Synthesis of 4-substituted 3-[3-(dialkylaminomethyl)indol-1-yl]maleimides and study of their ability to inhibit protein kinase C-α, prevent development of multiple drug resistance of tumor cells and cytotoxicity

*A. Yu. Simonov,[a] S. A. Lakatosh,[a] Yu. N. Luzikov,[a] M. I. Reznikova,[a] O. Yu. Susova,[b] A. A. Shtil',[b] S. M. Elizarov,[c] V. N. Danilenko,[d] and M. N. Preobrazhenskaya[a]**

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

[b]*N. N. Blokhin Russian Cancer Scientific Center, Russian Academy of Medical Sciences, 24 Kashirskoe sh., 115478 Moscow, Russian Federation. Fax: +7 (495) 324 1114*

[c]*A. N. Bakh Institute of Biochemistry, Russian Academy of Sciences, 33, str. 2 Leninsky prosp., 119071 Moscow, Russian Federation. Fax: +7 (495) 954 2732*

[d]*N. I. Vavilov Institute of General Genetics, Russian Academy of Sciences, 3 ul. Gubkina, 119991 Moscow, Russian Federation. Fax: +7 (495) 132 8962*

A series of 3-[3-(dialkylaminomethyl)indol-1-yl]maleimides containing the indole, dihydroindole, mercaptophenol, or tetrahydroquinoline residues at position 4 of the maleimide ring, as well as 3-(dialkylaminomethyl)indole derivatives have been synthesized. Their ability to inhibit *in vitro* protein kinase C-α (PKC-α) has been studied. Cytotoxicity of new compounds and their ability to constrain activation of multiple drug resistance (MDR) have been studied in the human tumor cell line. Both the toxic and the low-toxic PKC-α inhibitors prevent the activation of MDR in the tumor cells. Among compounds under study, a number of substances have been found that prevent the activation of MDR but do not inhibit PKC-α.

Key words: bisindolylmaleimides, protein kinase C, target-specific therapy, antitumor medicines, transformed cells, cytotoxicity, multiple drug resistance.

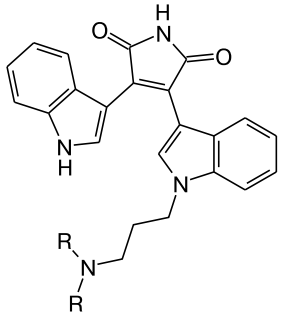
Currently, the search for inhibitors of protein kinases became one of important directions in the quest for new medicinal agents.[1] Protein kinases C (PKC) is a family of serine-threonine protein kinases playing a key role in the regulation of cellular processes in eukaryotes. The involvement of some PKC isoforms, in particular, the α-isoform (PKC-α) in the survival of tumor cells is especially important.[1,2] It was found that it mediates the intracellular signals regulating expression of the gene *MDR*1 (Multidrug Resistance 1). The product of this gene, the transmembrane protein P-glycoprotein, participating in the active outbreak of chemotherapeutic drugs from the cells into the intercellular medium, is the factor inducing multiple drug resistance (MDR) in tumor cells.[3,4] The gene *MDR*1 can be activated by the action of antitumor drugs. The acute (during the first hours of action) activation of *MDR*1 leads to the accumulation of P-glycoprotein and development of MDR.[5] Since the activation of *MDR*1 and MDR itself are mediated by PKC-α, the inhibition of this isoform prevents the emergence of MDR. In fact, bis(indolyl)-maleimide I, which is an inhibitor of PKC-α, prevents the activation of *MDR*1 by antitumor drugs.[6]

Bis(indol-3-yl)maleimides, which nowadays are under various stages of clinical trials, belong to inhibitors of protein kinases.[1,7] Among bis(indol-3-yl)maleimides and related fused compounds, *e.g.*, derivatives of indolocarbazole such as Rebeccamicin and Staurosporin, the highly active inhibitors of serine-threonine protein kinases and topoisomerase I, triggering tumor cell apoptosis, have been found.[1,8] Compounds Bis-I and Bis-III are well-known PKC inhibitors, they derive from unsubstituted bis(indol-3-yl)maleimide (Bis-IV), which is the inhibitor of PKC, too. The active derivatives contain the maleimide core unsubstituted at the nitrogen atom and aminoalkyl substitu-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 9, pp. 1977—1985, September, 2008.

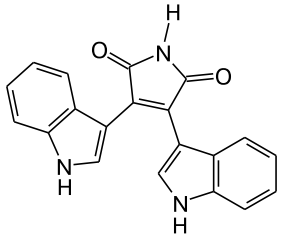
1066-5285/08/5709-2011

ent at position 1 of one of the indole rings in which the substituted or unsubstituted amino group is separated from the indole core by three carbon atoms.[8]



Bis-I, Bis-III

R = Me (Bis-I), H (Bis-III)



Bis-IV

Earlier,[9,10] methods for the preparation of 3,4-bis(indol-1-yl)maleimides and 3-(indol-1-yl)maleimide derivatives have been developed. These compounds are the structural analogs of 3,4-bis(indol-3-yl)maleimides, therefore, it can be assumed that new efficient inhibitors of PKC can be designed on their basis.

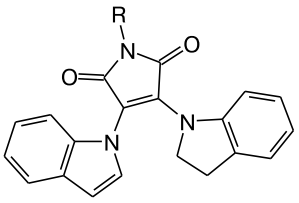
Results and Discussion

In the present work, a series of heterocycles **1—7** were converted into derivatives containing 3-dialkylaminomethyl group in the indole ring (Scheme 1), and their properties were assessed aimed at the evaluation of their prospects for the use in oncology.

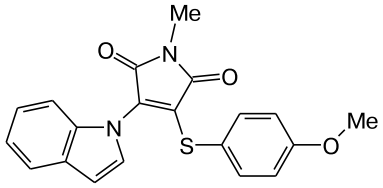
The Mannich reaction with 3,4-bis(indol-1-yl)-1-methylmaleimide (**5**) gave rise to mono- and bisaminomethylation products, which were separated by chromatography.

In contrast to the known PKC inhibitors, the indole fragment is bound in the compounds under study to the maleimide ring through the nitrogen atom, whereas the dialkylamino group is separated from the indole ring by one carbon atom. However, the distances between the nitrogen atoms of the side chain and the indole ring in compound Bis-I and in the aminomethyl derivatives obtained are close and are equal to 4.64 and 4.57 Å, respectively.[11]

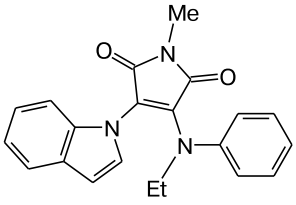
For the new compounds, their ability to inhibit PKC-α a cell-free test system, as well as cytotoxicity in the human leucosis cell line, and the ability to prevent activation of gene *MDR*1 under the action of antitumor drug Cytosar (cytosine β-D-arabinofuranoside) have been investigated. A relationship between the amount of radioactive phosphate transferred by the kinase from [γ-^{32}P]ATP to its protein substrate and the concentration of the inhibitor has been studied. The cytotoxicity is expressed as InC_{50} (inhibitory concentration that causes death of 50% of cells



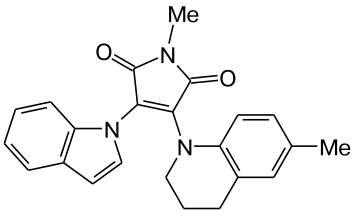
1a—d



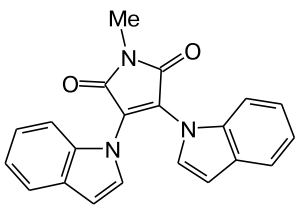
2



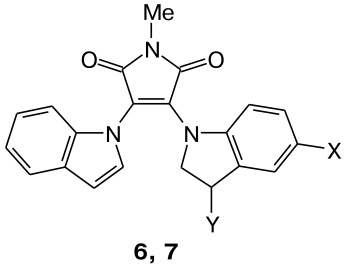
3



4



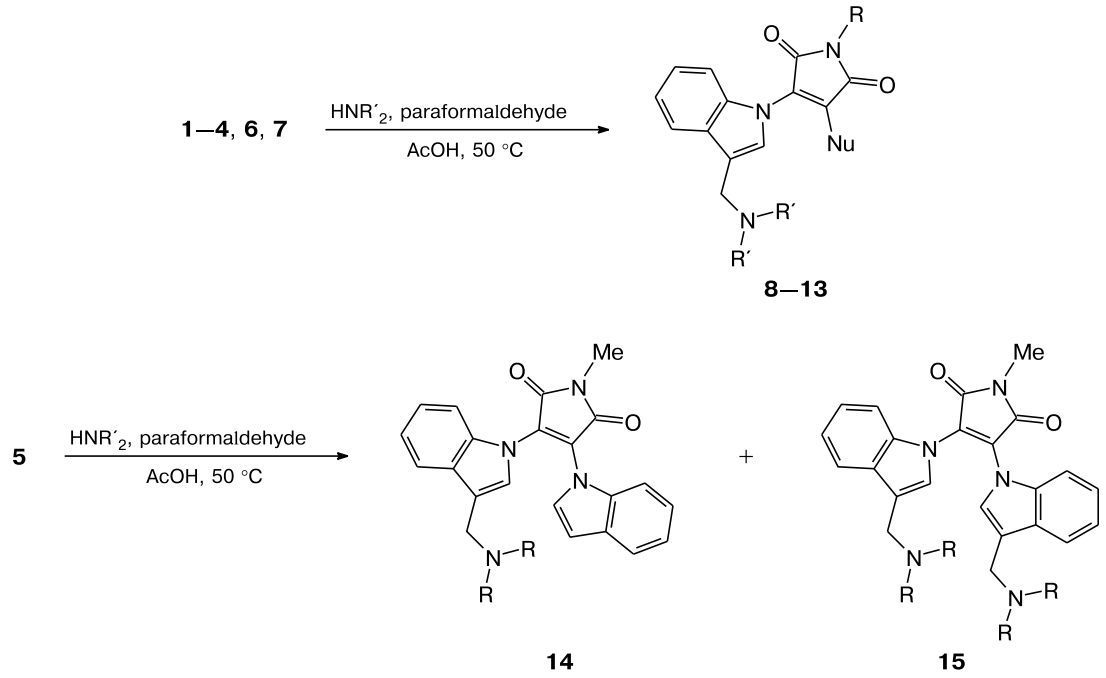
5



6, 7

1: R = H (**a**), Me (**b**), Et (**c**), $(CH_2)_3NMe_2$ (**d**)
6: Y = H, X = Cl
7: Y = COOMe, X = H

Scheme 1



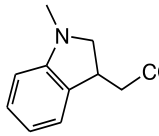
8: Nu = indolin-1-yl, R = H, R´ = Et (**a**); R = H, NR´$_2$ = pyrrolidino (**b**); R = R´ = Me (**c**); R = Me, NR´$_2$ = pyrrolidino (**d**); R = Me, NR´$_2$ = morpholino (**e**); R = Me, NR´ = 4-methylpiperazino (**f**); R = R´ = Et (**g**); R = —$(CH_2)_3NMe_2$, R´ = Et (**h**)

9: Nu = 4-methoxyphenylthio, R = Me; NR´$_2$ = NMe_2 (**a**), NEt_2 (**b**), pyrrolidino (**c**), morpholino (**d**), 4-methylpiperazino (**e**)

10: Nu = *N*-ethylanilino, R = Me; NR´$_2$ = NMe_2 (**a**), NEt_2 (**b**), pyrrolidino (**c**), morpholino (**d**), 4-methylpiperazino (**e**)

11: Nu = 6-methyl-1,2,3,4-tetrahydroquinolino, R = Me, R´ = Et

12: Nu = 5-chloroindolino, R = Me, R´ = Et

13: Nu =  , R = Me, R´ = Et

14: NR_2 = NMe_2 (**a**), NEt_2 (**b**), pyrrolidino (**c**), morpholino (**d**), 4-methylpiperazino (**e**)

15: NR_2 = NMe_2 (**a**), pyrrolidino (**b**), morpholino (**c**), 4-methylpiperazino (**d**)

after 72 h of incubation) for the K562 leucosis cells line. Compounds with $InC_{50} < 10$ μmol L^{-1} were considered as cytotoxic.

Prevention of MDR was determined from the decrease in activation of the gene *MDR*1 in the K562 cells treated with Cytosar (10 μmol L^{-1}, 16 h) in combination with a compound under study (PC_{50}).[5,6]

The data obtained are summarized in Table 1.

From the data given in Table 1, it follows that the overwhelming part of compounds synthesized are capable of suppressing the MDR induction at concentrations comparable or less than the concentration of Bis-I, the standard PKC-α inhibitor. Compounds **8b**, **9b**, and **9c** are the exceptions, they are efficient at higher concentrations.

Compounds under study can be divided into three groups with regard to their cytotoxicity. Moderately cytotoxic compounds are marked with an asterisk (*) in Table 1. The main part of compounds synthesized belongs to this group. Low-toxic compounds (**) are less numerous and are mainly represented by 3-(2,3-dihydroindol-1-yl)-4-(indol-1-yl)-1-methylmaleimide derivatives (compounds **8c—f**). It is essential that all of them suppress induction of MDR and are potent PKC-α inhibitors. Some of them exhibit activity comparable with, or exceeding, the activity of the standard inhibitor Bis-I. Mono- and bis(dialkylaminomethyl) derivatives of 3,4-bis(indol-1-yl)-1-methylmaleimide possessed high cytotoxicity (***).

A considerable difference of PKC-α inhibitors obtained from the known bis(indol-3-yl)maleimide derivatives should be emphasized: the introduction of alkyl (aminoalkyl) substituents at the maleimide nitrogen atom has no noticeable effect on the activity, which follows, for example, from the comparison of compounds **8a** and **8g**. In the case of bis(indol-3-yl)maleimide derivatives, sub-

Table 1. Biological properties of maleimide derivatives

Compound	IC_{50} /nmol L^{-1}	InC_{50}	PC_{50}
		μmol L^{-1}	
8a*	90	16±4	5.4
8b*	—	18±5	7.3
8c**	94	>50	5.2
8d**	125	>50	1.9
8e**	—	>50	3.3
8f**	—	>50	2.2
8g*	117	20±4	1.8
8h*	135	10+3	2.1
9b*	>250	12±4	10
9c*	—	12±4	7.3
9d*	—	16±4	6.6
9e*	—	16±3	4.6
10b*	150	12±3	3.7
10c*	>250	19+2	6.6
10d*	—	14±3	6.2
10e*	>250	18+2	5.4
11*	130	17+3	5.4
12***	>250	7±3	5.4
15a***	121	5±3	3.3
15b**	—	>50	5.4
15c***	—	12±3	6.1
15d**	—	>50	4.6
Bis-I	102	5±1	4.5

Note. IC_{50} is the concentration of compound inducing a decrease in activity of PKC-α by 50% (in a number of cases, (–) IC_{50} was not determined); InC_{50} is the concentration of compound inducing apoptosis of 50% of cells (in all the experiments, the corresponding values in the samples under monitoring without addition of compounds under study were taken as 100%); PC_{50} is the concentration of compound decreasing activation of gene *MDR*1 with Cytosar by 50% (the cells treated only with Cytosar were taken as having 100% activation of *MDR*1).

* Moderately toxic, ** low toxic, *** highly toxic compounds (for explanation, see text).

stitution at the maleimide nitrogen atom leads to a sharp decrease in activity. This allows us to suggest that the interaction of new inhibitors with the active site of the enzyme can differ from the binding in the bis(indol-3-yl)maleimide derivatives since it is known that the NH group of the maleimide fragment is involved in the formation of one of important hydrogen bonds between the inhibitor and the enzyme.[1,8]

It is of note that the data given in Table 1 suggest the absence of direct correlation between the inhibitory activity with respect to PKC-α *in vitro* and suppression of the MDR induction in cells under the action of Cytosar. Thus the values of IC_{50} for **8c** and **8d** are virtually the same, whereas the values of PC_{50} differ by a factor of about 3. In addition, compounds **10c**, **10e**, and **14a** are not PKC-α inhibitors being at the same time efficient suppressors of the MDR induction. This allows one to suppose that not only PKC-α, but also other enzymes involved into the process of MDR induction are the intracellular target (targets) of the compounds obtained, which makes them valuable instruments for the study of molecular mechanisms of the MDR induction in tumor cells under the action of chemotherapeutic drugs. The practical value of the new series of compounds consists also in the possibility of choosing a particular derivative depending on the properties required. Cytotoxic compounds can serve as the prototypes of new antitumor drugs, which not only suppress the growth of tumor cells, but also prevent the emergence of MDR. The low-toxic compounds can be used in combination with the known cytostatic drugs for prevention of the MDR emergence during chemotherapy.

Experimental

Melting points were measured on a Buchi SMP-20 apparatus. ^1H and ^{13}C NMR spectra were recorded on a Varian VXR-400 spectrometer (^1H, 400 MHz; ^{13}C, 75 MHz) using the solvent signal as the standard. Mass spectra were obtained on a Finnigan SAQ 710 instrument (70 eV, direct inlet, temperature of the ions source, 150 °C) using the electron impact technique (EI-MS). Analytical HPLC was performed on a Shimadzu LC10 chromatograph on a Gemini C18 column (4.6×250 mm) with the particle size of 5 μm (Phenomenex, USA). Detection was performed on a Shimadzu UV—Vis 10A spectrophotometer at the wavelength of 254 nm. The mobile phase was composed of 0.2% of $HCOONH_4$ (*A*) and acetonitrile (*B*). The elution was carried out in a gradient regime during which the percentage of acetonitrile (*B*) increased from 30 to 90% in 20 min and kept at 90% for 10 min at a flow rate of 1 mL min^{-1}. The volume of the injector loop was 10 μm, the samples were injected up to concentrations of 0.01—0.05 mg mL^{-1} in aqueous acetonitrile (1 : 1). Analytical TLC was performed on Silica gel F254 plates (Merck), column chromatography, on Silica gel Merck 60. Extracts were dried with anhydrous Na_2SO_4, solvents were evaporated under reduced pressure. Reagents and solvents, unless otherwise stated, were obtained from commercial sources. Data from HPLC, mass spectrometry, and NMR spectroscopy, as well as melting points of crystalline products are given in Tables 2 and 3.

1-(3-Dimethylaminopropyl)-3-(2,3-dihydroindol-1-yl)-4-(indol-1-yl)maleimide (8h). Excess of 3-(dimethylamino)propyl chloride hydrochloride and K_2CO_3 (5 equiv.) were added to a solution of 3-(2,3-dihydroindol-1-yl)-4-(indol-1-yl)maleimide (1 g) in dioxane (30 mL), the mixture was refluxed with stirring for ~16 h and filtered, the filtrate was concentrated. The residue was dissolved in ethyl acetate (100 mL), the solution was washed with brine (50 mL) and dried, the solvent was evaporated. After chromatography ($CHCl_3$—MeOH—Et_3N, 6 : 1 : 0.1), compound **8h** was obtained as a red solid; the yield was 0.86 g (68%).

Aminomethylation of compounds 1—4, 6, and 7 (general procedure). Paraformaldehyde (500 mg) and the corresponding amine (8 mmol) were added to a solution of the starting indolylmaleimide (2 mmol) in acetic acid (50 mL). For the preparation of dimethylamino derivatives, 40% aq. Me_2NH was used. The

Table 2. Analytical properties of compounds obtained

Compound	Molecular formula (M_w)	M.p./°C	HPLC data	
			R_t /min	Content (%)
8a	$C_{25}H_{26}N_4O_2$ (414.5)	253—255	11.05	98.6
8b	$C_{25}H_{24}N_4O_2$ (412.48)	Amorphous	10.75	97.6
8c	$C_{24}H_{24}N_4O_2$ (400.47)	Amorphous	12.33	97.1
8d	$C_{26}H_{26}N_4O_2$ (426.51)	145—146	12.98	98.0
8e	$C_{26}H_{26}N_4O_3$ (442.51)	205—207 (fumarate)	16.64	97.8
8f	$C_{27}H_{29}N_5O_2$ (455.55)	Amorphous	12.56	98.8
8g	$C_{27}H_{30}N_4O_2$ (442.55)	Amorphous	14.28	97.9
8h	$C_{30}H_{37}N_5O_2$ (499.65)	Amorphous	8.58	98.3
9a	$C_{23}H_{23}N_3O_3$ (421.51)	Amorphous	12.81	97.9
9b	$C_{25}H_{27}N_3O_3$ (449.57)	199—200 (fumarate)	8.4	99.0
9c	$C_{25}H_{25}N_3O_3$ (447.55)	212—213 (fumarate)	18.33	98.5
9d	$C_{25}H_{25}N_3O_4$ (463.55)	118—119	13.52	98.6
9e	$C_{26}H_{28}N_4O_3$ 476.59	228—230 (fumarate)	12.77	98.8
10a	$C_{24}H_{26}N_4O_2$ (402.49)	Amorphous	12.66	98.0
10b	$C_{26}H_{30}N_4O_2$ (430.54)	95—96	13.77	97.4
10c	$C_{26}H_{28}N_4O_2$ (428.53)	Amorphous	16.70	98.1
10d	$C2_6H_{28}N_4O_3$ (444.53)	Amorphous	13.61	98.2
10e	$C_{27}H_{31}N_5O_2$ (457.57)	125—126	12.87	97.9
11	$C_{28}H_{32}N_4O_2$ (456.58)	Amorphous	14.95	98.1
12	$C_{26}H_{27}ClN_4O_2$ (462.97)	115—117	14.68	98.5
13	$C_{29}H_{32}N_4O_4$ (500.59)	Amorphous	13.29	97.7
14a	$C_{24}H_{22}N_4O_2$ (398.46)	Amorphous	13.04	97.0
14b	$C_{26}H_{26}N_4O_2$ (426.51)	107—108 (fumarate)	14.07	97.6
14c	$C_{26}H_{24}N_4O_2$ (424.49)	95—97 (fumarate)	13.65	97.8
14d	$C_{26}H_{24}N_4O_3$ (440.49)	204—205	18.64	98.7
14e	$C_{27}H_{27}N_5O_2$ (453.54)	110—111 (fumarate)	12.57	97.9
15a	$C_{27}H_{29}N_5O_2$ (455.55)	Amorphous	3.65	98.3
15b	$C_{31}H_{33}N_5O_2$ (507.63)	Amorphous	3.47	98.2
15c	$C_{31}H_{33}N_5O_4$ (539.62)	Amorphous	15.24	97.6
15d	$C_{33}H_{39}N_7O_2$ (565.71)	Amorphous	8.11	99.0

reaction mixture was stirred for 20 h at 50 °C and concentrated, the residue was dissolved in ethyl acetate (100 mL), the solution was washed with saturated aq. $NaHCO_3$ (2×30 mL) and water and dried, the solvent was evaporated. The residue was subjected to chromatography (EtOAc—Pr^iOH—25% aq. NH_3, 24 : 8 : 1) to isolate the corresponding dialkylaminomethyl derivatives as red solids in 50—75% yield.

Aminomethylation of *N*-methyl-3,4-bis(indol-1-yl)maleimide (5). Paraformaldehyde (500 mg) and the corresponding amine (25 mmol) were added to a solution of compound **5** (1 g, 2.9 mmol) in acetic acid (50 mL). Under conditions described above, the corresponding mono- and bis(dialkylaminomethyl) derivatives were obtained as red solids in 50 and 20% yields, respectively.

Synthesis of fumarates of aminomethyl derivatives. Equimolar amount of fumaric acid (saturated solution in anhydrous diethyl ether) was added to a solution of compounds **1—4** and **6—9** (200—300 mg) in anhydrous diethyl ether (100 mL). The precipitate that formed was filtered off, washed with anhydrous ether (20 mL), and dried *in vacuo* to obtain fumarates of the corresponding compounds (**1—4** and **6—9**) as orange crystalline powders in 50—70% yield.

Inhibition of activity of PKC-α by new bis(indolyl)maleimide derivatives. The ativity of the human recombinant PKC-α (Calbiochem, USA) was assayed based on the incorporation of ^{32}P from ATP into the total histone from calf tymus* in the optimized cell-free test system.

The reaction was carried out in a buffer solution of the following composition: tris-HCl (20 mmol L^{-1}), $MgCl_2$ (5 mmol L^{-1}), $CaCl_2$ (1 mmol L^{-1}), NaCl (200 mmol L^{-1}), 2-mercaptoethanol (6 mmol L^{-1}), 0.01% Triton X-100, pH 7.5 (at 30 °C). The reaction mixture was prepared from the buffer solution (0.1 mL) by addition of the inhibitor under study dissolved in DMSO to the final concentrations in a mixture of 50, 100, and 250 nmol L^{-1}.

* Generous gift from the Academician L. L. Kiselev laboratory at the V. A. Engel'hardt Institute of Molecular Biology of the Russian Academy of Sciences.

Table 3. Spectral data of compounds obtained

Compound	MS, m/z (I_{rel} (%))	NMR, δ (J/Hz)	
		^{1}H	^{13}C
8a	414 $[M]^+$ (25), 342 $[M - NEt_2]^+$ (100)	0.97—1.01 (m, 6 H); 2.40—2.46 (m, 4 H); 3.08 (t, 2 H, J = 8.1); 3.66 (s, 2 H); 4.27 (t, 2 H, J = 8.1); 6.01 (d, 1 H); 6.41 (t, 1 H, J = 7.1); 6.61 (t, 1 H, J = 7.4); 6.93, 6.99 (both t, 1 H each, J = 6.9); 7.02 (d, 1 H, J = 8.3); 7.14 (d, 1 H, J = 7.9); 7.24 (s, 1 H); 7.55 (d, 1 H, J = 7.2); 10.8 (s, 1 H)	11.7 (2 C), 28.8, 45.8 (2 C), 47.6, 52.3, 108.9, 110.8, 111.6, 114.9, 119.3, 119.7, 121.9, 122.0, 124.2, 125.8, 127.1, 127.5, 131.7, 132.3, 136.8, 142.6, 167.5, 168.3
8b	412 $[M]^+$ (40), 342 $[M - N(C_4H_8)]^+$ (100)	1.83 (t, 4 H, J = 2.1); 2.90 (t, 4 H, J = 4.0); 3.09 (t, 2 H, J = 7.1); 4.23 (s, 2 H); 4.29 (t, 2 H, J = 8.0); 6.02 (d, 1 H, J = 8.1); 6.47 (t, 1 H, J = 7.5); 6.63 (t, 1 H, J = 7.4); 6.99—7.07 (m, 3 H); 7.21 (d, 1 H, J = 7.5); 7.51 (s, 1 H); 7.64 (d, 1 H, J = 7.2); 10.5 (s, 1 H)	22.5 (2 C), 28.8, 47.2, 51.7 (2 C), 52.5, 107.9, 111.2, 111.8, 118.7, 120.4, 122.3, 122.4, 124.3, 125.9, 127.0, 129.7, 132.1, 133.2, 136.5, 142.4, 167.3, 168.2
8c	400 $[M]^+$ (30), 356 $[M - NMe_2]^+$ (100)	2.13 (s, 6 H); 3.01 (s, 3 H); 3.10 (t, 2 H, J = 8.0); 3.52 (s, 4 H); 4.29 (t, 2 H, J = 7.9); 6.05 (d, 1 H, J = 8.1); 6.44, 6.62 (both t, 1 H each, J = 7.4); 6.93 (t, 1 H, J = 7.5); 6.99 (t, 1 H, J = 7.9); 7.03 (d, 122.3, 124.3, 126.0, 127.2, 1 H, J = 7.0); 7.14 (d, 1 H, J = 8.0); 7.26 (s, 1 H); 7.52 (d, 1 H, J = 7.7)	23.6, 28.8, 44.7 (2 C), 52.5, 53.9, 108.0, 110.9, 111.8, 119.8, 114.8, 119.4, 122.0, 122.3, 124.3, 126.0,127.2, 127.4, 131.9, 132.4, 136.9, 142.5, 166.4, 167.5
8d	426 $[M]^+$ (35), 356 $[M - NC_4H_8]^+$ (100)	1.66, 2.39 (both s, 4 H each); 3.01 (s, 3 H); 3.09 (t, 2 H, J = 8.0); 3.69 (s, 2 H); 4.27 (t, 2 H, J = 8.1); 6.02 (d, 1 H, J = 8.1); 6.42 (t, 1 H, J = 7.6); 6.63 (t, 1 H, J = 7.3); 6.94 (t, 1 H, J = 7.5); 6.99 (t, 1 H, J = 7.0); 7.03 (d, 1 H, J = 7.3); 7.14 (d, 1 H, J = 7.7); 7.24 (s, 1 H); 7.52 (d, 1 H, J = 7.3)	23.1 (2 C), 23.7, 28.8, 49.7, 52.5, 110.9, 53.2 (2 C), 108.1, 111.8, 115.3, 119.1, 119.8, 122.0, 122.3, 124.3, 125.9, 126.8, 127.3, 131.9, 132.4, 136.8, 142.5, 166.4, 167.5
8e	442 $[M]^+$ (53), 356 $[M - NC_4H_8O]^+$ (100)	2.29 (s, 4 H); 3.01 (s, 3 H); 3.10 (t, 2 H, J = 8.0); 3.54 (s, 4 H); 3.58 (s, 2 H); 4.30 (t, 2 H, J = 7.9); 5.96 (d, 1 H, J = 8.0); 6.39 (t, 1 H, J = 7.7); 6.62 (t, 1 H, J = 7.3); 6.96 (t, 1 H, J = 7.7); 7.02 (t, 1 H, J = 7.5); 7.04 (d, 1 H, J = 6.9); 7.18 (d, 1 H, J = 8.1); 7.22 (s, 1 H); 7.56 (d, 1 H, J = 7.6)	23.7, 28.8, 52.5, 52.8 (2 C), 52.9, 66.3 (2 C), 107.9, 111.0, 111.8, 113.4, 119.3, 120.0, 122.1, 122.4, 124.3, 125.9, 127.5 (2 C), 132.0, 132.6, 136.8, 142.5, 166.5, 167.5
8f	455 $[M]^+$ (100), 356 $[M - NC_4H_8NMe]^+$ (42)	2.43 (s, 3 H); 2.50—2.51, 2.73—2.74 (both m, 4 H each); 3.01 (s, 3 H); 3.10 (t, 2 H, J = 7.9); 3.69 (s, 2 H); 4.30 (t, 2 H, J = 7.9); 5.98 (d, 1 H, J = 7.9); 6.42 (t, 1 H, J = 7.7); 6.55 (s, 2 H); 6.63 (t, 1 H, J = 7.4); 6.97 (t, 1 H, J = 7.7); 7.03 (t, 1 H, J = 8.1); 7.04 (d, 1 H, J = 7.4); 7.19 (d, 1 H, J = 8.1); 7.27 (s, 1 H); 7.57 (d, 1 H, J = 7.5) (fumarate)	23.7, 28.9, 43.7, 50.3 (2 C), 51.8, 52.6, 53.1 (2 C), 107.7, 111.1, 111.8, 112.7, 119.3, 120.1, 122.2, 122.4, 124.4, 125.9, 127.4, 128.0, 132.1, 132.8, 134.7 (2 C), 136.8, 142.4, 166.4, 167.2 (2 C), 167.5 (fumarate)
8g	442 $[M]^+$ (25), 370 $[M - NEt_2]^+$ (100)	0.86—0.99 (m, 6 H); 1.18—1.21 (m, 3 H); 2.40—2.45 (m, 4 H); 3.24 (t, 2 H, J = 8.0); 3.57—3.59 (m, 2 H); 3.65 (s, 2 H); 4.30 (t, 2 H, J = 8.1); 6.00 (d, 1 H, J = 8.0); 6.42 (t, 1 H, J = 7.7); 6.63 (t, 1 H, J = 7.5); 6.94 (t, 1 H, J = 7.1); 6.99 (t, 1 H, J = 7.0); 7.03 (d, 1 H, J = 7.3); 7.14 (d, 1 H, J = 8.0); 7.23 (s, 1 H); 7.56 (d, 1 H, J = 7.6)	11.7 (2 C), 13.7, 28.8, 32.3, 45.8 (2 C), 47.6, 52.4, 107.9, 110.9, 111.7, 115.2, 119.3, 119.7, 121.9, 122.2, 124.2, 125.8, 127.0, 127.52, 131.8, 132.0, 136.9, 142.5, 166.1, 167.1
8h	413 $[M - (CH_2)_3NMe_2]^+$ (40), 342 $[M - (CH_2)_3NMe_2, NEt_2]^+$ (100)	0.92—1.00 (m, 12 H); 1.71—1.74 (m, 2 H); 2.38—2.48 (m, 10 H); 3.09 (t, 2 H, J = 7.9); 3.56 (t, 2 H, J = 6.8); 3.64 (s, 2 H); 4.30 (t, 2 H, J = 8.0); 5.95 (d, 1 H, J = 8.1); 6.40 (t, 1 H, J = 8.0); 6.61 (t, 1 H, J = 7.3); 6.93 (t, 1 H, J = 7.7); 6.99 (t, 1 H, J = 8.3); 7.30 (d, 1 H, J = 7.3); 7.12 (d, 1 H, J = 8.1); 7.22 (s, 1 H); 7.55 (d, 1 H, J = 7.3)	11.5 (2 C), 11.8 (2 C), 25.6, 28.8, 36.1, 45.9 (2 C), 46.1 (2 C), 47.5, 49.9, 52.5, 107.8, 110.9, 111.6, 115.2, 119.4, 119.7, 122.00, 122.3, 124.3, 125.9, 127.0, 127.5, 131.8, 131.9, 136.9, 142.5, 166.3, 167.4

(*to be continued*)

Table 3 (*continued*)

Compound	MS, m/z (I_{rel} (%))	NMR, δ (J/Hz)	
		^{1}H	^{13}C
9a	421 $[M]^+$ (56), 377 $[M - NMe_2]^+$ (100)	2.11 (s, 6 H); 3.02 (s, 3 H); 3.39 (s, 2 H); 3.54 (s, 3 H); 6.38, 6.96 (both d, 2 H each, J = 8.8); 7.07 (s, 1 H); 7.07 (t, 1 H, J = 6.5); 7.19 (t, 1 H, J = 7.1); 7.29 (d, 1 H, J = 8.2); 7.49 (d, 1 H, J = 7.7)	24.2, 44.9 (2 C), 53.8, 54.9, 112.5, 113.8 (2 C), 116.2, 117.4, 119.3, 120.8, 122.2, 126.1, 126.3, 128.2, 132.1 (2 C), 132.4, 135.2, 159.0, 166.4, 167.6
9b	449 $[M]^+$ (35), 377 $[M - NEt_2]^+$ (100)	1.10—1.16 (m, 6 H); 2.69—2.71 (m, 4 H); 3.02, 3.55 (both s, 3 H each); 3.93 (s, 2 H); 6.39 (d, 2 H, J = 8.9); 6.57 (s, 2 H); 6.98 (d, 2 H, J = 8.8); 7.11 (t, 1 H, J = 7.1); 7.22 (t, 1 H, J = 7.2); 7.29 (s, 1 H); 7.33 (d, 1 H, J = 8.2); 7.57 (d, 1 H, J = 7.9) (fumarate)	10.1 (2 C), 24.1, 45.1 (2 C), 46.1, 55.0, 112.6, 113.8 (2 C), 117.0, 118.9, 121.0, 122.5, 128.0, 128.0, 128.2, 131.8, 132.3 (2 C), 134.6 (2 C), 135.2, 159.1, 166.3, 167.1 (2 C), 167.3 (fumarate)
9c	47 $[M]^+$ (15), 377 $[M - NC_4H_8]^+$ (100)	1.83, 2.84 (both s, 4 H each); 3.02, 3.55 (both s, 3 H each); 4.02 (s, 2 H); 6.39 (d, 2 H, J = 8.9); 6.55 (s, 2 H); 6.97 (t, 2 H, J = 8.9); 7.11 (t, 1 H, J = 6.9); 7.22 (t, 1 H, J = 7.1); 7.29 (s, 1 H); 7.31 (d, 1 H, J = 8.2); 7.58 (d, 1 H, J = 7.9) (fumarate)	22.7 (2 C), 24.1, 47.7, 52.3 (2 C), 55.0, 112.5, 113.8 (2 C), 116,9, 118.9, 118.8, 121.1, 122.5, 127.7, 128.2, 134.7 (2 C), 128.3, 131.6, 132.3 (2 C), 135.1, 159.1 (2 C), 166.3, 167.3 (2 C) (fumarate)
9d	463 $[M]^+$ (37), 377 $[M - NC_4H_8O]^+$ (100)	2.32 (s, 4 H); 3.02 (s, 3 H); 3.48 (s, 2 H); 3.54 (s, 3 H); 3.57 (s, 2 H); 6.37, 6.96 (both d, 2 H each, J = 9.0); 7.08 (t, 1 H, J = 7.0); 7.09 (s, 1 H); 7.19 (t, 1 H, J = 7.1); 7.29 (d, 1 H, J = 8.1); 7.54 (d, 1 H, J = 7.8)	24.2, 51.8, 53.1 (2 C), 55.0, 66.2 (2 C), 112.5, 113.8 (2 C), 115.1, 117.3, 119.3, 120.8, 122.3, 126.3 (2 C), 126.5, 128.2, 132.1 (2 C), 132.2, 135.3, 159.0, 166.5, 167.6
9e	476 $[M]^+$ (20), 377 $[M - NC_4H_8NMe]^+$ (100)	2.27 (s, 3 H); 2.43—2.51 (m, 8 H); 3.02 (s, 3 H); 3.51 (s, 2 H); 3.56 (c, 3 H); 6.38 (d, 2 H, J = 8.9); 6.97 (t, 2 H, J = 8.8); 7.07 (t, 1 H, J = 7.8); 7.09 (s, 1 H); 7.19 (t, 1 H, J = 7.1); 7.29 (d, 1 H, J = 8.3); 7.53 (d, 1 H, J = 7.7)	24.1, 44.8, 51.6 (2 C), 52.4, 54.1 (2 C), 55.0, 112.4, 113.7 (2 C), 115.2, 117.3, 119.2, 120.7, 122.2, 126.3, 126.7, 128.1, 132.1 (2 C), 132.2, 135.3, 159.0, 166.3, 167.4
10a	402 $[M]^+$ (25), 358 $[M - NMe_2]^+$ (100)	1.05 (m, 3 H); 2.46 (s, 6 H); 2.98 (s, 3 H); 4.00—4.06 (m, 4 H); 6.56 (s, 2 H); 6.74 (t, 1 H, J = 7.3); 6.85 (t, 2 H, J = 8.2); 6.98 (d, 2 H, J = 8.7); 7.03 (t, 1 H, J = 7.8); 7.12 (t, 1 H, J = 7.1); 7.19 (s, 1 H); 7.23 (d, 1 H, J = 8.2); 7.55 (d, 1 H, J = 7.8) (fumarate)	13.8, 23.7, 41.5 (2 C), 46.5, 50.4, 105.8, 107.2, 111.5, 118.5, 120.4, 122.1, 123.0 (2 C), 124.8, 127.5, 127.9 (2 C), 130.7, 134.8 (2 C), 136.3, 138.5, 141.4, 166.0, 167.5, 167.6 (2C) (fumarate)
10b	430 $[M]^+$ (25), 358 $[M - NEt_2]^+$ (100)	0.98 (t, 6 H, J = 7.1); 1.04 (t, 3 H, J = 0.9); 2.39 (m, 4 H); 2.97 (s, 3 H); 3.52 (s, 2 H); 3.89 (m, 2 H); 6.78 (t, 1 H, J = 7.3); 6.88 (t, 2 H, J = 8.1); 6.94 (s, 1 H); 6.96—7.01 (m, 3 H); 7.07 (t, 1 H, J = 7.1); 7.17 (t, 1 H, J = 8.1); 7.49 (d, 1 H, J = 7.8)	11.8 (2 C), 13.7, 23.6, 45.9 (2 C), 46.1, 47.8, 108.0, 111.1, 114.4, 119.2, 119.5, 121.7, 122.3 (2 C), 124.2, 127.1, 127.8, 127.8 (2 C), 136.6, 137.3, 141.8, 166.2, 167.6
10c	428 $[M]^+$ (21), 358 $[M - NC_4H_8]^+$ (100)	1.04 (t, 3 H, J = 6.3); 1.67, 2.37 (both s, 4 H each); 2.97 (s, 3 H); 3.58 (s, 2 H); 3.89—3.92 (m, 2 H); 6.78 (t, 1 H, J = 7.4); 6.88 (t, 2 H, J = 7.5); 6.95 (s, 1 H); 6.97—7.00 (m, 3 H); 7.07 (t, 1 H, J = 7.1); 7.17 (d, 1 H, J = 8.2); 7.47 (d, 1 H, J = 7.8)	13.7, 23.1 (2 C), 23.6, 46.2, 49.9, 53.4 (2 C), 107.9, 111.1, 114.6, 119.0, 119.6, 121.7, 122.4 (2 C), 124.3, 127.0, 127.6, 127.8 (2 C), 136.5, 137.4, 141.8, 166.2, 167.6

(*to be continued*)

Table 3 (*continued*)

Compound	MS, m/z (I_{rel} (%))	NMR, δ (J/Hz)	
		^{1}H	^{13}C
10d	444 $[M]^+$ (30), 358 $[M - NC_4H_8O]^+$ (100)	1.10 (t, 3 H, J = 6.9); 2.29 (s, 4 H); 3.00 (s, 3 H); 3.48 (s, 2 H); 3.58 (s, 4 H); 3.97 (m, 2 H); 6.78 (t, 1 H, J = 7.4); 6.74 (t, 1 H, J = 7.3); 6.84 (t, 2 H, J = 7.5); 6.94 (s, 1 H); 6.97—7.02 (m, 3 H); 7.10 (t, 1 H, J = 7.9); 7.53 (d, 1 H, J = 7.8)	13.7, 23.5, 46.2, 53.0 (2 C), 53.1, 66.3 (2 C), 107.5, 111.1, 112.8, 119.1, 119.6, 121.7, 122.4 (2 C), 124.3, 127.5, 127.6, 127.8 (2 C), 136.5, 137.4, 141.5, 166.2, 167.4
10e	457 $[M]^+$ (20), 358 $[M - NC_4H_8NMe]^+$ (100)	1.06 (t, 3 H, J = 6.3); 2.16 (s, 3 H); 2.31 (s, 4 H); 2.97 (s, 3 H); 3.45 (s, 6 H); 3.94 (m, 2 H); 6.75 (t, 1 H, J = 7.3); 6.85 (t, 2 H, J = 8.2); 6.91 (s, 1 H); 6.96—6.99 (m, 3 H); 7.07 (t, 1 H, J = 7.1); 7.17 (d, 1 H, J = 8.1); 7.48 (d, 1 H, J = 7.8)	13.6, 23.5, 45.6, 46.1, 52.3 (2 C), 52.7, 54.7 (2 C), 107.5, 111.0, 113.2, 119.0, 119.5, 121.6, 122.4 (2 C), 124.2, 127.3, 127.6, 127.7 (2 C), 136.5, 137.5, 141.6, 166.1, 167.4
11	456 $[M]^+$ (37), 384 $[M - NEt_2]^+$ (100)	0.96—1.02 (m, 6 H); 1.93 (t, 2 H, J = 5.7); 1.98 (s, 3 H); 2.38—2.43 (m, 4 H); 2.63 (t, 2 H, J = 6.6); 3.00 (s, 3 H); 3.59 (s, 2 H); 3.72 (t, 2 H, J = 5.3); 6.23 (d, 1 H, J = 8.3); 6.49 (d, 1 H, J = 8.2); 6.60 (s, 1 H); 6.28 (t, 1 H, J = 7.2); 7.00 (s, 1 H); 7.05 (t, 1 H, J = 7.3); 7.18 (d, 1 H, J = 8.1); 7.54 (d, 1 H, J = 7.7)	11.7 (2 C), 20.0, 22.4, 23.5, 25.5, 45.7 (2 C), 47.4, 47.9, 111.2, 111.4, 114.8, 119.0, 119.1, 119.7, 121.6, 125.4, 126.4, 126.9, 128.0, 128.6, 131.1, 134.5, 135.2, 136.0, 166.7, 167.2
12	C464 $[M]^+$ (10), 462 $[M]^+$ (25), 391 $[M - NEt_2]^+$ (40), 390 $[M - NEt_2]^+$ (100)	1.01—1.04 (m, 6 H); 2.46—2.51 (m, 4 H); 3.02 (s, 3 H); 3.11 (t, 2 H, J = 8.1); 3.44 (s, 2 H); 4.31 (t, 2 H, J = 7.1); 5.95, 6.41 (both d, 1 H each, J = 8.6); 6.99 (t, 1 H, J = 7.1); 7.04 (t, 1 H, J = 7.9); 7.07 (d, 1 H, J = 7.2); 7.17 (d, 1 H, J = 7.8); 7.25 (s, 1 H); 7.61 (d, 1 H, J = 7.7)	11.4 (2 C), 23.4, 28.5, 45.8 (2 C), 47.4, 52.4, 109.1, 110.8, 112.4, 119.3, 119.8, 122.0, 124.1, 125.4, 125.7, 127.0 (2 C), 127.4, 131.7, 134.1, 136.5, 141.5, 166.1, 167.0
13	500 $[M]^+$ (60), 428 $[M - NEt_2]^+$ (100)	0.95—1.03 (m, 6 H); 2.38—2.44 (m, 4 H); 2.68—2.87 (m, 2 H); 3.01 (s, 3 H); 3.66 (s, 2 H); 3.67 (s, 3 H); 3.67—3.73 (m, 2 H); 4.55—4.60 (m, 1 H); 5.87 (d, 1 H, J = 8.1); 6.41 (t, 1 H, J = 7.8); 6.64 (t, 1 H, J = 7.3); 6.96 (t, 1 H, J = 7.2); 7.00 (t, 1 H, J = 6.6); 7.06 (d, 1 H, J = 7.5); 7.20 (d, 1 H, J = 7.1); 7.21 (s, 1 H); 7.57 (d, 1 H, J = 8.1)	11.7 (2 C), 23.5, 37.0, 37.7, 45.8 (2 C), 47.5, 51.4, 58.0, 108.0, 111.1, 111.6, 115.2, 119.4, 119.8, 121.9, 122.3, 123.6, 126.5, 126.9, 127.5, 131.9, 133.8, 136.7, 142.2, 166.5, 167.4, 171.8
14a	398 $[M]^+$ (53), 354 $[M - NMe_2]^+$ (100)	2.16 (s, 6 H); 3.12 (s, 3 H); 3.55 (s, 2 H); 6.57 (t, 2 H, J = 9.4); 6.67 (t, 1 H, J = 7.2); 6.73 (d, 1 H, J = 7.3); 6.79 (t, 1 H, J = 3.4); 6.90 (d, 2 H, J = 5.5); 7.46 (d, 1 H, J = 10.1); 7.47 (s, 1 H); 7.52 (d, 1 H, J = 7.8); 7.65 (d, 1 H, J = 3.5)	24.1, 44.7 (2 C), 106.6, 110.7, 110.8, 117.3, 119.7, 120.6, 121.1, 121.4, 122.3, 122.6 (2 C), 122.6, 123.0, 126.1, 127.8, 128.2, 128.32, 134.9, 135.4, 166.6, 166.6
14b	426 $[M]^+$ (20), 354 $[M - NEt_2]^+$ (100), 339 $[M - NEt_2 - Me]^+$ (12)	1.00 (m, 6 H); 2.46 (m, 4 H); 3.12 (s, 3 H); 3.69 (s, 2 H); 6.55 (d, 1 H, J = 8.4); 6.65 (d, 1 H, J = 6.2); 6.67 (t, 1 H, J = 7.7); 6.75 (d, 1 H, J = 8.3); 6.78 (d, 1 H, J = 4.1); 6.91 (t, 1 H, J = 4.0); 6.93 (t, 1 H, J = 4.0); 7.44 (s, 1 H); 7.47 (d, 1 H, J = 7.8); 7.56 (d, 1 H, J = 7.7); 7.64 (d, 1 H, J = 3.4)	11.7 (2 C), 24.0, 46.0 (2 C), 47.5, 108.5, 110.7, 110.8, 117.9, 119.6, 120.5, 121.0, 121.3, 122.1, 122.5 (2 C), 123.1, 125.8, 127.7, 128.2, 128.3, 134.9, 135.4, 166.5, 166.5
14c	424 $[M]^+$ (22), 354 $[M - NC_4H_8]^+$ (100)	1.69, 2.44 (both s, 4 H each); 3.10 (s, 3 H); 3.75 (s, 2 H); 6.50 (d, 1 H, J = 7.6); 6.59 (d, 1 H, J = 8.4); 6.62 (t, 1 H, J = 7.2); 6.72 (t, 1 H, J = 8.3); 6.74 (d, 1 H, J = 3.5); 6.88 (t, 1 H, J = 7.0); 6.91 (t, 1 H, J = 7.9); 7.42 (d, 1 H, J = 6.9); 7.43 (s, 1 H); 7.51 (d, 1 H, J = 7.8); 7.60 (d, 1 H, J = 3.5)	23.1 (2 C), 23.7, 52.5, 53.2 (2 C), 108.1, 110.9, 111.8, 115.3, 119.1, 119.8, 122.0, 122.3, 124.3, 125.9, 126.8 (2 C), 127.3 (2 C), 131.95, 132.4, 136.8, 142.5, 166.4, 167.5

(*to be continued*)

Table 3 (*continued*)

Compound	MS, m/z (I_{rel} (%))	NMR, δ (*J*/Hz)	
		^{1}H	^{13}C
14d	440 $[M]^+$ (25), 354 $[M - NC_4H_8O]^+$ (100)	2.31—2.35 (m, 4 H); 3.12 (s, 3 H); 3.44—3.85 (m, 4 H); 3.62 (s, 2 H); 6.52 (d, 1 H, *J* = 7.6); 6.64 (t, 1 H, *J* = 7.0); 6.71 (d, 1 H, *J* = 8.2); 6.78 (t, 1 H, *J* = 3.3); 6.80 (d, 1 H, *J* = 5.5); 6.91 (t, 1 H, *J* = 8.1); 6.95 (t, 1 H, *J* = 7.1); 7.39 (s, 1 H); 7.46 (d, 1 H, *J* = 7.8); 7.58 (d, 1 H, *J* = 7.9); 7.64 (d, 1 H, *J* = 3.5)	24.1, 51.5 (2 C), 52.8, 66.1 (2 C), 106.6, 110.7, 111.0, 116.1, 119.6, 120.7, 121.2, 121.4, 122.3, 122.6, 122.9, 123.1, 126.3, 127.8, 128.3, 128.3, 134.9, 135.4, 166.5, 166.2
14e	458 $[M]^+$ (100), 354 $[M - NC_4H_8NMe]^+$ (47)	2.16 (s, 3 H); 2.22—2.40 (m, 8 H); 3.12 (s, 3 H); 3.61 (s, 2 H); 6.53 (d, 1 H, *J* = 8.2); 6.65 (d, 1 H, *J* = 7.4); 6.69 (d, 1 H, *J* = 7.9); 6.77 (t, 1 H, *J* = 7.4); 6.78 (t, 1 H, *J* = 3.8); 6.91 (t, 1 H, *J* = 7.5); 6.94 (t, 1 H, *J* = 7.7); 7.39 (s, 1 H); 7.46, 7.55 (both d, 1 H each, *J* = 7.7); 7.64 (d, 1 H, *J* = 3.4)	24.0, 45.6, 52.2 (2 C), 52.4, 54.7 (2 C), 106.5, 110.7, 110.9, 126.7, 119.6, 120.5, 121.1, 121.3, 122.2, 122.5, 122.7, 123.0, 126.0, 127.7, 128.2, 128.3, 134.9, 135.4, 166.4, 166.5
15a	455 $[M]^+$ (25), 411 $[M - NMe_2]^+$ (100), 368 $[M - 2\ NMe_2]^+$ (50)	2.16 (s, 12 H); 3.11 (s, 3 H); 3.56 (s, 4 H); 6.56 (d, 2 H, *J* = 8.2); 6.67 (t, 2 H, *J* = 8.2); 6.89 (t, 2 H, *J* = 7.9); 7.49 (s, 2 H); 7.51 (d, 2 H, *J* = 7.9)	24.1, 44.7 (4 C), 53.6 (2 C), 110.7 (2 C), 117.2 (2 C), 119.7 (2 C), 121.1 (2 C), 122.4 (2 C), 122.7 (2 C), 126.1 (2 C), 128.2 (2 C), 135.4 (2 C), 166.6 (2 C)
15b	507 $[M]^+$ (30), 436 $[M - NC_4H_8]^+$ (50), 367 $[M - 2\ NC_4H_8O]^+$ (100)	1.69, 2.43 (both s, 8 H each); 3.10 (s, 3 H); 3.73 (s, 4 H); 6.60 (d, 2 H, *J* = 8.3); 6.69 (d, 2 H, *J* = 7.7); 6.92 (t, 2 H, *J* = 6.9); 7.45 (s, 2 H); 7.52 (d, 2 H, *J* = 8.0)	23.1 (4 C), 24.0, 49.6 (2 C), 53.2 (4 C), 110.9 (2 C), 117.8 (2 C), 119.4 (2 C), 121.1 (2 C), 122.4 (2 C), 122.8 (2 C), 125.7 (2 C), 128.2 (2 C), 135.3 (2 C), 166.6 (2 C)
15c	539 $[M]^+$ (23), 453 $[M - NC_4H_8O]^+$ (33), 368 $[M - 2\ NC_4H_8O]^+$ (100)	2.34 (s, 8 H); 3.11 (s, 3 H); 3.56 (s, 8 H); 3.63 (s, 4 H); 6.65 (d, 2 H, *J* = 8.2); 6.70 (d, 2 H, *J* = 7.8); 6.93 (t, 2 H, *J* = 7.4); 7.42 (s, 2 H); 7.56 (d, 2 H, *J* = 7.8)	24.0, 52.7 (2 C), 52.8 (4 C), 66.2 (4 C), 119.6 (2 C), 110.9 (2 C), 116.0 (2 C), 121.2 (2 C), 122.5 (2 C), 123.0 (2 C), 126.7 (2 C), 128.3 (2 C), 135.3 (2 C), 116.9 (2 C)
15d	565 $[M]^+$ (8), 466 $[M - NC_4H_8NMe]^+$ (100), 368 $[M - 2\ (NC_4H_8NMe)]^+$ (55)	2.46 (s, 6 H); 2.54, 2.77 (both s, 8 H each); 3.11 (s, 3 H); 3.72 (s, 4 H); 6.56 (s, 2 H); 6.62 (d, 2 H, *J* = 8.2); 6.72 (t, 2 H, *J* = 7.8); 6.94 (t, 2 H, *J* = 7.3); 7.47 (s, 2 H); 7.55 (d, 2 H, *J* = 7.8) (fumarate)	24.0, 43.5 (2 C), 50.3 (4 C), 51.7 (2 C), 53.1 (4 C), 110.9 (2 C), 115.7 (2 C), 119.6 (2 C), 121.3 (2 C), 122.5 (2 C), 122.9 (2 C), 126.6 (2 C), 128.2 (2 C), 134.6 (2 C), 135.3 (2 C), 166.4 (2 C), 167.3 (2 C) (fumarate)

1 min after addition of the inhibitor, [γ-^{32}P]ATP (with specific activity of 500 imp L min^{-1} mol^{-1}, Phosphor Production Association, RF), histones from calf tymus (50 μg), phosphatidylserine (2.0 μg), 1,2-dioleine (0.25 μg) were added to the reaction mixture (phosphatidylserine and diacylglycerol were suspended in Tris-HCl (20 *M*, pH 7.5), sonicated in a UZDN-1, U-42 ultrasonic disintegrator for 1 min at a frequency of 15 kHz, and the micellar solution was added to the reaction mixture). The reaction was triggered by addition of enzyme PKC (0.05 μg, 2040 Units (mg of protein)$^{-1}$). The background activity of PKC-α was determined in the mixture described above in the presence of ethylene glycol tetraacetate (0.5 *M*) instead of Ca^{2+}. After 4 min of incubation at 30 °C, the reaction was stopped by addition of cold 10% trichloroacetic acid (TCA) (1.5 mL). The acid-insoluble material was collected on Whatman GF/A glass fiber filters, washed with cold TCA, and the radioactivity was measured using a LS 6500 Cherenkov liquid scintillation counter (Beckman Coulter, USA). The final concentration of DMSO in the reaction mixture did not exceed 0.5%. The maximum consumption of ATP in the reaction was less than 5%. The IC_{50} values were determined graphically from the relationship of reciprocal values of PKC-α activity and concentration of the inhibitor under study (Fig. 1). The difference between the starting and the background incorporation levels of ^{32}P into histones was taken as the 100% of activity.

Investigation of cytotoxicity. The cells of the K562 and HCT116 lines were cultured in the Dulbecco's modified Eagle's

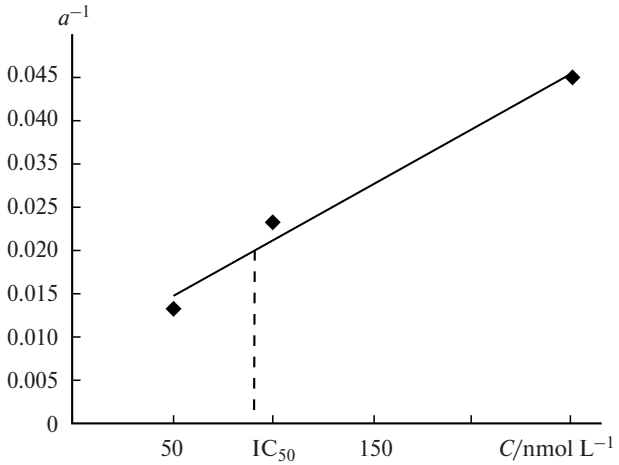


Fig. 1. Reciprocal values of the PKC-α activity versus concentration of inhibitor **8a** (*C*); *a* is activity of PKC.

medium with addition of 5% of embryonal calf serum at 37 °C, in the atmosphere containing 5% of CO_2. Cytotoxicity was determined in the MTT-test after incubation for 72 h.[12]

This work was financially supported by the Russian Foundation for Basic Research (Project No. 06-03-32233) and the European Community in the framework of the "Protein Kinases, New Drug Targets for Post-Genomic Era" Program (Contract No. LSHB-CT-2004-503467).

References

1. A. J. Bridges, *Chem. Rev.*, 2001, **101**, 2541.
2. M. Serova, A. Ghoul, K. A. Benhadji, E. Cvitkovic, S. Faivre, F. Calvo, F. Lokiec, E. Raymond, *Semin Oncol.*, 2006, **33**, 466.
3. S. V. Ambudkar, I. W. Kim, Z. E. Sauna, *Eur. J. Pharm. Sci.*, 2006, **27**, 392.
4. R. Pérez-Tomás, *Curr. Med. Chem.*, 2006, **13**, 1859.
5. P. Chaudhary, I. Roninson, *J. Natl. Cancer Inst.*, 1993, **85**, 632.
6. A. A. Shtil, J. Azare, *Int. Rev. Cytol.*, 2005, **246**, 1.
7. P. Cohen, *Perspectives*, 2002, **1**, 309.
8. P. G. Goekjian, M. R. Jirousek, *Expert Opin. Invest. Drugs*, 2001, **10**, 2117.
9. S. A. Lakatosh, Y. N. Luzikov, M. N. Preobrazhenskaya, *Org. Biomol. Chem.*, 2003, **1**, 826.
10. S. A. Lakatosh, A. Yu. Simonov, Yu. N. Luzikov, M. N. Preobrazhenskaya, *Khim. Farm. Zh.*, 2006, **40**, 435 [*Pharm. Chem. J.*, 2006, **40** (Engl. Transl.)].
11. E. E. Bykov, S. A. Lakatosh, M. N. Preobrazhenskaya, *Izv. Akad. Nauk, Ser. Khim.*, 2006, 754 [*Russ. Chem. Bull., Int. Ed.*, 2006, **55**, 781].
12. A. E. Shchekotikhin, V. A. Glazunova, Yu. N. Luzikov, V. N. Buyanov, O. Yu. Susova, A. A. Shtil, M. N. Preobrazhenskaya, *Bioorg. Med. Chem.*, 2006, **14**, 5241.

Received October 19, 2007;
in revised form July 22, 2008