New Organoselenium Compounds Active against Pathogenic Bacteria, Fungi and Viruses

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Different *N*-substituted benzisoselenazol-3(2*H*)-ones, analogues of ebselen were designed as new antiviral and antimicrobial agents. We report their synthesis, chemical properties as well as study on biological activity against broad spectrum of pathogenic microorganisms (*Staphylococcus aureus*, *Staphylococcus simulans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans*, *Aspergillus niger*) and viruses (herpes simplex virus type 1 (HSV-1), encephalomyocarditis virus (EMCV), vesicular stomatitis virus (VSV)), *in vitro*. Most of them exhibited high activity against viruses (HSV-1, EMCV) and gram-positive bacteria strains (*S. aureus*, *S. simulans*), while their activity against gram-negative bacteria strains (*E. coli*, *P. aeruginosa*, *K. pneumoniae*) was substantially lower. Some of tested compounds were active against yeast *C. albicans* and filamentous fungus *A. niger*.

Key words benzisoselenazol-3(2*H*)-one; antiviral agent; antimicrobial agent; organoselenium compound; ebselen

Organoselenium compounds are currently an area of interest for many chemists and medicinal biologists due to their broad spectrum of biological activity and prospective applications as enzyme inhibitors, antiinflammatory and antitumor agents.[1—3] It has been revealed that a simple organoselenium compound 2-phenylbenzisoselenazol-3(2*H*)-one called ebselen (**1a**) could act against oxidative stress in similar way as a common enzyme—glutathione peroxidase (Gpx).[4—6]

In our previous papers we reported that various 2-substituted benzisoselenazol-3(2*H*)-ones and related diaryl diselenides exhibited high activity as immunostimulants inducing cytokines such as interferon, tumor necrosis factor and interleukin (IL-2) in human peripheral blood leukocytes.[7,8] Moreover, some of these compounds, particularly their 7-azaanalogues, exhibited high inhibitory activity against pathogenic bacteria, fungi and viruses.[9]

In this paper we report synthesis, chemical properties as well as inhibitory activity against viruses, bacteria and fungi of ebselen analogues (**2**—**6**), having in 2-position alkyl groups (**2**) or containing oxaalkyl or azaalkyl functions (**3**—**6**). We wanted to answer the question whether the introduction of additional polar functions to alkyl substituent in 2-position can have the influence on biological activity of tested compounds. It was predicted that in this case an extra intermolecular interaction, like hydrogen bonds, between the molecule and active centre of the biological receptor can occur. Moreover, insertion of the polar group can increase the solubility in water, thus increase the bioavailability and make compounds more accessible for *in vivo* study.

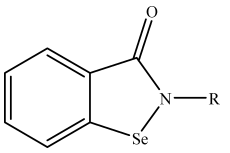

Fig. 1. General Formula of the Analogues of Ebselen **1a**

1: **1a**: R=Ph, **1b**: R=H
2: **2a**: R=Me; **2b**: R=Et; **2c**: R=*n*-Pr; **2d**: R=*i*-Pr; **2e**: R=*n*-Bu; **2f**: R=*t*-Bu; **2g**: R=c-Hex; **2h**: adamantyl; **2i**: R=$(CH_2)_{11}CH_3$; **2j**: R=$(CH_2)_{17}CH_3$
3: **3a**: R=CH_2OH; **3b**: R=CH_2CH_2OH; **3c**: R=$CH(CH_2OH)_2$ **3d**: R=$CH_2CH(OEt)_2$; **3e**: R=$CH(CH_3)CH(OMe)_2$
4: **4a**: R=CH_2COOH ; **4b**: R=CH_2COOCH_3; **4c**: $COOCH_2CH_3$
5: **5a**: R=$CH_2CH_2N(CH_3)_2$; **5b**: $CH_2CH_2N(CH_3)_3{}^+I^-$
6: **6a**: R=C=O(NHPr); **6b**: C=O(NH-c-Hex), **6c**: C=O(NHPh); **6d**: C=O(NHPh-3Cl)

For this purpose several organoselenium compounds: 2-alkylbenzisoselenazol-3(2*H*)-ones (**2**), 2-hydroxyalkylbenzisoselenazol-3(2*H*)-ones and 2-(2,2-dialkoxyalkyl)benzisoselenazol-3(2*H*)-ones (**3**), 2-carboxymethylbenzisoselenazol-3(2*H*)-one (**4a**) and the esters (**4b**, **c**), 2-aminoalkylbenzisoselenazol-3(2*H*)-ones (**5**) and 2-(*N*-substituted carbamoyl)benzisoselenazol-3(2*H*)-ones (**6**) were synthesized. All these analogues were screened in the antiviral, antibacterial and antifungal assays *in vitro*.

Results and Discussion

Chemistry Since it is believed that Se–N bond in benzisoselenazol-3(2*H*)-ones is responsible for their biological activity we wanted to check the influence of different substituents at nitrogen atom on biological activity. We supposed that character of substituent, mainly polarity and shape could have a significant influence on biological activity. Thus, among designed *N*-substituted benzisoselenazol-3(2*H*)-ones there were derivatives with short non-polar groups such as methyl, ethyl, propyl and butyl, long non-polar chains like dodecyl and octacedyl which make molecule more lipophilic, branched non-polar isopropyl, *tert*-butyl and adamantyl substituents which can be a steric hinderence. Compounds with polar substituents containing more polar groups such as hydroxyl, carboxyl, amino and carbamoyl and the less polar like acetal and ester groups fell to the second series. These groups can act as proton donors or acceptors in hydrogen bond formation. Some of them like carboxyl and amino group also can take part in ionic interations, similarly as polar ammonium salt. In control experiment we used nonsubstituted benzisoselenazol-3(2*H*)-one and ebselen which were proved to exhibit biological activity. All considered compounds contain endocyclic Se–N bond which is responsible

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for transformation of thiols to selenosulphides or disulphides.[10)]

In this work we present two synthetic approaches to *N*-substituted benzisoselenazol-3(2*H*)-ones. First method was based on the selenenylation-acylation of the primary amines with chloride **10**, and the second new one, on the substitution of hydrogen at 2-position of benzisoselenazol-3(2*H*)-one (**1b**) in reactions with formaldehyde, ethyl chloroformate and isocyanates. Ebselen (**1a**) and unsubstituted benzisoselenazol-3(2*H*)-one (**1b**) were prepared by the treatment of 2-chloroselenobenzoyl chloride (**10**) with aqueous ammonia or aniline, respectively, the same way as reported in our previous works.[7)] Chloride **10** was obtained in a four-step synthesis starting from anthranilic acid.[11)] The compound **10** treated with various primary alkylamines produced 2-alkylbenzisoselenazol-3(2*H*)-ones **2a**—**j**, while treated with 2-aminoethanol and serinol gave corresponding 2-hydroxyalkylbenzisoselenazol-3(2*H*)-ones **3b** and **3c**. The reaction of chloride **10** with 2-aminoacetaldehyde diethylacetal and 2-aminopropionaldehyde dimethylacetal produced compounds **3d** and **3e**. 2-Carboxymethylbenzisoselenazol-3(2*H*)-one **4a** and its methyl ester **4b** were obtained in the reaction of chloride **10** with glycine and methyl ester of its hydrochloride, respectively. The reaction of chloride **10** with *N*,*N*-dimethylethylenediamine resulted in 2-aminoethylbenzisoselenazol-3(2*H*)-one **5a**, which heated in excess of methyl iodide gave compound **5b**. For the synthesis of compounds **3a** and **6a**—**d** benzisoselenazol-3(2*H*)-one (**1b**) was used as an initial material. The reaction of **1b** with formaldehyde gave another hydroxy derivative—2-hydroxymethylbenzisoselenazol-3(2*H*)-one (**3a**). In the reaction of the compound **1b** with different isocyanates new carbamoyl derivatives **6a**—**d** were obtained. Potassium salt of **1b** treated with ethyl chloroformate gave ester **4c** (Fig. 2).

Biological Activity The compounds **1**—**6** were tested as potential antimicrobial and antiviral agents using *in vitro* assays.

Antimicrobial Activity The compounds **1**—**6** were tested against pathogenic bacteria, yeast and filamentous fungus. The results expressed as minimal inhibitory concentration (MIC) values, are shown in Table 1.

The majority of tested compounds **2**—**6** was highly active against gram-positive bacteria strains, particularly *S. aureus*, having MIC values in a range 2.0—32.0 μg/ml, close to these evaluated for control compounds **1a**, **b** and penicillin G (MIC=1.0 μg/ml). Generally, the compounds **2**—**6** were inactive or weakly active against gram-negative bacteria strains (*E. coli*, *P. aeruginosa* and *K. pneumoniae*). Only **3a** and **3b**, having in 2-position of heterocyclic ring hydroxyl group as substituent were moderately active against *E. coli* (MIC=14.0 μg/ml and 29.0 μg/ml). Strong fungicidal activity against yeast strain *C. albicans* was shown by benzisoselenazol-3(2*H*)-ones **2a**—**h** substituted in 2-position with alkyl groups (MIC=1.0—3.0 μg/ml). The same compounds **2a**—**h** were moderately active against *A. niger* with MIC values in a range of 8.0—28.0 μg/ml.

Antiviral Activity and Cytotoxicity All compounds **1**—**6** were tested as inhibitors of cytophatogenicity of encephalomyocarditis virus (EMCV, noneveloped RNA virus), herpes simplex virus type 1 (HSV-1, enveloped DNA virus) and vesicular stomatitis virus (VSV, enveloped RNA virus). The virus titer was measured in human cell line A549 and MIC (μg/ml) was determined. The results are given in Table 2.

High activity against HSV-1 and EMCV (MIC=4.0—8.0 μg/ml) similar to the activity of the reference compounds **1a**, **b** was observed for majority of tested 2-substituted benzisoselenazol-3(2*H*)-ones (**2a**—**h**, **3a**—**d**, **4b**, **c**, **5a**, **6a**, **c**, **d**). The 2-(2,2-dialkoxyethyl)benzisoselenazol-3(2*H*)-ones **3d**, **e** and carbamoyl derivatives were moderately active against EMCV virus (MIC=20—60 μg/ml). The long alkyl chains $-(CH_2)_{11}CH_3$ and $-(CH_2)_{17}CH_3$ made compounds **2i** and **2j** practically inactive against these viruses, probably because of their low solubility in culture medium. Almost all tested compounds **1**—**6** were completely inactive against VSV, the only one exception was compound **4c**.

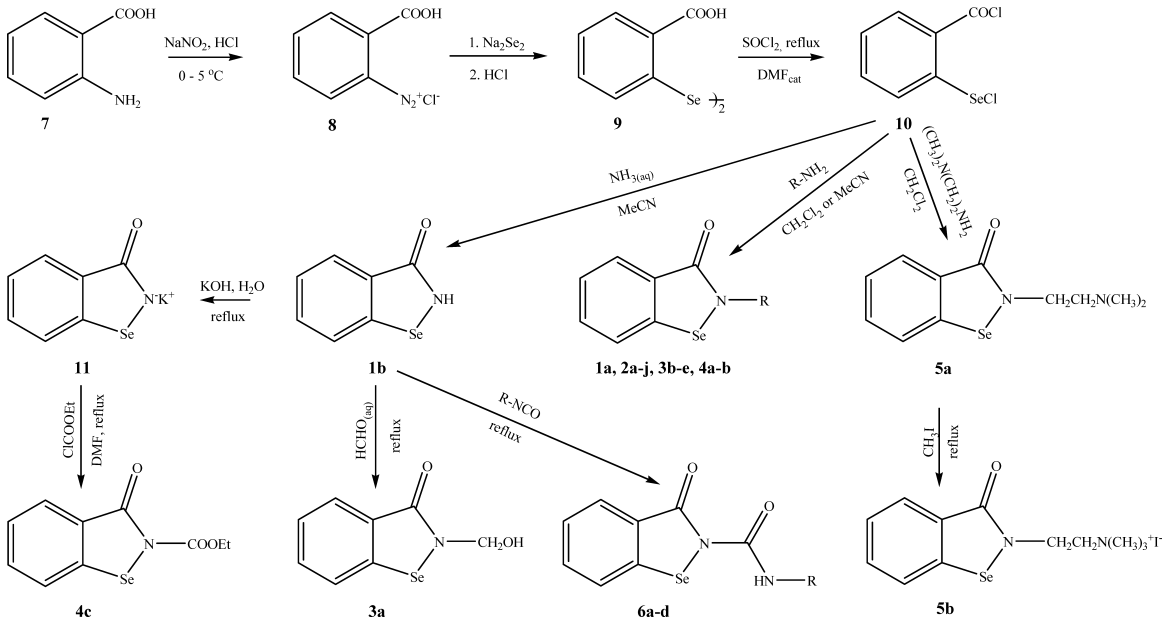

Fig. 2. Synthesis of the Compounds **1**—**6**

Table 1. Antimicrobial Activity of the Organoselenium Compounds **1**—**6**

Compound No.	Bacteria					Yeasts *C. albicans*	Fungi *A. niger*
	Gram-(+)		Gram-(−)				
	S. aureus	*S. simulans*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*		
1a	34	55	274	n.a	n.a	8.0	17
1b	6.0	12	25	99	50	3.0	396
2a	7.0	13	53	424	106	3.0	27
2b	7.0	14	57	452	226	2.0	28
2c	7.0	8.0	120	n.a	480	2.0	15
2d	7.0	15	120	n.a	n.a	2.0	15
2e	8.0	8.0	32	n.a	508	1.0	16
2f	32	8.0	127	n.a	n.a	2.0	16
2g	9.0	4.0	280	n.a	n.a	2.0	9.0
2h	332	42	n.a	n.a	n.a	5.0	10
2i	n.a	46	n.a	n.a	n.a	11	n.a
2j	n.a	n.a	n.a	n.a	n.a	n.a	n.a
3a	2.0	7.0	14	n.a	27	228	228
3b	15	8.0	29	n.a	121	242	242
3c	8.0	32	68	n.a	272	272	n.a
3d	9.0	39	n.a	n.a	n.a	314	63
3e	9.0	75	n.a	n.a	n.a	n.a	300
4a	32	128	512	512	n.a	n.a	512
4b	4	34	135	n.a	270	135	17
4c	32	64	129	n.a	n.a	129	129
5a	34	67	67	135	135	n.a	n.a
5b	51	26	51	206	206	n.a	n.a
6a	71	71	283	n.a	n.a	9	1
6b	10	10	n.a	n.a	n.a	10	162
6c	40	10	n.a	n.a	n.a	79	127
6d	2.7	11	22	88	176	n.a	352

MIC=minimal inhibitory concentration (μg/ml). n.a: non-active, MIC>512 μg/ml.

Table 2. Antiviral Activity and Cytotoxicity of the Organoselenium Compounds **1**—**6**

Compound No.	$TCCD_{50}$	HSV-1		EMCV		VSV	
		MIC	I	MIC	I	MIC	I
1a	12.0	8.0	1.5	10.0	1.2	>1000	<0.012
1b	3.0	8.0	0.4	4.0	0.8	>1000	<0.003
2a	2.5	8.0	0.3	4.0	0.6	>1000	<0.003
2b	3.0	8.0	0.4	4.0	0.8	>1000	<0.003
2c	3.5	6.0	0.6	6.0	0.6	>1000	<0.004
2d	3.5	6.0	0.6	6.0	0.6	>1000	<0.004
2e	5.0	6.0	0.8	6.0	0.8	>1000	<0.005
2f	6.0	6.0	1.0	4.0	1.5	>1000	<0.006
2g	3.0	6.0	0.5	8.0	0.4	>1000	<0.003
2h	78	100	0.8	>1000	<0.1	>1000	<0.078
2i	6.0	4.0	1.5	>1000	<0.006	>1000	<0.006
2j	12.0	>500	<0.02	>500	<0.02	>1000	<0.012
3a	4.0	4.0	1.0	4.0	1.0	>1000	<0.004
3b	4.0	4.0	1.0	4.0	1.0	>1000	<0.004
3c	15.0	4.0	3.8	8.0	1.9	>1000	<0.015
3d	7.3	6.0	1.2	20.0	0.4	>1000	<0.007
3e	2.5	8.0	0.3	60.0	0.04	>1000	<0.003
4a	78	40	2.0	40	2.0	>1000	<0.078
4b	6.1	6	1.0	8	0.8	>1000	<0.006
4c	17	10	1.7	10	1.7	600	0.028
5a	1.22	2	0.6	10	0.1	>1000	<0.001
5b	156	40	3.9	20	7.8	>1000	<0.156
6a	9.8	4	2.5	40	0.2	>1000	<0.01
6b	34	60	0.6	100	0.3	>1000	<0.034
6c	58.5	10	5.9	10	5.9	>1000	<0.059
6d	4.3	6	0.7	20	0.2	>1000	<0.004
Acyclovir	>230	>1000[a)]	—	—	—	—	—

$TCCD_{50}$=tissue culture cytotoxic dose determined on human A549 cells (μg/ml). MIC=minimal inhibitory concentration (μg/ml). I=$TCCD_{50}$/MIC. *a*) Acyclovir was inactive in virucidal assay, but it inhibited viral replication at 20 μg/ml.

The cytotoxic effect of the compounds **1**—**6** was determined in human cell line A549 and minimal concentration which was toxic to approximately 50% of cells was taken as $TCCD_{50}$ (μg/ml). Values of the cytotoxicity of synthesized compounds are presented in Table 2. Generally, they were high (2.5—15 μg/ml) with few exceptions for compounds **2h**, **4a**, **5b**, **6b**, **c**.

In consequence, although most of compounds tested against HSV-1 and EMCV exhibited appreciable antiviral activity, their high cytotoxicity caused that chemotherapeutic indices (I) definied as $TCCD_{50}$/MIC ratio, in most cases were below 1.0 which means that cytotoxic dose was lower than inhibitory dose. The compounds **3c**, **4a**, **4c**, **5b** and **6c** acted more selectively and their I values were above 1.0 that made them more prospective antiviral agents.

Conclusions

It has been shown that the structural modification of parent ebselen structure by a replacement of phenyl substituent in 2-position of heterocyclic ring by a small non-polar alkyl group, plays a crucial role in strong enhancement of antimicrobial and antiviral activity. However, high cytotoxicity of these compounds resulted in undesirable low chemotherapeutic indices (I) values. Generally, heteroatom (nitrogen, oxygen) built in the 2-substituent didn't enhance antimicrobial or antiviral activity significantly, but sometimes diminished cytotoxicity that resulted in indices above 1.

Experimental

Chemistry All reagents and solvents were purchased from Aldrich and Fluka. Melting points were determined with a digital melting point apparatus Electrothermal IA 9100. IR spectra were measured on a Perkin-Elmer 2000 FT spectrometer in KBr pellets. ^{1}H-, ^{13}C- and ^{77}Se-NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Bruker DRX spectrometer 300 MHz or 600 MHz. Chemical shifts are reported in ppm relative to TMS or dimethyl selenide. Reaction progress was monitored by a thin layer chromatography (TLC) on silica gel 60F254 coated aluminium TLC plates from Merck.

Reaction of Chloride 10 with Amines. General Procedure A solution of chloride **10** (1.27 g, 5 mmol) in dry acetonitrile (**1b**, **2a**—**h**, **3b**—**e**, **4a**, **b**) or dichloromethane (**1a**, **2i**, **j**, **5a**) was added dropwise at room temperature or in ice/NaCl bath (**4b**) over 30 min to a stirred solution of corresponding amine (16.5 mmol) (or its hydrochloride (5 mmol) and triethylamine 1.67 g, 16.5 mmol for **2h**, **4b**) in dry acetonitrile (**1b**, **2a**—**h**, **3b**—**e**, **4a**, **b**) or dichloromethane (**1a**, **2i**, **j**, **5a**) and the reaction was continued for additional 2—16 h. When the reaction was completed, the solvent was evaporated *in vacuo* and the crystalline residue was treated with water (100 ml) and stirred for 2—3 h. The insoluble solid was filtered off, washed with water and dried in the air. Crude products were recrystallized from methanol (**1a**, **2h**, **4a**), dioksan (**1b**), acetonitrile (**2a**, **3c**), cyclohexane (**2b**, **d**, **5a**), hexane (**2c**), hexane/toluene (**2e**—**f**), methanol/H_2O (**2g**) ethyl acetate/heksan (**3d**, **e**) or purified by chromatography on silica gel with chloroform (**2i**), dichloromethane/ethyl acetate (20 : 1) (**2j**) or chloroform/acetone (20 : 1) (**4b**) as the eluent and then recrystallized from hexane (**2i**, **j**) or hexane/chloroform (**4b**).

Compounds **1a** (88% yield, mp 181—182 °C),[7] **1b** (86% yield, mp 231—232 °C),[7] **2a** (84% yield, mp 156—158 °C),[7] **2b** (68% yield, mp 98—99 °C),[12] **2d** (83% yield, mp 99—101 °C),[13] **2e** (55% yield, mp 90—91 °C),[14] **2f** (94% yield, mp 151 °C),[7] **2g** (96% yield, mp 148—151 °C),[13] **2i** (96% yield, mp 80—82 °C),[7] **2j** (95% yield, mp 87—89 °C),[7] **3b** (85% yield, mp 148—150 °C),[15] **4a** (85% yield, mp 185—187 °C)[7] have been known and are reported in references cited.

2-Propylbenzisoselenazol-3(2*H*)-one (2c) 82% yield, yellow powder, mp 78—79 °C, ^{1}H-NMR (DMSO-d_6) δ: 0.90 (3H, t, J=7.4 Hz), 1.59—1.71 (2H, m), 3.68 (2H, t, J=7.1 Hz), 7.41 (1H, t, J=7.4 Hz), 7.60 (1H, t, J=7.6, 1.3 Hz), 7.80 (1H, d, J=7.6 Hz), 8.04 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 11.0, 23.2, 44.8, 125.6, 125.8, 127.2, 128.0, 131.3, 139.0. ^{77}Se-NMR (DMSO-d_6) δ: 868. IR (KBr) cm^{-1}: 3090, 2959,1640, 1591, 1444, 1355, 741.

2-(1-Adamantyl)benzisoselenazol-3(2*H*)-one (2h) 75% yield, yellow prisms, mp 218—221 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.65—1.74 (6H, m), 2.11 (3H, s), 2.33 (6H, d, J=2.0 Hz), 7.38 (1H, t, J=7.4 Hz), 7.57 (1H, t, J=7.6), 7.73 (1H, d, J=7.4 Hz), 7.99 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 29.3, 35.7, 40.7, 58.0, 125.1, 125.5, 126.9, 130.4, 130.9, 138.1, 165.6. ^{77}Se-NMR (DMSO-d_6) δ: 843. IR (KBr) cm^{-1}: 3054, 2906, 1596, 1445, 1328, 731.

2-(1,3-Dihydroxyisopropyl)benzisoselenazol-3(2*H*)-one (3c) 79% yield, orange prisms, mp 174 °C, ^{1}H-NMR (DMSO-d_6) δ: 3.59—3.63 (2H, m), 3.66—3.70 (2H, m), 4.52 (1H, q, J=5.2 Hz), 5.06 (2H, t, J=5.4 Hz), 7.38 (1H, t, J=7.4 Hz), 7.57 (1H, t, J=7.6 Hz), 7.82 (1H, d, J=7.7 Hz), 8.01 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 56.2, 59.9, 125.2, 125.3, 127.0, 127.6, 131.0, 140.9, 166.8. ^{77}Se-NMR (DMSO-d_6) δ: 877. IR (KBr) cm^{-1}: 3217, 2890, 1604, 1451, 1343, 738.

2-(2,2-Diethoxyethyl)benzisoselenazol-3(2*H*)-one (3d) 87% yield, colorless crystals, mp 102—103 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.16 (6H, t, J=7.0 Hz), 3.51—3.56 (2H, m), 3.65—3.70 (2H, m), 3.83 (2H, d, J=5.2 Hz), 4.62 (1H, t, J=5.2 Hz), 7.40 (1H, t, J=7.4 Hz), 7.60 (1H, t, J=7.6 Hz), 7.83 (1H, d, J=7.7 Hz), 8.03 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 15.2, 46.0, 62.2, 99.4, 100.7, 125.4, 125.5, 127.2, 127.3, 131.4, 140.2, 166.6.^{77}Se-NMR (DMSO-d_6) δ: 903. IR (KBr) cm^{-1}: 3069, 2975, 2884, 1587, 1442, 1370, 1122, 1062, 745.

2-(2,2-Dimethoxy-1-methylethyl)benzisoselenazol-3(2*H*)-one (3e) 84% yield, colorless crystals, mp 117 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, d, J=6.8 Hz), 3.38 (3H, s), 3.41 (3H, s), 4.43 (1H, d, J=4.0 Hz), 4.69—4.73 (1H, m), 7.40 (1H, t, J=7.4 Hz), 7.58 (1H, t, J=7.6 Hz), 7.81 (1H, d, J=7.6 Hz), 8.07 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 15.2, 49.8, 55.1, 55.6, 105.7, 125.4, 125.6, 127.1, 127.8, 131.2, 140.1, 166.3. ^{77}Se-NMR (DMSO-d_6) δ: 855. IR (KBr) cm^{-1}: 3089, 2931, 2830, 1596, 1446, 1346, 1124, 1055, 740.

2-Carboxymethylbenzisoselenazol-3(2*H*)-one Methyl Ester (4b) 75% yield, white powder, mp 141—145 °C, ^{1}H-NMR (DMSO-d_6) δ: 3.68 (3H, s), 4.56 (2H, s), 7.43 (1H, t, J=7.4 Hz), 7.64 (1H, t, J=7.6 Hz), 7.84 (1H, d, J=7.7 Hz), 8.07 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 45.0, 52.4, 126.2, 126.4, 127.4, 127.8, 132.6, 140.8, 167.5, 170.0. ^{77}Se-NMR (DMSO-d_6) δ: 909. IR (KBr) cm^{-1}: 3073, 2952, 1747, 1595, 1444,1208, 733.

2-[2-(*N*,*N*-Dimethylamino)ethyl]benzisoselenazol-3(2*H*)-one (5a) 68% yield, pale yellow powder, mp 89—93 °C, ^{1}H-NMR (DMSO-d_6) δ: 2.28 (6H, s), 2.53 (2H, t, J=5.7 Hz), 3.82 (2H, t, J=5.6 Hz), 7.37 (1H, t, J=7.4 Hz), 7.55 (1H, t, J=7.6 Hz), 7.80 (1H, d, J=7.7 Hz), 8.00 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 40.8, 44.6, 58.1, 125.3, 125.4, 126.8, 127.7, 130.9, 142.1, 166.5. ^{77}Se-NMR (DMSO-d_6) δ: 890. IR (KBr) cm^{-1}: 3085, 2935, 2852, 2818, 1600, 1445, 751.

The compound **5b** was obtained by heating of **5a** with methyl iodide, used in excess, during 1h. After this time the yellow solid precipitated.

2-[2-(*N*,*N*-Dimethylamino)ethyl]benzisoselenazol-3(2*H*)-one Methyl Iodide (5b) 92% yield, yellow powder, mp 210 °C, ^{1}H-NMR (DMSO-d_6) δ: 3.15 (9H, s), 3.59 (2H, t, J=6.8 Hz), 4.19 (2H, t, J=6.7 Hz), 7.44 (1H, t, J=7.3 Hz), 7.63 (1H, t, J=7.6 Hz), 7.83 (1H, d, J=7.5 Hz), 8.23 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 37.3, 52.6, 63.4, 125.9, 126.7, 127.1, 127.3, 131.6, 139.4, 166.7. ^{77}Se-NMR (DMSO-d_6) δ: 881. IR (KBr) cm^{-1}: 3012, 2959, 1618, 1442, 742.

Reaction of Benzisoselenazol-3(2*H*)-one (1b) with Formaldehyde Benzisoselenazol-3(2*H*)-one (**1b**) (0.39 g; 2 mmol) and 38% aqueous solution of formaldehyde in excess (1.5 ml) were placed in a hermetically closed test-tube. The reaction was carried out in reflux for 20 min until all **1b** completely dissolved. After cooling, water (2 ml) was added. The insoluble solid was filtered off, washed with water, dried and recrystallized from chloroform to give pure **3a**.

2-Hydroxymethylbenzisoselenazol-3(2*H*)-one (3a) 81% yield, white crystals, mp 227—228 °C, ^{1}H-NMR (DMSO-d_6) δ: 5.13 (2H, d, J=7.3 Hz), 6.45 (1H, t, J=7.3 Hz), 7.42 (1H, t, J=7.4 Hz), 7.61 (1H, t, J=7.6 Hz), 7.83 (1H, d, J=7.7 Hz), 8.05 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 67.0, 125.7, 125.8, 127.4, 128.2, 131.6, 139.5, 165.9. ^{77}Se-NMR (DMSO-d_6) δ: 859. IR (KBr) cm^{-1}: 3261, 3059, 2950, 1635, 1597, 1450, 1348, 731.

Reaction of Benzisoselenazol-3(2*H*)-one Potassium Salt (11) with Ethyl Chloroformate Benzisoselenazol-3(2*H*)-one potassium salt (**11**) (1.18 g; 5 mmol), ethyl chloroformate in excess (5 ml) and DMF (5 ml) were heated in reflux for 16 h. Then the mixture was poured into water (100 ml). The insoluble product was filtered off, dried and recrystallized from ethyl acetate.

2-Carboxybenzisoselenazol-3(2*H*)-one Ethyl Ester (4c) 81% yield, colorless crystals, mp 180.5—181.5 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.31 (3H, t, J=7.1 Hz), 4.30 (2H, q, J=7.1 Hz), 7.46 (1H, t, J=7.4 Hz), 7.72 (1H, t,

J=7.6 Hz), 7.87 (1H, d, J=7.6 Hz), 8.00 (1H, d, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 14.1, 62.9, 125.8, 126.3, 128.1, 128.3, 133.8, 139.0, 151.3, 164.4. ^{77}Se-NMR (DMSO-d_6) δ: 926. IR (KBr) cm^{-1}: 3086, 2992, 1751, 1639, 1446, 1259, 731.

Reaction of Benzisoselenazol-3(2*H*)-one (1b) with Isocyanates. General Procedure Benzisoselenazol-3(2*H*)-one (**1b**) (0.2 g; 1 mmol), corresponding liquid isocyanate in excess (2 ml) and toluene (3 ml) for **6b**, **d** were placed in a hermetically closed test-tube. The reaction was carried out in reflux for 1 h until all **1b** completely dissolved. Then the mixture was cooled down and the insoluble product was filtered off, washed with hexane, dried and recrystallized from methanol (**6a**, **b**) or DMSO/H_2O (**6c**, **d**).

2-Propylcarbamoylbenzisoselenazol-3(2*H*)-one (6a) 99% yield, orange crystals, mp 112—114 °C, ^{1}H-NMR (DMSO-d_6) δ: 0.91 (3H, t, J=7.4 Hz), 1.52—1.58 (2H, m), 3.26—3.29 (2H, m), 7.48 (1H, t, J=7.5 Hz), 7.72 (1H, t, J=7.6 Hz), 7.91 (1H, d, J=7.7 Hz), 8.07 (1H, d, J=8.0 Hz), 8.88 (1H, t, J=5.7 Hz). ^{13}C-NMR (DMSO-d_6) δ: 11.1, 22.3, 41.4, 125.9, 126.1, 128.0, 128.9, 133.4, 139.3, 152.7, 165.8. ^{77}Se-NMR (DMSO-d_6) δ: 906. IR (KBr) cm^{-1}: 3043, 2934, 1693, 1631, 1444, 1317, 1226, 732.

2-Cyclohexylcarbamoylbenzisoselenazol-3(2*H*)-one (6b) 96% yield, white powder, mp 187.5—191.5 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.21—1.24 (1H, m), 1.29—1.37 (4H, m), 1.50—1.52 (1H, m), 1.63—1.65 (2H, m), 1.84—1.86 (2H, m), 3.65—3.71 (1H, m), 7.46 (1H, t, J=7.5 Hz), 7.70 (1H, t, J=7.7 Hz), 7.89 (1H, d, J=7.7 Hz), 8.06 (1H, d, J=8.0 Hz), 8.86 (1H, d, J=7.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 23.9, 24.9, 32.1, 48.6, 125.9, 126.1, 128.0, 128.9, 133.4, 139.2, 151.7, 166.0. ^{77}Se-NMR (DMSO-d_6) δ: 907. IR (KBr) cm^{-1}: 3098, 2921, 1699, 1614, 1533, 1444, 1319, 736.

2-Phenylcarbamoylbenzisoselenazol-3(2*H*)-one (6c) 87% yield, colorless crystals, mp 222—224 °C, ^{1}H-NMR (DMSO-d_6) δ: 7.14 (1H, t, J=7.3 Hz), 7.38 (2H, t, J=7.8 Hz), 7.52 (1H, t, J=7.5 Hz), 7.59 (2H, d, J=8.1 Hz), 7.76 (1H, t, J=7.6 Hz), 7.97 (1H, d, J=7.8 Hz), 8.10 (1H, d, J=8.1 Hz), 11.08 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 118.1, 119.7, 124.0, 126.0, 126.4, 128.3, 128.6, 128.7, 129.0, 133.8, 137.1, 139.3, 150.3, 166.4. ^{77}Se-NMR (DMSO-d_6) δ: 919. IR (KBr) cm^{-1}: 3076, 1699, 1441, 1301, 744.

2-(3-Chlorophenylcarbamoyl)benzisoselenazol-3(2*H*)-one (6d) 98% yield, beige powder, mp 215—218 °C, ^{1}H-NMR (DMSO-d_6) δ: 7.20 (1H, d, J=7.9 Hz), 7.37—7.55 (3H, m), 7.74—7.83 (2H, m), 7.97 (1H, d, J=7.4 Hz), 8.10 (1H, d, J=8.1 Hz), 11.16 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 116.8, 117.7, 121.6, 125.5, 126.2, 127.1, 127.5, 130.3, 131.4, 133.1, 140.9, 141.4, 152.2, 168.6. ^{77}Se-NMR (DMSO-d_6) δ: 807. IR (KBr) cm^{-1}: 3058, 2894, 1602, 1559, 1440, 1348, 737.

Evaluation of Biological Activity. Antimicrobial Assay Antimicrobial activity of the compounds **1**—**6** was determined by serial dilution method using gram-positive bacterial strains: *Staphylococcus aureus* ATCC 25923, *Staphylococcus simulans* 103P, gram-negative bacterial strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 258243, *Klebsiella pneumoniae* ATCC 700603, filamentous fungus *Aspergilus niger* and yeast *Candida albicans* obtained from the Collection of Microorganisms of Wrocław Medical University. Tested compounds were disolved in dimethyl sulfoxide, its final concentration in broth medium never exceeded 5% (v/v). The final concentration of tested compounds was in a range 0.25—512 μg/ml. The cultures of bacteria, fungi and yeast (in appropriate liquid medium) were incubated with tested compounds at 28 °C for 24 h. Afterwards, small amounts of assay medium were transfered with platinum loop on Petri dishes containing solid agar medium and incubated at 28 °C for 24 or 48 h. The microbial activity was characterized by MIC value (minimal concentration of the compound in μg/ml which inhibits growth of the microorganisms by 100%).

Antiviral Assay The compounds **1**—**6** at various concentrations (1—1000 μg/ml) were incubated with following viruses: HSV-1 (herpes simplex virus type 1, Herpesviridae, enveloped virus), EMCV (encephalomyocarditis virus, Picornaviridae, non-enveloped virus) and VSV (vesicular stomatitis virus, Rhabdoviridae, enveloped virus). Viruses EMCV and VSV were used at the dose of 10^5 tissue culture infectious dose for 50% ($TCID_{50}$)/ml, HSV-1 at the dose of 10^4 $TCID_{50}$/ml. After 1 h incubation at room temperature, the virus titer was measured in human cell line A549. Concentration of compound causing 100 times (for HSV-1) or 1000 times (for EMCV and VSV) decrease of virus titer was taken as minimal inhibitory concentration, MIC (μg/ml).

Cytotoxicity Cytotoxicity of the compounds **1**—**6** was determined in human lung adenocarcinoma cell line A549 (ATCC 185). The experiment was performed in 96-well microplates. The cells were treated with various doses of compounds and cultivated for 48 h at 37 °C in the atmosphere of 5% CO_2 in air. Then, cultures were examined under microscope. The minimal concentration which was toxic to approximately 50% of cells was taken as $TCCD_{50}$.

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