Tris(1-alkylindol-3-yl)methylium salts as a novel class of antitumor agents

*E. V. Stepanova,[a] A. A. Shtil´,[a] S. N. Lavrenov,[b] V. M. Bukhman,[a] A. N. Inshakov,[a] E. P. Mirchink,[b] A. S. Trenin,[b] O. A. Galatenko,[b] E. B. Isakova,[b] V. A. Glazunova,[a] L. G. Dezhenkova,[b] E. Sh. Solomko,[a] E. E. Bykov,[b] and M. N. Preobrazhenskaya[b]**

[a]*N. N. Blokhin Russian Oncological Scientific Center, Russian Academy of Medical Sciences, 24 Kashirskoe sh., 115478 Moscow, Russian Federation. Fax: +7 (495) 324 1205*

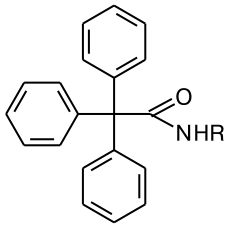
[b]*G. F. Gause Research Institute of New Antibiotics, Russian Academy of Medical Sciences, 11 ul. Bol´shaya Pirogovskaya, 119021 Moscow, Russian Federation. Fax: +7 (499) 245 0295. E-mail: mnp@space.ru*

Tris(1-alkylindol-3-yl)methanes were obtained and oxidized into tris(1-alkylindol-3-yl)methylium salts. The resulting salts are more toxic to cultured tumor cells than to non-tumor ones. The cytotoxicity of tris(1-alkylindol-3-yl)methylium salts depends on the length of the substituent at the N atom of the heterocycle, increasing from an *N*-unsubstituted derivative toward *N*-butyl- and *N*-pentyl derivatives. A further increase in the length of the *N*-alkyl substituent lowers the cytotoxicity. The cytotoxicity of tris(1-alkylindol-3-yl)methylium salts for tumor cells correlates with their antibacterial and antifungal activity. Tris(1-alkylindol-3-yl)methylium salts produced a cytocide effect on Gram-positive microorganisms and the most active compounds, on Gram-negative microorganisms as well. Similar patterns of the structure—activity relationship of *N*-alkylated tris(indol-3-yl)methylium derivatives, which was observed for various lines of tumor cells, bacteria, and fungi, suggest the general character of the mechanisms of the death of prokaryotic and eukaryotic cells induced by these compounds.

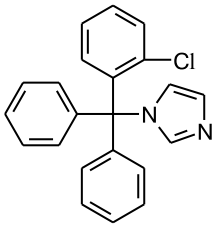
Key words: antitumor agents, tris(1-alkylindol-3-yl)methanes, tris(1-alkylindol-3-yl)-methylium salts, propeller compounds, cytotoxicity, turbomycin A, antibacterial activity, antifungal activity.

In the last few years, the attention of researchers is attracted by compounds containing a triphenylmethane fragment.[1—5] Some compounds of this type have been found to exhibit anti-proliferation activity. Researchers make an emphasis on the multifunctional role of the triphenylmethyl structural motif in antitumor compounds. For instance, it has been demonstrated that triphenylacetamides (TPMAs) stop the cell cycle at the phase G1 and induce the apoptosis of melanoma cells in the culture.[1—4] Triphenylacetamides substantially reduce the level of the nuclear factor NFkB in cells; NFkB is constitutively active in melanoma and its activity is crucial for proliferation of melanoma cells and triggering of their apoptosis.

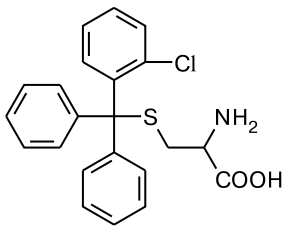
We found it interesting to move from compounds containing a triphenylmethyl fragment to trihetarylmethyl derivatives. Since the investigation of TPMAs has revealed the biological activity of compounds with electron-donating substituents in the phenyl rings, it was reasonable to study first indole-containing compounds. Earlier,[6] we have developed a method for the synthesis of symmetrical tris-(1-alkylindol-3-yl)methanes and the corresponding tris-(1-alkylindol-3-yl)methylium salts.



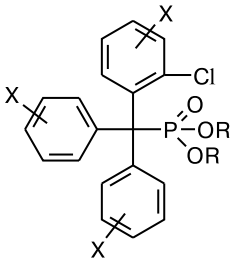
TPMAs



Clotrimazole



S-Trityl-L-cysteine



Triphenylphosphonic acid

These compounds are homologs of the antibiotic turbomycin A (**1a**), which is chemically a tris(indol-3-yl)-

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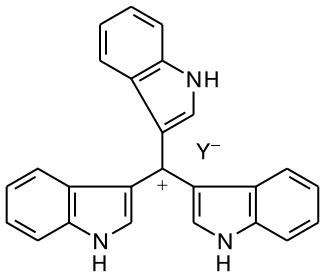
methylium salt. Turbomycin A and turbomycin B (bis-(indol-3-yl)(phenyl)methylium salt) were isolated from soil by metagenomics using 24 546-membered DNA expressed in *Escherichia coli*.[7] Turbomycins A and B exhibit some activity against Gram-negative and Gram-positive microorganisms.



Turbomycin A (**1a**)

Tris(1-alkylindol-3-yl)methylium salts have been found[6] to be cytotoxic. The cytotoxicity of these compounds depends on the length of the alkyl chain, passing through a maximum at $C_{alk} = 4—5$. The most active compounds of this series induce the apoptosis of tumor cells.[6] In the present work, we developed convenient methods for the preparation of tris(1-alkylindol-3-yl)methylium salts, studied their physicochemical properties, structure—activity correlations (cytotoxicity and antitumor activity), and antibacterial and antifungal properties, and examined some parameters of the mechanism of action of these compounds.

Results and Discussion

Synthesis and physicochemical properties of tris(1-alkylindol-3-yl)methylium salts

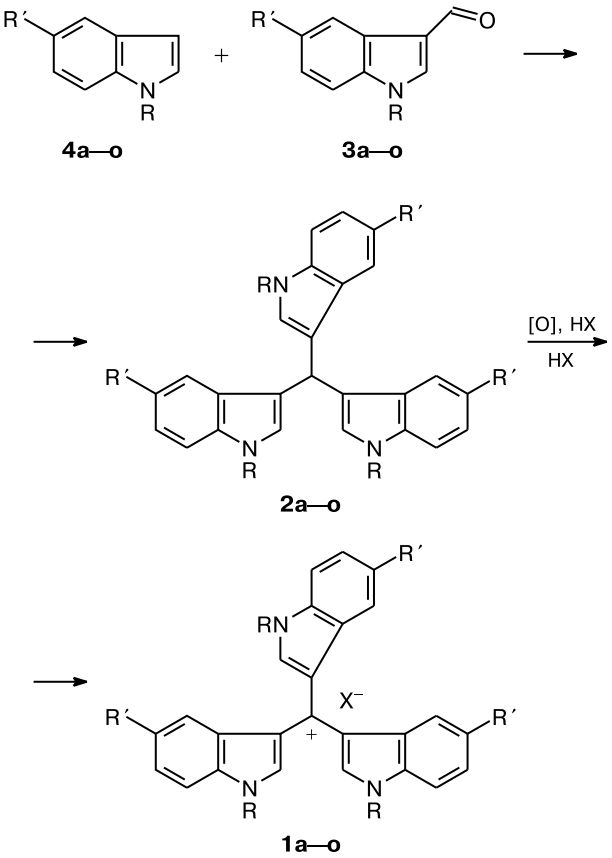
Earlier,[6] symmetrical tris(1-alkylindol-3-yl)methanes **2** have been obtained by acid-catalyzed condensation of 1-alkylindole-3-carbaldehydes **3** with the respective 1-alkylindoles **4** (Scheme 1).

It turned out that tris(1-alkylindol-3-yl)methanes can conveniently be prepared by alkylation **2a** with alkyl halides in DMSO in the presence of KOH as described[8] for N-alkylation of indoles (Scheme 2). According to data from HPLC, NMR spectroscopy, and mass spectrometry, the resulting *N*-alkylindolylmethanes **2b—m** are identical with the compounds described earlier.[6]

Tris(1-alkylindol-3-yl)methylium salts **1** are prepared by oxidation of tris(1-alkylindol-3-yl)methanes **2** (*e.g.*, with dichlorodicyanoquinone (DDQ), chloranil,[1,2] tritylium perchlorate,[9] or $FeCl_3$.[10]

Earlier,[6] we have oxidized tris(1-alkylindol-3-yl)-methanes into tris(1-alkylindol-3-yl)methylium salts in butanol in the presence of the corresponding acid using activated charcoal saturated with atmospheric oxygen.

Scheme 1

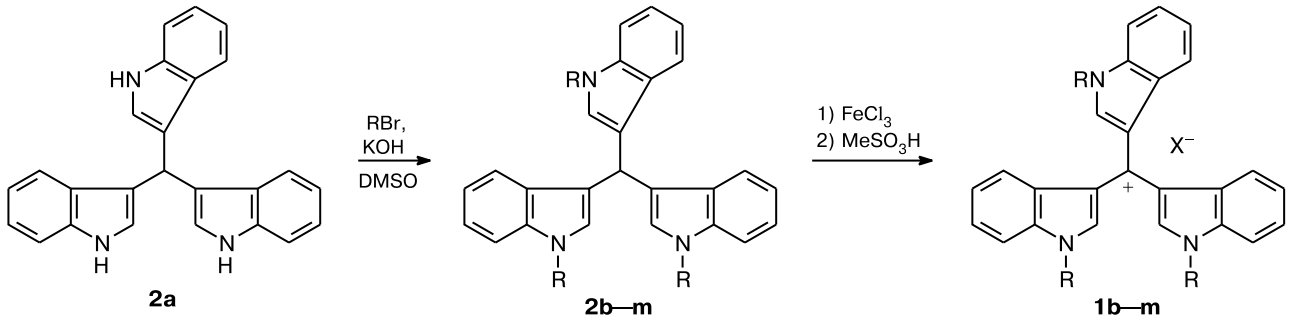


R = H (**a**), Me (**b**), Et (**c**), Pr^n (**d**), Bu^n (**e**), n-C_5H_{11} (**f**), n-C_6H_{13} (**g**), CH_2Ph (**h**), n-C_7H_{15} (**i**), n-$C_{10}H_{21}$ (**j**), $CH_2CH_2CHMe_2$ (**k**), Bu^s (**l**), Ph (**m**); R = n-C_5H_{11} (**n**, **o**);
R′ = H (**a**—**m**), Br (**n**), OMe (**o**)
X = Cl, $MeSO_3$

Here we carried out the oxidation of tris(1-alkylindol-3-yl)methanes with Fe^{III} salts in a homogeneous medium (see Scheme 2). Triindolylmethylium salts of strong acids are intensely colored ionic compounds soluble in water. Like triphenylmethane dyes, they turn colorless in basic solutions (formation of carbinols). Acidification transforms the carbinol again into a colored triindolylmethylium salt (Scheme 3). Using this reaction, we converted one triindolylmethylium salt ($Y^- = Cl^-$) into others ($X^- = MeSO_3^-$, AcO^-). Triindolylmethylium methanesulfonates are best soluble in water among the salts studied.

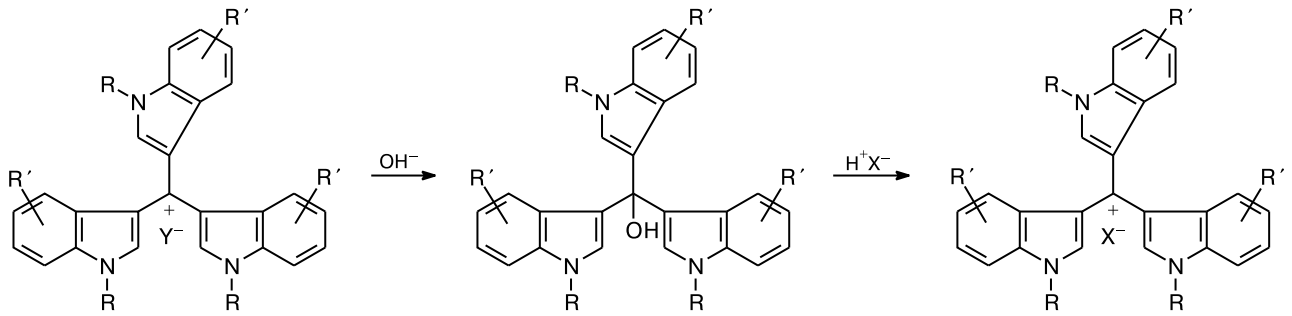
The room-temperature ^1H NMR spectra of tris(1-alkylindol-3-yl)methylium salts show two sets of signals with an intensity ratio of 1 : 2 for the H(2) proton; above 100 °C, only one set of signals is observed.[6] The hetaryl rings in the propeller compounds under study are not symmetrical: they can exist as a mixture of isomeric propeller conformations because addition of three aromatic (indole) rings to the central atom (a hub) in triindolylmethylium salts makes up a three-blade propeller.[11] Rotation of the indole

Scheme 2



$X^- = MeSO_3^-$

Scheme 3

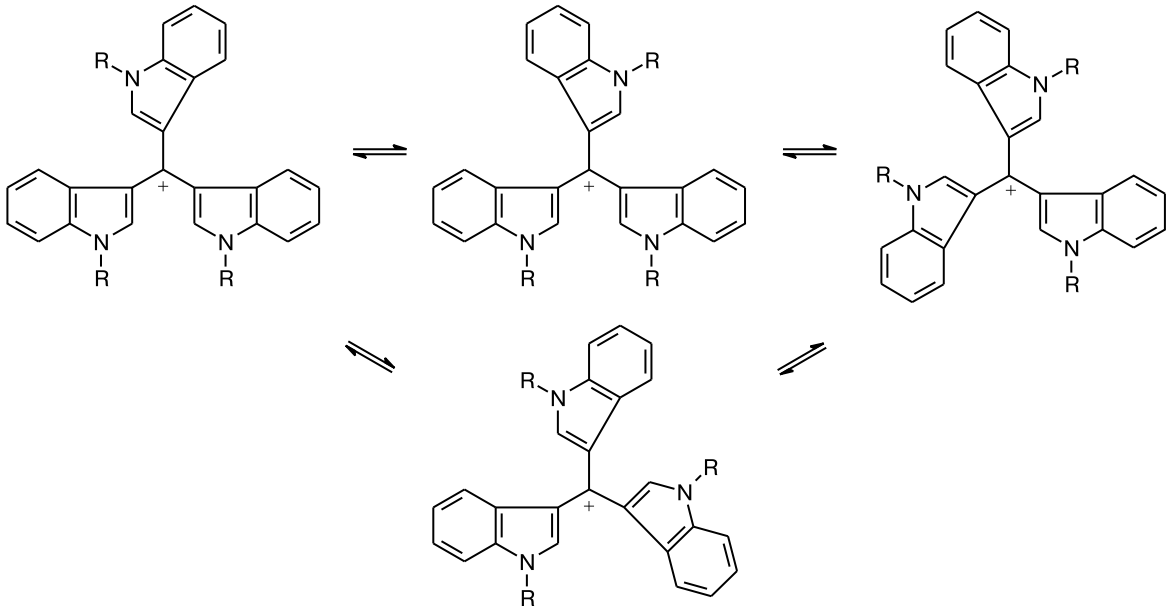


rings clockwise and anticlockwise gives rise to several conformations (Scheme 4).

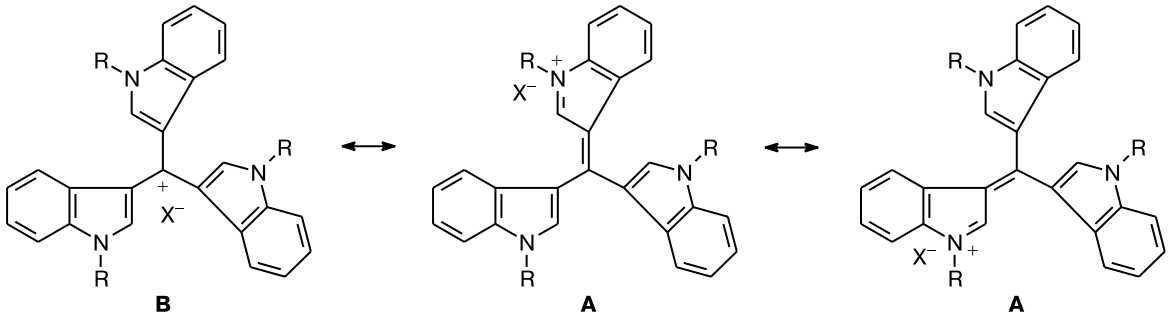
The resonance stabilization (Scheme 5) facilitates the rotation of the indole rings about the imaginary plane. The fact that the spectrum at 100 °C contains the signals of only one form suggests rapid interconversions of the conformers.

Quantum-chemical computations using the semiempirical AM1 method and the DFT method (B3LYP/6-

Scheme 4



Scheme 5



31G(d)) revealed not only a local minimum on the potential energy surface that corresponds to the degenerate, delocalized form of triindolyl cation **B** but also a local minimum corresponding to cation **A** with the indolenine ring. It should be noted that the total energy difference between the two cation forms is small (the delocalized form of triindolyl cation **B** is more stable than indolenine form **A** only by 1.4 kcal mol^{-1} (B3LYP/6-31G(d)).

Biological tests

The cytotoxicity of tris(1-alkylindol-3-yl)methylium methanesulfonates was estimated in an MTT assay for such human tumor cell lines as HCT116 intestine carcinoma, K562 and Jurkat leukoses, Mel Kor melanoma, SKOV-3 (ovarian cancer), and SK-BR-3 and MCF-7 (breast cancer) (Table 1). The cytotoxicity of novel compounds was also studied with non-tumor cells: the lymphocytes of healthy donors and the endothelial cell line of mouse blood vessels SVEC-4-10. The average IC_{50} values (IC_{50} is an inhibitor´s concentration required to suppress the cell proliferation by 50%) decreased with an increase in the number of carbon atoms in the unbranched alkyl substituent from *N*-methyl (**1b**) to *N*-butyl (**1e**) and 1-pentyl derivatives (**1f**). This trend was true for all the tumor cell lines studied. The antibiotic turbomycin A (**1a**) was comparably active with the *N*-ethyl derivative (**1c**). The compounds with substituents longer than *N*-pentyl (**1f**) exhibit lower activity (higher IC_{50} values). The most active compounds **1e** and **1f** are more toxic for the cell line obtained from a disseminated melanoma sample of human skin (Mel Kor) than for the breast cancer SK-BR-3 and the ovarian cancer SKOV-3 (see Ref. 6 and Table 1);

Table 1. Cytotoxic effect of tris(1-alkylindol-3-yl)methylium methanesulfonates **1a—o** on tumor cells, lymphocytes of donors, and the endothelial cells of mouse blood vessels

Compound	IC_{50}/μmol L^{-1}								
	SVEC-4-10	Lymphocytes of donors	HCT116	K562	Jurkat	Mel Kor	SKOV-3	MCF-7	SK-BR-3
1a	—	—	2.30±0.20	1.60±0.40	—	—	—	—	—
1b	—	—	3.20±0.30	3.20±0.30	—	—	—	—	—
1c	>10	2.43±0.99	1.30±0.30	0.20±0.10	0.50±0.19	0.22±0.04	2.26±0.42	1.31±0.16	2.17±0.94
1d	>10	1.72±0.06	1.00±0.30	0.20±0.10	0.27±0.01	0.23±0.06	0.81±0.03	1.84±0.66	1.76±0.05
1e	0.37±0.00	0.94±0.25	0.30±0.10	0.09±0.04	0.22±0.03	0.07±0.01	0.25±0.04	0.65±0.25	0.26±0.12
1f	0.36±0.00	0.66±0.10	0.15±0.04	0.05±0.03	0.20±0.06	0.05±0.01	0.28±0.07	0.78±0.11	0.43±0.19
1g	0.76±0.03	1.64±0.00	0.30±0.10	0.07±0.03	0.49±0.01	0.61±0.18	3.81±2.64	>10	>10
1h	0.18±0.01	0.14±0.00	0.10±0.04	0.03±0.01	0.03±0.01	0.06±0.02	0.32±0.06	0.48±0.15	2.47±0.08
1i	0.64±0.14	2.97±0.00	1.00±0.20	0.20±0.04	1.13±0.14	0.42±0.07	4.11±2.18	4.66±1.12	6.39±1.85
1j	—	—	16.40±2.30	12.4±2.10	—	—	—	—	—
1k	—	—	<0.1	—	0.14±0.02	0.36±0.08	0.39±0.08	1.33±0.52	0.42±0.03
1l	>10	—	2.50±0.70	5.9±1.50	—	—	—	—	—
1m	1.60±0.0	—	—	—	—	0.51±0.13	0.88±0.29	3.24±0.25	0.87±0.02
1n	>10	—	7.30±2.00	—	—	>10	>10	>10	>10
1o	4.86±0.42	>10	0.80±0.20	0.15±0.03	>10	0.94±0.06	4.58±1.64	>10	>10

Note. IC_{50} is the concentration required to suppress the proliferation of tumor cells by 50%. Here and in Tables 2 and 3, the standard errors of the measurements were determined from at least three independent experiments.

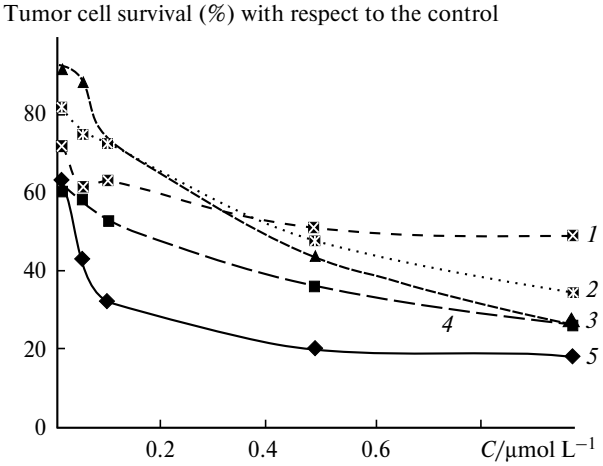


Fig. 1. Cytotoxicity of compound **1h** against various tumor cells: Jurkat leukemia (*1*), Mel Kor melanoma (*2*), SKOV-3 ovarian cancer (*3*), MCF-7 breast cancer (*4*), and SK-BR-3 (*5*).

Table 2. Apoptotic effects of compounds **1c—i,k,o** on the cell line of the T-cell lymphoblastic Jurkat leukemia in humans

Compound	Number of apoptotic cells	
	1.0	0.1
1c	19.64±7.82	5.27±1.39
1d	30.48±7.55	5.71±1.52
1e	50.83±0.47	21.21±5.14
1f	66.27±4.64	6.99±1.84
1g	52.54±6.84	13.39±0.70
1h	84.64±6.20	45.71±1.67
1i	24.89±2.40	15.70±1.87
1k	96.95±0.56	22.53±4.06
1o	24.51±2.50	17.8±0.45

Note. The number of apoptotic cells (% of the control test) is given for compounds **1** (c = 1.0 and 0.1 μmol L^{-1}).

compounds **1g,h,l** are most cytotoxic for Jurkat and K562 leukemia cells (Fig. 1).

It is worth noting that the most active compounds **1f** and **1e** are less toxic for the lymphocytes of healthy donors and the endothelial cell line of mouse blood vessels SVEC-4-10 than for the tumor cells studied; this suggests that compounds **1f** and **1e** are mainly toxic for proliferating cells. The presence of an isopentyl residue in the indole ring somewhat decreases the cytotoxicity (compound **1k**). Derivative **1l** containing a *sec*-butyl residue is much less cytotoxic than compound **1e** containing an *n*-butyl group. The presence of substituents in the indole fragments of the most active tris(1-pentylindol-3-yl)-methylium makes the resulting 5-bromoindole (**1n**) and 5-methoxyindole derivatives (**1o**) substantially less active. *N*-Aryl derivatives (1-benzyl (**1h**) and 1-phenyl (**1m**)) are only slightly inferior in activity to 1-pentyl derivative **1f**; these compounds also exhibit high submicromolar activity.

The cell death (in particular, the induced apoptosis of the Jurkat cell line) under the action of the compounds obtained was studied (Table 2). Cells were incubated together with the inhibitors for 48 h and stained with a conjugate of Annexin V with fluorescein isothiocyanate (FITC) for estimation of their apoptosis using flow cytofluorimetry. Compounds **1e,f** in a concentration of 1 μmol L^{-1} caused the apoptosis of approximately half the tumor cells (50.2 and 57%, respectively). The best apoptotic effects were exhibited by tris(1-benzylindol-3-yl)-methylium (**1h**) and tris(1-isopentylindol-3-yl)methylium (**1k**) (Figs 2, 3).

Compound **1f** caused the DNA disintegration characteristic of apoptosis. First, after the 24-h incubation of Jurkat cells with compound **1f** (c = 1 μmol L^{-1}), the percentage of cell nuclei in the sub-G1 region increased on the cell cycle histogram: 25±4% versus 4±1% in intact cells. After the 48-h incubation with compound **1f**, the percentage of cell nuclei in the sub-G1 region increased to 45±6%. Second, the internucleosomal DNA degradation took place, which was identified as a set of DNA fragments differing in length by 140—170 nucleotide pairs). It is essential that the death of cells under the action of compound **1f** did not depend on the status of pro-apoptotic protein p53, because this compound was equally cytotoxic for the line HCT116 ($p53^{+/+}$) and the subline HCT116p53KO with the inactivated protein p53 (the data are omitted).

The heterodimeric protein NFkB regulates the expression of various genes (including some proto-oncogenes) involved in the apoptosis inhibition, increases the cell survival and proliferative activity, and stimulates angiogenesis and metastasis, which play a key role in tumor development and progression. The activation of the protein NFkB is associated with the tumor resistance to various chemotherapeutical agents and radiation therapy. Constitutive expression and/or activation of the protein NFkB is found in many types of malignant tumors, including breast and lung cancers, melanomas, lymphomas, *etc*. When activated, the protein NFkB is translocated to the cell nucleus to activate the transcription of some genes, including antiapoptotic ones.[13]

We studied the influence of the newly obtained compounds in nontoxic concentrations on the amount of the activated subunit p65 of protein NFkB. The compounds reduced the level of the subunit p65 in cells and blocked its translocation to the cell nucleus. For instance, compound **1h** in concentrations below 10 nmol L^{-1} substantially decreased the amount of p65 in Jurkat cells. Jurkat line cells were incubated with compound **1h** ($c = 10^{-8}$ mol L^{-1}) for 48 h, stained with antibodies for activated NFkB, and analyzed on a fluorescence microscope. The best inhibi-

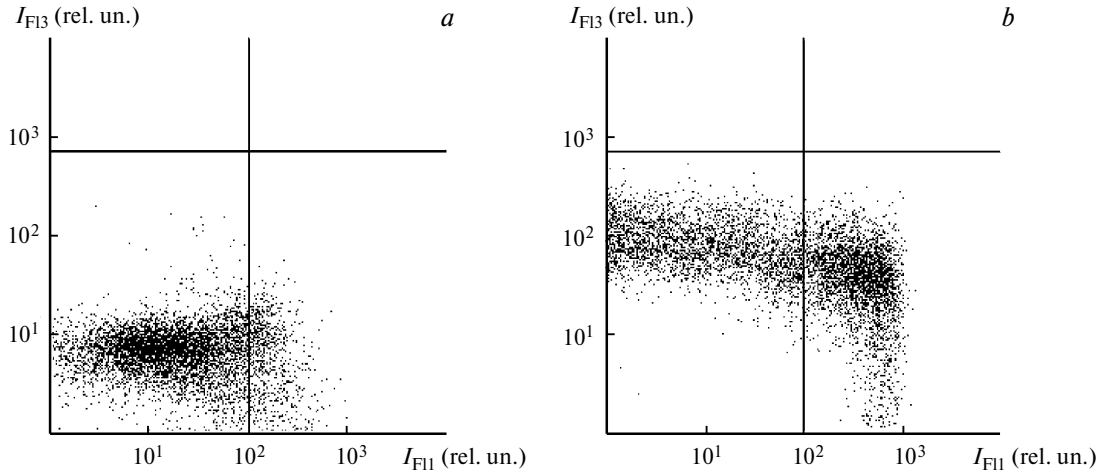


Fig. 2. Apoptotic effect of compound **1h** on the Jurkat cell line (stained with Annexin V). Jurkat leukemia cells before (*a*) and after 48-h incubation (*b*) with compound **1h** ($c = 0.1$ μmol L^{-1}).

tion of activated NFkB was achieved with compounds **1f** and **1e** in a dose of 500 nmol L^{-1}.

The antitumor activity was tested for animals with transplantable tumors. When male mice B6D2F1 were injected subcutaneously with melanoma B16 (10^6 cells) at day 0 and then compound **1f** was administered nine times in doses of 7.5 mg kg^{-1} and 12.5 mg kg^{-1}, the tumor growth inhibition at observations´ day 25 was 60—70% (Fig. 4).

Compounds **1a**—**h,j,o** were also tested for the ability to suppress the growth of prokaryotic cells. In tests against Gram-positive and Gram-negative bacteria, vancomycin (Vm) and gentamicin (Gm) were employed as reference drugs (Table 3).

The structure—antibacterial activity correlation pattern for the compounds studied is similar to that for mammalian cells. Compounds **1a**—**f** and **1h** were more active than the natural antibiotic turbomycin A; *N*-propyl (**1d**), *N*-butyl (**1e**), and *N*-pentyl derivatives (**1f**) were most active. The compounds were active against both vancomycin-susceptible and vancomycin-resistant microorganisms. *N*-Butyl derivative **1e** was effective against the Gram-negative cell lines *Escherichia coli*, *Salmonella cholerae*, and *Pseudomonas aeruginosa* in minimum inhibitory concentrations (MIC) of 4 μg mL^{-1}.

The antifungal activity of compounds **1a**—**h,k** was tested against the yeast strains *Candida albicans* and *Cryptococcus humicolus* and the mycelial fungi *Aspergillus niger* and *Fusarium oxysporum* with amphotericin B as a reference drug (Table 4).

The tests of the triindolylmethylium methanesulfonates against the above fungi revealed a structure—activity correlation similar to that found for bacterial cells. Turbomycin A (**1a**) and *N*-methyl derivative **1b** were ineffective against these fungi; *N*-butyl and *N*-pentyl derivatives (**1e** and **1f**, respectively) showed enhanced antifungal activity, which decreased in compounds with longer alkyl substituents. Compounds **1e** and **1f** approximate in antifungal activity to the antifungal antibiotic amphotericin B (see Table 4).

Interestingly, tris(2-methylindol-3-yl)methylium methanesulfonate (**1p**) is much less active against bacteria

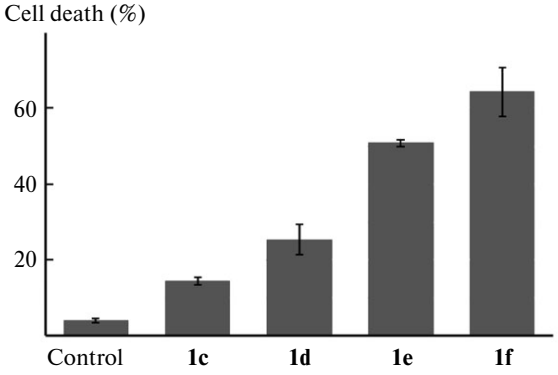


Fig. 3. Apoptotic index of Jurkat cells upon the 48-h incubation with compounds **1c**—**f** ($c = 1$ μmol L^{-1}), measured with a flow cytofluorimeter for the cells stained with Annexin V-FITC.

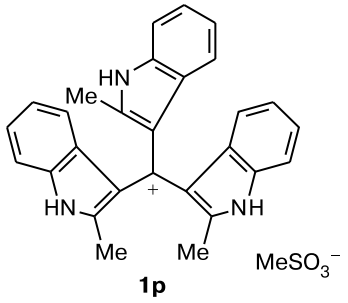
Fig. 4. Inhibition of the growth of the transplantable tumor melanoma B16 in the presence of compound **1f** added nine times every third day: $c = 7.5$ (*1*) and 12.5 mg kg^{-1} (*2*).

Table 3. Antibacterial activity of triindolylmethylium methanesulfonates **1a—h,j,p**

Compound	MIC/μg mL^{-1}										
	533 *S. epidermidis*	602 *S. haemolyticus*	3797 *S. aureus* (GISA)	3798 *S. aureus* (GISA)	559 *E. faecalis* (GSE)	560 *E. faecalis* (GRE)	569 *E. faecium* (GRE)	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27583	*K. pneumonia* 3213	*S. cholerae suis No. 100*
Control	0.25 (Vm)	0.25 (Vm)	8.0 (Vm)	8.0 (Vm)	0.13 (Vm)	>64 (Vm)	>64 (Vm)	0.5 (Gm)	4.0 (Gm)	0.5 (Gm)	0.5 (Gm) >64
1a	4	4	4	—	4	4	4	>64	>64	>64	—
1b	2	2	2	1	8	8	8	—	—	—	>64
1c	0.5	1	1	—	2	4	2	>64	>64	>64	>64
1d	0.25	0.5	0.5	0.5	1	1	1	8	32	64	16
1e	0.25	0.5	0.5	0.5	0.5	0.5	0.5	4	4	4	8
1f	1	1	1	1	2	2	2	4	8	16	>64
1g	8	16	16	16	16	32	32	32	>64	>64	>64
1h	1	1	1	1	2	2	2	64	>64	>64	—
1j	>32	>32	>32	>32	—	—	—	—	—	—	—
1p	8	16	16	16	—	—	—	—	—	—	

Note. MIC is the minimum inhibitory concentration, Vm is vancomycin (for Gram-positive bacteria), and Gm is gentamicin (for Gram-negative bacteria).

than isomeric tris(1-methylindol-3-yl)methylium methanesulfonate (**1b**). Apparently, this is due to hindered rotation of the indole ring around the hub and the prevalence of the conformations that do not interact with an intracellular target.



1p

Table 4. Activity of tris(1-indol-3-yl)methylium methanesulfonates **1a—h,j** against yeast and mycelial fungi

Compound	MIC/μg mL^{-1}			
	Candida albicans ATCC 14053	*Cryptococcus humicolus* ATCC 9949	*Aspergillus niger* ATCC 16404	*Fusarium oxysporum* VKM F-140
Control	1	1	1	4
1a	>16	>16	>16	>16
1b	>16	>16	>16	>16
1c	4	>16	16	>16
1d	1	2	2	8
1e	1	2	1	2
1f	1	1	1	2
1g	2	2	2	4
1h	2	4	2	4
1j	>16	>16	>16	>16

Note. Amphotericin B was used as a control antibiotic.

To sum up, a similar character of the structure—activity correlations for tris(1-alkylindol-3-yl)methylium salts against various types of tumor cells, bacteria, mycelial fungi, and yeast suggests their general cytotoxicity mechanism pattern for prokaryotic and eukaryotic cells.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a VXR-400 instrument (400 MHz) in DMSO-d_6 with the solvent signals as the internal standards (δ_H 2.51, δ_C 39.50). Analytical TLC was carried out on aluminum plates with fixed silica gel F_{254} (layer thickness 0.2 mm, Merck). Mass spectra were measured on Finnigan SSQ 710 and Bruker BIFLEX III MALDI TOF instruments. The purity of the compounds obtained was checked using microanalysis and HPLC. Analytical HPLC was carried out on a Shimadzu LC10 chromatograph (Kromasil-100 C18 column, 4×250 mm, 5 μm (BioKhimmak, Russia), sample concentration 0.05—0.025 mg mL^{-1}, detection at a wavelength of 286 nm).

Tris(1-pentyl-1*H*-indol-3-yl)methane (2f). Finelly ground KOH (5.13 g, 90 mmol) was added to a solution of compound **2a** (3.61 g, 10 mmol) in DMSO (100 mL). After active stirring for 40 min, 1-bromopentane (6 g, 40 mmol) was added. The reaction mixture was stirred for 3 h and diluted with water (300 mL). The precipitate that formed was filtered off and recrystallized from methanol. The yield of compound **2f** was 4.6 g (80%), colorless crystalline powder. The powder is identical with an authentic sample[6] (HPLC, NMR spectroscopy, and mass spectrometry).

Compounds **2b—e,g—m** were obtained in a similar way from compound **2a** and appropriate bromoalkanes; their identity with authentic samples[6] was confirmed by HPLC, NMR spectroscopy, and mass spectrometry.

Tris(1-pentyl-1*H*-indol-3-yl)methylium methanesulfonate (1f). Activated charcoal (0.5 g) and methanesulfonic acid (1.43 mL,

20 mmol) were added to a suspension of compound **2f** (5.72 g, 10 mmol) in butanol (100 mL). The mixture was stirred for 48 h with access to air. The charcoal was filtered off and the filtrate was twice washed with water and concentrated. The residue was triturated with ether, filtered off, and washed with ether. The yield of compound **1f** was 5.32 g (80%), red amorphous powder. Its physicochemical and spectroscopic characteristics are identical with those of an authentic sample.[6]

Tris(1-pentyl-1*H*-indol-3-yl)methylium chloride. A solution of $FeCl_3$ (4.86 g, 30 mmol) in water (50 mL) was added to a solution of compound **2f** (5.72 g, 10 mmol) in THF (200 mL). The mixture was stirred for 24 h and concentrated *in vacuo*. The residue was diluted with water and the resulting precipitate was filtered off and dried. The yield of tris(1-pentyl-1*H*-indol-3-yl)methylium chloride was 4.43 g (75%), red amorphous powder. Its physicochemical and spectroscopic characteristics are identical with those of an authentic sample.[6]

Tris(1-pentyl-1*H*-indol-3-yl)methylium acetate. A 10% solution of NaOH (30 mL) was added to a suspension of compound **1f** (6.65 g, 10 mmol) in ether (40 mL). The mixture was stirred for 30 min, whereupon the organic layer was separated and concentrated. The yield of tris(1-pentyl-1*H*-indol-3-yl)methanol was 5.75 g (98%), light yellow amorphous solid.

Acetic acid (1.2 mL, 20 mmol) was added to a solution of tris(1-pentyl-1*H*-indol-3-yl)methanol (5.87 g, 10 mmol) in THF. The mixture was stirred for 20 min and evaporated to dryness *in vacuo*. The residue was triturated with ether and the resulting precipitate was filtered off and washed with ether. The yield of tris(1-pentyl-1*H*-indol-3-yl)methylium acetate was 5.6 g (90%), red powder. Its physicochemical and spectroscopic characteristics are identical with those of an authentic sample.[6]

The cytotoxic activity of the compounds obtained was tested in a concentration range from 0.1 to 50 μmol L^{-1}. The percentage of viable cells was determined after a 48-h incubation. The average inhibiting doses required for the compounds to block the viability of 50% of cells (IC_{50}) are given in Table 1.

Cell lines. Cells were cultured in RPMI-1640 with addition of 10% fetal calf serum, $2 \cdot 10^{-3}$ *M* L-glutamine, penicillin (100 IU mL^{-1}), and streptomycin (100 μg mL^{-1}). The Mel Kor cell line was obtained from a disseminated skin melanoma sample at the Laboratory for Experimental Diagnostics and Biotherapy of Tumors of the N. N. Blokhin Russian Oncological Scientific Center (Russian Academy of Medical Sciences).[14] The subline HCT116p53KO was obtained at the laboratory headed by B. Fogelstein (Johns Hopkins University) and provided by B. P. Kopnin. Other cell lines were taken from the American Type Culture Collection. The chemicals were purchased from Sigma—Aldrich, unless otherwise specified.

Estimation of cell death. Cells were stained with a conjugate of Annexin V with FITC using an Annexin V Apoptosis Detection kit (BD Pharmingen). Jurkat cells ($6 \cdot 10^4$ cells mL^{-1}) were incubated together with the test compounds for 48 h, washed with a cold physiological buffer, and transferred to an Annexin V-binding buffer. The conjugate (5 μL) and propidium iodide (5 μL) were added and the mixture was incubated for 15 min. Cell fluorescence was determined on a flow cytofluorimeter at wavelengths of 525 (green channel) and 675 nm (red channel). At least 10 000 cells were analyzed. After the incubation with a test compound was completed, part of cells were lysed in a buffer containing 0.1% sodium citrate, the 0.3% detergent NP-40, RNAse A (100 μg mL^{-1}), and propidium iodide (50 μg mL^{-1}). To study the internucleosomal DNA degradation, the cells incubated with compound **1f** (c = 1 μmol L^{-1}) for 24 h were precipitated at 1000×g, the supernatant was centrifuged for 15 min at 12 000×g, the precipitates were combined and lysed in a buffer (pH 7.4) containing 0.02 *M* Tris-HCl, 0.35 *M* NaCl, 0.5% NP-40, 0.002 *M* $MgCl_2$, and 0.001 *M* dithiothreitol. DNA was extracted with phenol—chloroform (1 : 1) and precipiated with ethanol in the presence of 0.3 *M* sodium acetate at −20 °C. The precipitate was treated with RNAse A at 65 °C for 20 min and subjected to electrophoresis in a 1.5% agarose gel. Experiments were carried out at least three times.

Immunocytochemical determination of NFkB. Jurkat cells ($6 \cdot 10^4$ cells mL^{-1}) were incubated for 48 h with the test compounds in nontoxic concentrations, washed twice with a cold physiological buffer, and centrifuged onto the slide surfaces. Cells were fixed with ethanol and acetone and made permeable in a buffer containing 1% Triton X-100 for 5 min. Bovine serum albumin (final concentration 5%) was added. Primary antibodies for the active subunit NFkB p65 (MAB3026, Chemicon) were applied and kept for 1 h. Antibodies conjugated with Alexa Fluor 594 (A11005, Invitrogen) were used as secondary antibodies. The preparations were stained up with Hoechst-33258 (2 μg mL^{-1}) and analyzed on a Nikon 80i fluorescence microscope (400x magnification).

Estimation of the antibacterial and antifungal activities. The minimum inhibitory concentrations (MIC) for Gram-positive and Gram-negative bacteria were determined by the microdilution method in a cation-adjusted Müller—Hinton medium (Becton Dickinson and Company, Cockeysville (MD), USA) as described earlier.[15] The activity of the test compounds against various cultures of yeast and mycelial fungi was estimated using the previously described[16] micromethod by twofold serial dilutions in the nutrient medium RPMI 1640 (liquid, with L-glutamine, without sodium bicarbonate).

The data obtained were statistically processed using a *t*-test procedure.

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