residue was dissolved in MeCN (20 mL) and treated with a solution of *p*-toluenesulfonic acid (0.45 g, 5 mmol) in MeOH (5 mL). Slow removal of the solvents resulted in tosylate **3a**, colorless crystals.

Oxalate **3b** was obtained similarly with the use of oxalic acid.

Hydrochlorides **3c—f**,**h**,**i** were obtained as follows: gaseous HCl was bubbled through a solution of the corresponding neutral compound in anhydrous diethyl ether, the crystals of thus obtained hydrochloride were washed with diethyl ether and dried. Yields and spectral data for compounds **3a—f**,**h**,**i** are given in Tables 3 and 4.

***N*-[6,8-Dibromo-2-(trifluoromethyl)-3,4-dihydro-2*H*-chromen-3-yl]hydroxylamine (4)** was isolated from acidified aqueous layer obtained in the synthesis of **2c** by extraction with diethyl ether. Removal of the solvent, purification of the residue by column chromatography, and subsequent recrystallization from methanol afforded compound **4** in 70% yield (based on the amount of the residue obtained by extraction), m.p. 142—144 °C. Found (%): C, 30.85; H, 2.08; N, 3,44. $C_{10}H_8Br_2F_3NO_2$. Calculated (%): C, 30.72; H, 2.06; N, 3.58. MS (ESI), m/z (I_{rel} (%)): 391 (42). ^{1}H NMR (DMSO-d_6), δ: 3.03 (d, 2 H, CH_2, J = 3.0 Hz); 3.60 (s, 1 H, CHNHOH); 5.05 (m, 1 H, $CHCF_3$); 5.71 (d, 1 H, NH, J = 6.7 Hz); 7.41 (s, 1 H, Ar); 7.50 (s, 1 H, Ar); 7.6 (d, 1 H, OH, J = 2.0 Hz). ^{19}F NMR (DMSO-d_6), δ: 6.13 (s, 3 F, CF_3).

Cell culturing technique. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 10 mmol L^{-1} HEPES (Sigma, USA), 2 mmol L^{-1} L-glutamine (Sigma, USA), 40 ng mL^{-1} gentamicin (ICN, USA), 0.1% amino acid solution, and 0.1% vitamin solution (PanEco, Russia) at 37 °C under a 5% CO_2 environment. In order to maintain log phase growth, the cells were passaged every 3—4 days. The primary adherent cells were detached from polystyrene culture vessels with Versene and washed with serum-free RPMI-1640 medium.

MTT assay. The SKOV-3, HCT-116, and A549 cell were planted in clear-bottom 96-well plate at a density of $4 \cdot 10^3$ cells per mL, and the Jurkat cells, at a density of $15 \cdot 10^3$ cells per mL in complete RPMI-1640 culture medium (180 μL, Costar, USA). For cytotoxicity evaluation, 20 μL of a solution containing 2 μL of a stock solution of the test compound in DMSO and 18 μL of complete RPMI-1640 medium were added in each well and the plates were incubated for 72 h at 37 °C under a 5% CO_2 environment.

The test compounds were examined at concentrations of $1 \cdot 10^{-7}$, $1 \cdot 10^{-6}$, $1 \cdot 10^{-5}$, and $1 \cdot 10^{-4}$ mol L^{-1} in triplicate. For negative control, 2 μL of DMSO and 18 μL of complete RPMI-1640 medium per well were added. After 72 h, 20 μL of a MTT solution (stock concentration 5 mg mL^{-1}, final concentration 1 mg mL^{-1}) was introduced into each well and the plates were incubated at 37 °C for 4 h under a 5% CO_2 environment.

After formazan formation, the medium was removed. The precipitate was dissolved by adding 150 μL of DMSO and the plates were incubated at 37 °C for 5—7 min followed by gentle shaking on an orbital shaker. Then, the absorbance were measured at λ = 530 nm with a photometric immunoassay analyzer AIFP-01 Uniplan (Pikon Ltd., Russia). The absorbance value is directly proportional to the number of viable cells.

Cell survival rate was calculated by the following equation:

$$\text{Cell survival rate (\%)} = (D_{exp}/D_{control}) \cdot 100\%,$$

where D_{exp} is an absorbance of the experimental wells, $D_{control}$ is an absorbance of the negative control wells.

References

1. Pat. EA 015364; *Byul. Izobret.* [*Invention Bull.*], 2011, No. 3; http://www.eapo.org/ru/patents/reestr/.
2. G. P. Ellis, in *The Chemistry of Heterocyclic Compounds*, Ed. G. P. Ellis, Wiley, New York, 1977, p. 31.
3. D. Dauzonne, R. Royer, *Eur. J. Med. Chem. Chim. Ther.*, 1984, **19**, 477.
4. G. J. Finn, B. S. Creaven, D. A. Egan, *Eur. J. Pharm. Sci.*, 2005, **26**, 16.
5. R. Bergmann, R. Gericke, *J. Med. Chem.*, 1990, **33**, 492.
6. G. Burrell, F. Cassidy, J. M. Evans, D. Lightowler, G. J. Stemp, *J. Med. Chem.*, 1990, **33**, 3023.
7. S. Purser, P. R. Moore, S. Swallow, V. Gouverneur, *Chem. Soc. Rev.*, 2008, **37**, 320.
8. W. Dmovski, K. Piasecka-Maciejewska, *Org. Prep. Proc. Int.*, 2002, **34**, 514.
9. C.-L. Wang, H.-Q. Li, W.-D. Meng, F.-L. Qing, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4456.
10. M. Medebielle, R. Keirouz, E. Okada, D. Shibata, W. R. Dolbier, *Tetrahedron Lett.*, 2008, **49**, 589.
11. V. Yu. Korotaev, I. B. Kutyashev, V. Ya. Sosnovskikh, *Heteroat. Chem.*, 2005, **16**, 492.
12. V. Yu. Korotaev, V. Ya. Sosnovskikh, I. B. Kutyashev, A. Yu. Barkov, E. G. Matochkina, M. I. Kodess, *Tetrahedron*, 2008, **64**, 5055.
13. E. N. Stukan´, S. V. Makarenko, V. M. Berestovitskaya, *Russ. J. Gen. Chem.* (*Engl. Transl.*), 2011, **81**, 155 [*Zh. Obshch. Khim.*, 2011, **81**, 157].
14. V. Yu. Korotaev, V. Ya. Sosnovskikh, I. B. Kutyashev, M. I. Kodess, *Russ. Chem. Bull.* (*Int. Ed.*), 2006, **55**, 317 [*Izv. Akad. Nauk*, *Ser. Khim.*, 2006, 309].
15. V. Yu. Korotaev, V. Ya. Sosnovskikh, I. B. Kutyashev, M. I. Kodess, *Russ. Chem. Bull.* (*Int. Ed.*), 2006, **55**, 2020 [*Izv. Akad. Nauk*, *Ser. Khim.*, 2006, 1945].
16. S. Iwata, Y. Ishiguro, M. Utsugi, K. Mitsuhashi, K. Tanaka, *Bull. Chem. Soc. Jpn.*, 1993, **66**, 2432.

Received June 5, 2014;
in revised form June 25, 2014