

Synthesis of new alkylated and methoxylated analogues of ebselen with antiviral and antimicrobial properties

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Dedicated to Prof. Jacek Młochowski on the occasion of his 80th anniversary

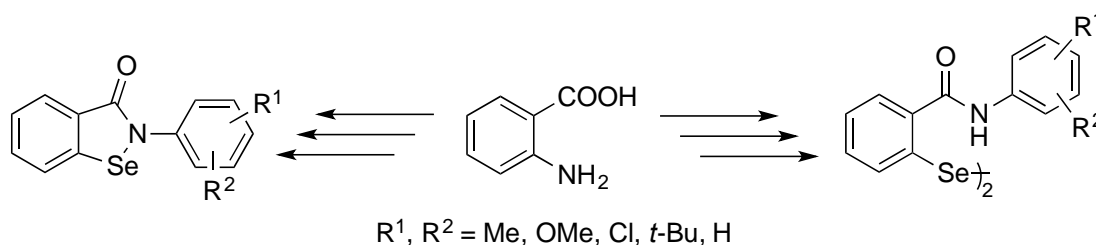
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Abstract

A series of new mono and disubstituted alkylated and methoxylated benzoselenazol-3(2*H*)-ones and bis(2-carbamoylaryl)diselenides were prepared in yields ranging from 55% to 95% starting from anthranilic acid and were evaluated for antiviral and antimicrobial activity. The compounds exhibited antiviral activity against Human herpes virus 1 and Encephalomyocarditis virus as well as antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*.



Keywords: Ebselen, organoselenium compounds, benzoselenazol-3(2*H*)-ones, diselenides, antiviral activity, antimicrobial activity

Introduction

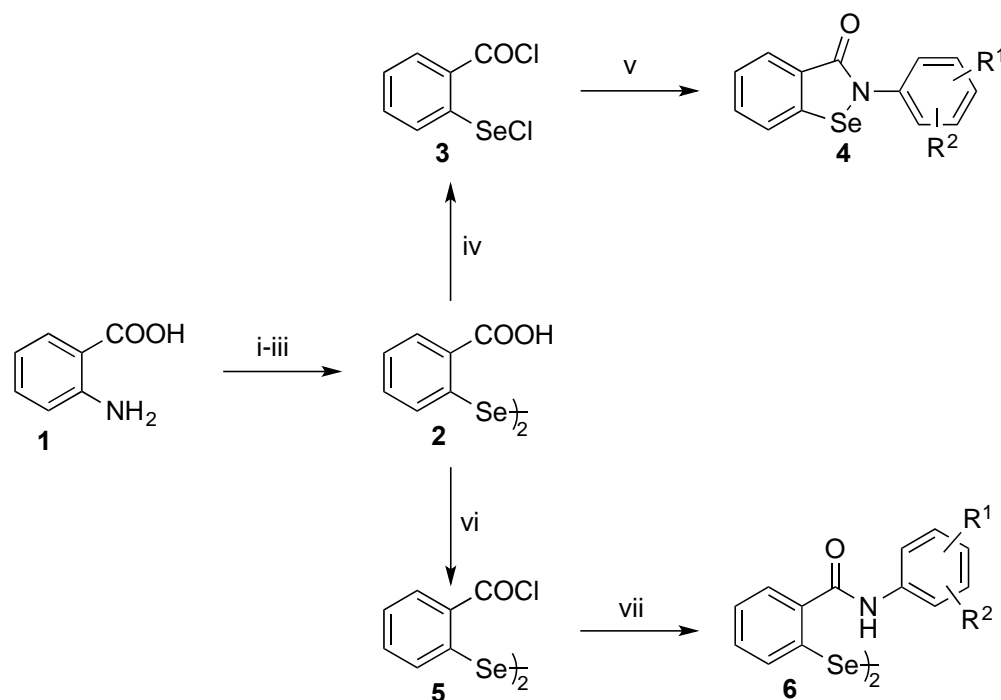
Over last few decades organoselenium compounds have been extensively studied in organic and medicinal chemistry.¹⁻¹⁵ Due to their unique properties, they attracted a wide interest as reagents in new synthetic routes,³ promising catalysts for various oxidation reactions^{1-7,16-19} and potential pharmaceuticals.^{8-15,20,21} Ebselen (2-phenylbenzisoselenazol-3(2*H*)-one) and its analogues have received particular attention as glutathione peroxidase mimics,^{8,11,15} antioxidant and anti-inflammatory agents.¹³⁻¹⁵ Furthermore, the continuing interest in this class of compounds has led to the development of various structures with antiviral²²⁻²⁶ and antimicrobial²⁴⁻²⁷ properties. In this work we report a convenient synthetic route to a series of new alkylated and methoxylated benzisoselenazol-3(2*H*)-ones **4** and bis(2-carbamoylaryl)diselenides **6** which were tested for antiviral and antimicrobial activity in biological studies.

Results and Discussion

Searching for new antiviral and antimicrobial agents, we report here the synthesis of new alkylated and methoxylated analogues of ebselen – an organoselenium compound well known for its variety of biological and oxygen transfer properties. 2-Phenylbenzisoselenazol-3(2*H*)-ones alkylated and methoxylated on the benzene ring **4a-l** were prepared through a tandem selenenylation-acylation of mono and disubstituted anilines with 2-(chloroseleno)benzoyl chloride (**3**), in the presence of triethylamine as a base, whereas corresponding bis(2-carbamoylaryl) diselenides **6c,e,g-h,j-l** were prepared via the reaction of the benzoyl chloride **5** with appropriate anilines in the presence of sodium carbonate (Scheme 1). Both benzoyl chlorides **3** and **5** were obtained in a three-step synthesis starting from anthranilic acid (**1**), which was diazotized and reacted with dilithium diselenide in aprotic medium (THF) to give after acidification 2,2'-diselenobisbenzoic acid **2** with 78 % yield.²⁸⁻³⁰ Efficient cooling is important to reduce by-product formation such as salicylic acid. Dilithium diselenide was generated *in situ* from elemental lithium and selenium in the presence of 4,4'-di(*t*-butyl)biphenyl, as a catalyst.³¹ Alternatively, disodium diselenide in protic medium (MeOH) can be used to introduce the diselenide moiety, however the yield was reported to be lower (18-67 %).^{24,32} The procedure for preparation of disodium diselenide is critical to obtain 2,2'-diselenobisbenzoic acid (**2**) with good yields. The type of reducing agent, such as hydrazine monohydrate,²⁴ and sodium tetrahydroborate²¹ as well as reaction times up to 48 hours, are of particular importance. Some authors report yields even up to 95% however no synthetic details were provided.²⁰

The acid **2** was easily converted into 2-(chloroseleno)benzoyl chloride (**3**) (85%) with thionyl chloride, used in excess, in the presence of catalytic amounts of *N,N'*-dimethylformamide.²⁹ When thionyl chloride was used in excess (2.5 equivalents), chloride **5** (64%) was produced.²⁹ The reaction of benzoyl chlorides **3** and **5** with anilines substituted at *ortho*, *meta* or *para* positions of the phenyl ring by electron-donating methyl and/or strong electron-donating methoxy groups, (in some cases with a chlorine substituent) provides a straightforward way to obtain new mono and disubstituted 2-phenylbenzisoselenazol-3(2*H*)-ones **4** and bis(2-carbamoylaryl) diselenides **6**, respectively. All products were prepared with good to excellent yields (Scheme 1). An *ortho* effect of methyl and methoxy groups, and a steric hindrance effect of the *tert*-butyl substituent in the case of formation of compound **4f**, were not observed, only in the cases of selenazolones **4d**, and **4j** were minor effects of electron-withdrawing chlorine substituent observed. This confirms that the general approach presented here can be successfully used for preparation of new substituted analogues of ebselen. To our best

knowledge compounds **4a-h**, **4j**, **6c**, **6e**, **6g**, **6h**, **6j**, **6k** are new structures and were not previously described in the literature. All compounds were fully characterized by spectroscopic methods (see Experimental Section).



4 / 6	a	b	c	d	e	f	g	h	i	j	k	l
R ¹	2-Me	2-OMe	3-Me	2-Me	2-OMe	2-OMe	2-Cl	2-Cl	4-Me	2-Me	3-OMe	4-OMe
R ²	4-OMe	5-Me	4-Cl	3-Cl	5-Cl	5- <i>t</i> -Bu	5-Me	4-Me	H	5-Cl	H	H
Yield 4 (%)	67	63	70	55	79	82	70	95	72	60	63	70
Yield 6 (%)	-	-	95	-	85	-	75	62	-	86	74	83

Reagents: **i:** NaNO₂, HCl, -7 – 5°C, **ii:** Li₂Se₂, NaOH_{aq}, -7 – 0°C, **iii:** HCl, **iv:** SOCl₂ (excess), DMF (cat.), Δ, **v:** R¹R²-C₆H₃-NH₂, MeCN, Et₃N, **vi:** 3,5 eq. SOCl₂, DMF (cat.), benzene, Δ, **vii:** R¹R²-C₆H₃-NH₂, CH₂Cl₂, Na₂CO₃.

Scheme 1. Synthesis of alkylated and methoxylated benzisoselenazol-3(2H)-ones **4** and bis(2-carbamoyl-aryl)diselenides **6**.

Antiviral and antimicrobial evaluation of organoselenium compounds

The alkylated and methoxylated analogues of ebselen **4** and **6** were evaluated for antiviral activity against Human herpes virus 1 (HHV-1, *Herpesviridae*, enveloped virus), Encephalomyocarditis virus (EMCV, *Picornaviridae*, non-enveloped virus) and Vesicular stomatitis virus (VSV, *Rhabdoviridae*, enveloped virus) as well as antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* by serial dilution techniques.²⁶ Their activities were compared with those of ebselen.

The compounds exhibited high antiviral activity against HHV-1 and EMCV but in almost all cases were inactive towards VSV (Table 1). A similar tendency was observed in our previous studies.²⁴⁻²⁶ Generally, benzisoselenazol-3(2H)-ones **4** were more active than corresponding diselenides **6**. The results of screening of benzisoselenazol-3(2H)-ones **4** toward HHV-1 are particularly interesting. These compounds were more active than ebselen, with minimal inhibitory concentration (MIC) values in a range of 2 – 6 μg/mL. The most

promising as antiviral agents were compounds **4b-d** and **6c** with higher chemotherapeutic indices (I), indicating that the concentration active against virus was lower than that showing a cytotoxic effect.

Table 1. Antiviral activity and cytotoxicity of compounds **4** and **6**

Compound	TCCD ₅₀ ^a [μg/mL]	HHV-1		EMCV		VSV	
		MIC ^b	I ^c	MIC ^b	I ^c	MIC ^b	I ^c
4a	7.3	4	1.8	20	0.4	n.a	-
4b	8.5	2	4.2	10	0.8	n.a	-
4c	19.5	4	4.9	60	0.3	n.a	-
4d	78	6	13	n.a	-	n.a	-
4e	8.5	4	2.1	20	0.4	n.a	-
4f	4.9	6	0.8	8	0.6	n.a	-
4g	11	4	2.7	8	1.4	n.a	-
4h	7.3	4	1.8	10	0.7	n.a	-
4i	3.7	2	1.8	40	0.09	n.a	-
4j	8.5	4	2.1	10	0.9	n.a	-
6c	58.5	10	5.8	n.a	-	n.a	-
6e	22	40	0.5	200	0.1	400	0.06
6g	58.5	20	2.9	60	1.0	n.a	-
6h	58.5	100	0.6	n.a	-	n.a	-
6j	19.5	200	0.1	800	0.02	n.a	-
Ebselen	12	8	1.5	10	1.2	n.a	-

^a TCCD₅₀ – Tissue Culture Cytotoxic Dose (μg/mL). ^b MIC – Minimal Inhibitory Concentration (μg/mL).

^c I = TCCD₅₀/MIC. n.a – non-active, MIC > 1000 μg/mL.

Table 2. Antimicrobial activity of compounds **4** and **6**

Compound	MIC ^a [μg/mL]				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
4a	20	10	80	n.a.	80
4b	20	20	80	n.a.	80
4e	85	85	85	n.a.	42
4f	33	33	33	n.a.	65
4k	76	38	38	n.a.	68
4l	10	152	152	n.a.	76
6e	n.a	340	n.a	n.a.	n.a
6k	305	n.a	n.a	n.a.	n.a
6l	n.a	n.a	n.a	n.a.	n.a
Ebselen	17	17	32	n.a.	128

^a MIC – Minimal Inhibitory Concentration (μg/mL). n.a. – non-active, MIC > 512 μg/mL.

Benzisoselenazol-3(2H)-ones **4** were found to be active against *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* (Table 2). None of compounds exhibited antibacterial activity against *P. aeruginosa* which is in agreement with previous observations.²⁶ Usually this gram-negative bacterial strain is not sensitive to benzisoselenazol-3(2H)-

ones and only a few structures were found to exhibit very low antibacterial activity towards it.^{24,26} Contrary to their cyclic analogues, diselenides appeared to be practically inactive against all tested microorganisms. Only **6e** and **6k** showed low activity against *B. subtilis* and *S. aureus*, respectively.

Conclusions

A series of new alkylated and methoxylated 2-phenylbenzisoselanzol-3(2*H*)-ones **4** and bis(2-carbamoylaryl)diselenides **6** were prepared in good to excellent yields starting from anthranilic acid. This general approach proved to be convenient for the preparation of various mono- and disubstituted analogues of ebselen. The studies of antiviral and antimicrobial activity revealed new potential antiviral agents against HHV-1 and EMCV, and antimicrobial agents against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*.

Experimental Section

General. Melting points were determined on an Electrothermal IA 91100 digital melting-point apparatus using the standard open capillary method. ¹H and ¹³C NMR spectra were recorded in CDCl₃ or DMSO-*d*₆ on a Bruker DRX 300 Spectrometer (¹H: 300.1 MHz, ¹³C: 75.4 MHz) or on a Bruker Avance 600 Spectrometer (¹H: 600.6 MHz, ¹³C: 151.0 MHz) at 295 K. Chemical shifts (δ) are given in parts per million (ppm) downfield relative to TMS, and coupling constants (J) are in Hz. Residual solvent central signals were recorded as follows: CDCl₃, δ_H = 7.263, δ_C = 77.00; DMSO-*d*₆, δ_H = 2.50, δ_C = 39.43. High-resolution mass spectra (HRMS) were recorded on a Waters LCD Premier XE instrument, and only the [M + H]⁺ or [M + Na]⁺ molecular species are reported. Selenium powder (100 mesh) with purity ≥99.5% used for Li₂Se₂ or Na₂Se₂ preparation was purchased from Aldrich. Freshly distilled acetonitrile (MeCN) was redistilled twice over P₂O₅ to obtain the anhydrous MeCN for preparation of the ebselen derivatives **4**. Methylene chloride (CH₂Cl₂) was distilled over P₂O₅ before preparation of diselenides **6**. Distilled triethylamine was stored over NaOH pellets. Other reagents and starting materials were directly used as obtained commercially. Purity of the products was confirmed by comparison of their melting point with data given in the literature and by spectroscopic methods.

2,2'-Diselenobisbenzoic acid (2) was prepared from anthranilic acid, using dilithium diselenide,³⁰ following the method previously reported²⁸ with some modifications. The crude product was recrystallized from 1,4-dioxane by slow evaporation under ca. 200 mmHg from a water bath at ca 75 °C to give 2,2'-diselenobisbenzoic acid (**2**) with 85% yield as pale crystals, mp 299-300 °C (lit.³⁰ 300-303 °C).

2-(Chloroseleno)benzoyl chloride (3) was prepared from 2,2'-diselenobisbenzoic acid (**2**) using excess of thionyl chloride, following the method previously reported.²⁹ Crude oily product was recrystallized from *n*-hexane and dried under vacuum (200 mmHg) to give 2-(chloroseleno)benzoyl chloride (**3**) as yellow needles, mp 65-67 °C (lit.²⁹ 65-66 °C).

Bis[(2-chlorocarbonyl)phenyl] diselenide (5) was prepared from 2,2'-diselenobisbenzoic acid (**2**), using thionyl chloride in excess of 2.5 equivalents, following the method previously reported.²⁹ The crude oily product was washed with *n*-hexane, recrystallized from CH₂Cl₂ and dried under vacuum to give bis[(2-chlorocarbonyl)phenyl] diselenide (**5**) as pale greenish yellow prisms, mp 180-181 °C (lit.²⁹ 174-175 °C).

General synthetic procedure for benzoselenazol-3(2H)-ones 4. To a stirred solution of substituted aniline (5.0 mmol) and dry triethylamine (1.8 ml, 1.02 g, 12.5 mmol) in anhydrous MeCN (50 mL) a solution of (2-chloroseleno)benzoyl chloride **3** (1.27 g, 5.0 mmol) in anhydrous MeCN (25 mL) was slowly added dropwise for 1 h. The reaction was continued for 1-48 h. The progress of reaction was monitored by TLC. After the reaction finished, the solvent was evaporated and water was added dropwise (100 mL) resulting in the product precipitation. Crude product was washed with water and 3.5% HCl if TLC indicated unreacted amine, and left to dry on the air.

2-(4-Methoxy-2-methylphenyl)benzoselenazol-3(2H)-one (4a). White flakes, yield 67%, mp 179–180 °C (from acetonitrile-water, 1:1 v/v); ^1H NMR (300 MHz, DMSO- d_6): δ_{H} 2.07 (s, 3H, CH₃), 3.78 (s, 3H, OCH₃), 6.84 (dd, J 8.6 Hz, J 2.9 Hz, 1H, H-5'), 6.92 (d, J 2.9 Hz, 1H, H-3'), 7.20 (d, J 8.6 Hz, 1H, H-6'), 7.47 (dd, J 8.3 Hz, J 6.9 Hz, 1H, H-6), 7.67 (ddd, J 8.0 Hz, J 6.9 Hz, J 1.3 Hz, 1H, H-5), 7.88 (dd, J 8.3 Hz, J 1.3 Hz, 1H, H-7), 8.08 (d, J 8.0 Hz, 1H, H-4). ^{13}C NMR (75 MHz, DMSO- d_6): δ_{C} 18.0, 55.2, 111.9, 115.7, 125.9, 126.0, 127.3, 127.8, 129.7, 129.9, 131.8, 137.9, 140.0, 158.8, 165.2. HRMS (TOF MS ESI): m/z for (C₁₅H₁₃NO₂Se + H)⁺ calculated: 320.0190; found: 320.0190.

2-(2-Methoxy-5-methylphenyl)benzoselenazol-3(2H)-one (4b). Pale brown needles, yield 63%, mp 172–173 °C (from water); ^1H NMR (300 MHz, DMSO- d_6): δ_{H} 2.27 (s, 3H, CH₃), 3.73 (s, 3H, OCH₃), 7.03 (d, 1H, J 6.4 Hz, H-3'), 7.16-7.18 (m, 2H, H-4', H-6'), 7.45 (ddd, J 7.9 Hz, J 7.5 Hz, J 0.8 Hz, 1H, H-6), 7.66 (ddd, J 8.2 Hz, J 7.0 Hz, J 1.4 Hz, 1H, H-5), 7.86 (dd, J 7.7 Hz, J 0.9 Hz, 1H, H-7), 8.06 (d, J 8.0 Hz, 1H, H-4). ^{13}C NMR (75 MHz, DMSO- d_6): δ_{C} 19.8, 55.7, 112.5, 125.7, 125.8, 126.8, 127.3, 127.7, 129.3, 129.4, 130.0, 131.8, 140.3, 153.0, 165.7. HRMS (TOF MS ESI): m/z for (C₁₅H₁₃NO₂Se + H)⁺ calculated: 320.0190; found: 320.0201.

2-(4-Chloro-3-methylphenyl)benzoselenazol-3(2H)-one (4c). Shiny orange crystals, yield 70%, mp 209–210 °C (from water); ^1H NMR (300 MHz, DMSO- d_6): δ_{H} 2.38 (s, 3H, CH₃), 7.47-7.53 (m, 3H, H-2',5',6'), 7.66-7.73 (m, 2H, H-5,6), 7.91 (d, J 7.7 Hz, 1H, H-7), 8.10 (d, J 8.0 Hz, 1H, H-4). ^{13}C NMR (75 MHz, DMSO- d_6): δ_{C} 19.6, 123.6, 125.8, 126.2, 127.0, 127.9, 128.3, 129.3, 130.0, 132.3, 136.3, 138.5, 138.8, 165.0. HRMS (TOF MS ESI): m/z for (C₁₄H₁₀ClNOSe + H)⁺ calculated: 323.9692; found: 323.9692.

2-(3-Chloro-2-methylphenyl)benzoselenazol-3(2H)-one (4d). Yellow crystals, yield 55%, mp 282–283 °C (from 1,2-dichloroethane); ^1H NMR (300 MHz, DMSO- d_6): δ_{H} 2.11 (s, 3H, CH₃), 7.29-7.36 (m, 2H, H-5',6'), 7.47-7.54 (m, 2H, H-6,4'), 7.70 (ddd, J 8.0 Hz, J 7.0 Hz, J 1.4 Hz, 1H, H-5), 7.90 (dd, J 7.7 Hz, J 1.2 Hz, 1H, H-7), 8.10 (d, J 8.0 Hz, 1H, H-4). ^{13}C NMR (75 MHz, DMSO- d_6): δ_{C} 15.3, 126.0, 126.1, 126.9, 127.5, 127.9, 128.0, 128.7, 132.1, 134.1, 134.8, 139.0, 140.2, 165.3. HRMS (TOF MS ESI): m/z for (C₁₄H₁₀ClNOSe + H)⁺ calculated: 323.9692; found: 323.9685.

2-(5-Chloro-2-methoxyphenyl)benzoselenazol-3(2H)-one (4e). Light yellow prisms, yield 79%, mp 205–206 °C (from acetonitrile-water, 85:15 v/v); ^1H NMR (300 MHz, DMSO- d_6): δ_{H} 3.79 (s, 3H, OCH₃), 7.19 (d, J 8.9 Hz, 1H, H-3'), 7.41-7.49 (m, 3H, H-6,4',6'), 7.67 (ddd, J 8.2 Hz, J 7.2 Hz, J 1.4 Hz, 1H, H-5), 7.87 (dd, J 7.8 Hz, J 0.8 Hz, 1H, H-7), 8.07 (d, J 8.1 Hz, 1H, H-4). ^{13}C NMR (75 MHz, DMSO- d_6): δ_{C} 56.6, 114.6, 124.1, 126.3, 126.4, 127.5, 128.4, 129.0, 129.1, 129.6, 132.6, 141.0, 154.6, 166.5. HRMS (TOF MS ESI): m/z for (C₁₄H₁₀ClNO₂Se + H)⁺ calculated: 339.9641; found: 339.9636.

2-(5-*t*-Butyl-2-methoxyphenyl)benzoselenazol-3(2H)-one (4f). Purple powder, yield 82%, mp 207–208 °C (purified by column chromatography, chloroform); ^1H NMR (300 MHz, CDCl₃): δ_{H} 1.31 (s, 9H, *t*-Bu), 3.82 (s, 3H, OCH₃), 6.94 (d, J 8.7 Hz, 1H, H-3'), 7.37 (dd, J 8.7 Hz, J 2.5 Hz, 1H, H-4'), 7.42-7.47 (m, 2H, H-6,6'), 7.62-7.65 (m, 2H, H-5,7), 8.12 (d, J 7.6 Hz, 1H, H-4). ^{13}C NMR (75 MHz, DMSO- d_6): δ_{C} 31.7, 33.7, 55.7, 112.2, 125.6 (2C), 125.7, 126.2, 126.5, 127.3, 127.7, 131.8, 140.3, 142.9, 152.9, 165.7. HRMS (TOF MS ESI): m/z for (C₁₈H₁₉NO₂Se + H)⁺ calculated: 362.0660; found: 362.0659.

2-(2-Chloro-5-methylphenyl)benzisoselenazol-3(2H)-one (4g). White powder, yield 70%, mp 184–185 °C (purified by column chromatography (chloroform/ethyl acetate, 5:1); ¹H NMR (300 MHz, CDCl₃): δ_H 2.35 (s, 3H, CH₃), 7.16 (d, *J* 8.2 Hz, 1H, H-4'), 7.27 (s, 1H, H-6), 7.38 (d, *J* 8.2 Hz, 1H, H-3'), 7.47 (dd, *J* 7.6 Hz, *J* 6.8 Hz, 1H, H-5), 7.62–7.70 (m, 2H, H-6,7), 8.13 (d, *J* 7.6 Hz, 1H, H-4). ¹³C NMR (75 MHz, CDCl₃): δ_C 20.5, 124.4, 125.8, 126.1, 129.0, 129.9, 130.4, 130.6, 131.4, 132.3, 135.1, 137.8, 139.5, 166.6. HRMS (TOF MS ESI): *m/z* for C₁₄H₁₀ClNOSe+H⁺ calculated: 323.9692; found: 323.9607.

2-(2-Chloro-4-methylphenyl)benzisoselenazol-3(2H)-one (4h). White powder, yield 95%, mp 208–209 °C (from ethyl acetate); ¹H NMR (300 MHz, CDCl₃): δ_H 2.36 (s, 3H, CH₃), 7.25 (dd, *J* 8.0 Hz, *J* 1.1 Hz, 1H, H-5'), 7.37 (d, *J* 8.0 Hz, 1H, H-6'), 7.45–7.50 (m, 2H, H-6,3'), 7.69 (ddd, *J* 8.0 Hz, *J* 7.3 Hz, *J* 1.3 Hz, 1H, H-5), 7.89 (dd, *J* 7.7 Hz, *J* 0.8 Hz, 1H, H-7), 8.09 (d, *J* 8.0 Hz, 1H, H-4). ¹³C NMR (75 MHz, DMSO-*d*₆): δ_C 20.3, 125.9, 126.0, 126.8, 127.9, 128.5, 130.3, 130.6, 132.1 (2C), 133.3, 139.8, 140.3, 165.6. HRMS (TOF MS ESI): *m/z* for C₁₄H₁₀ClNOSe+H⁺ calculated: 323.9692; found: 323.9694.

2-(4-Methylphenyl)benzisoselenazol-3(2H)-one (4i). Pale powder, yield 72%, mp 163–164 °C (from water) (lit.³³ 163–164 °C); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 2.32 (s, 3H, CH₃), 7.24 (d, *J* 8.1 Hz, 2H, H-2',6'), 7.47–7.53 (m, 3H, H-6,3',5'), 7.67 (ddd, *J* 8.0 Hz, *J* 7.5 Hz, *J* 1.3 Hz, 1H, H-5), 7.90 (d, *J* 7.0 Hz, 1H, H-7), 8.09 (d, *J* 8.0 Hz, 1H, H-4). ¹³C NMR (75 MHz, DMSO-*d*₆): δ_C 20.5, 124.5, 125.7, 126.2, 127.8, 128.4, 129.5, 132.0, 135.1, 137.0, 138.8, 164.8. HRMS (TOF MS ESI): *m/z* for (C₁₄H₁₁NOSe + H)⁺ calculated: 290.0085; found: 290.0084.

2-(5-chloro-2-methylphenyl)benzisoselenazol-3(2H)-one (4j). White powder, yield 60%, mp 189–190 °C (from chloroform); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 2.08 (s, 3H, CH₃), 7.36–7.42 (m, 3H, H-3',4',6'), 7.49 (dd, *J* 7.5 Hz, *J* 7.3 Hz, 1H, H-6), 7.69 (ddd, *J* 8.0 Hz, *J* 7.3 Hz, *J* 1.3 Hz, 1H, H-5), 7.89 (d, *J* 7.6 Hz, 1H, H-7), 8.10 (d, *J* 8.0 Hz, 1H, H-4). ¹³C NMR (151 MHz, DMSO-*d*₆): δ_C 17.8, 126.5, 126.6, 127.5, 128.4, 128.5, 128.6, 129.1, 130.8, 132.6, 132.7, 139.4, 140.8, 165.8. HRMS (TOF MS ESI): *m/z* for (C₁₄H₁₀ClNOSe + H)⁺ calculated: 323.9692; found: 323.9687.

2-(3-Methoxyphenyl)benzisoselenazol-3(2H)-one (4k). Pale yellow crystals, yield 63%, mp 165.5–167.5 °C (from acetonitrile) (lit.³⁴ 162–164 °C); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 3.77 (s, 3H, OCH₃), 6.82 (dd, *J* 8.3 Hz, *J* 0.8 Hz, 1H, H-6'), 7.15 (dd, *J* 8.0 Hz, *J* 0.8 Hz, 1H, H-4'), 7.31–7.36 (m, 2H, H-2',5'), 7.45 (ddd, *J* 7.8 Hz, *J* 7.2 Hz, *J* 0.9 Hz, 1H, H-6), 7.64 (ddd, *J* 8.0 Hz, *J* 7.2 Hz, *J* 1.4 Hz, 1H, H-5), 7.88 (dd, *J* 7.7 Hz, *J* 0.9 Hz, 1H, H-7), 8.36 (d, *J* 8.0 Hz, 1H, H-4). ¹³C NMR (75 MHz, DMSO-*d*₆): δ_C 55.2, 110.3, 111.3, 116.6, 125.7, 126.2, 127.9, 128.5, 129.9, 132.2, 138.8, 140.8, 159.6, 164.9. HRMS (TOF MS ESI): *m/z* for (C₁₄H₁₁NO₂Se + H)⁺ calculated: 306.0034; found: 306.0030.

2-(4-Methoxyphenyl)benzisoselenazol-3(2H)-one (4l). Pale needles, yield 70%, mp 180.5–181.5 °C (from water) (lit.²⁸ 181–184 °C); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 3.78 (s, 3H, OCH₃), 7.01 (dd, *J* 9.0 Hz, *J* 2.3 Hz, 2H, H-3',5'), 7.47 (ddd, *J* 7.8 Hz, *J* 7.1 Hz, *J* 1.0 Hz, 1H, H-6), 7.50 (dd, *J* 9.0 Hz, *J* 2.3 Hz, 2H, H-2',6'), 7.67 (ddd, *J* 7.9 Hz, *J* 7.2 Hz, *J* 1.4 Hz, 1H, H-5), 7.89 (dd, *J* 7.8 Hz, *J* 0.8 Hz, 1H, H-7), 8.08 (d, *J* 7.9 Hz, 1H, H-4). ¹³C NMR (75 MHz, DMSO-*d*₆): δ_C 55.3, 114.3 (2C), 125.7, 126.1, 126.5 (2C), 127.8, 128.2, 131.9, 132.1, 138.9, 157.2, 164.9. HRMS (TOF MS ESI): *m/z* for (C₁₄H₁₁NO₂Se + H)⁺ calculated: 306.0034; found: 306.0039.

General synthetic procedure for bis(2-carbamoylaryl) diselenides 6. To a stirred solution of substituted aniline (1.0 mmol) in dry CH₂Cl₂ (5 mL) Na₂CO₃ (2.5 mmol, 0.265 g) was added. Next, the solution of chloride 5 in dry CH₂Cl₂ was added dropwise for 30 min. The desired product, diselenide precipitated immediately. The reaction progress was controlled by TLC. The stirring was continued for 1–48 h. The product was filtered off and washed with dry CH₂Cl₂ (2 x 5 mL), water (1 x 5 mL) and 3.5% HCl (3 x 5 mL).

Bis[2-(4-chloro-3-methylphenylcarbamoyl)]phenyl diselenide (6c). White solid, yield 95%, mp 239–240 °C (from 1,2-dichloroethane); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 2.35 (s, 6H, CH₃), 7.38–7.50 (m, 6H, ArH-5, PhH-

4,5), 7.63 (d, *J* 8.6 Hz, 2H, ArH-6), 7.76-7.82 (m, 4H, PhH-6, ArH-2), 7.96 (d, *J* 7.2 Hz, 2H, PhH-3), 10.61 (s, 2H, NH). ¹³C NMR (151 MHz, DMSO-*d*₆): δ_c 19.81 (CH₃), 119.64 (CH), 122.83 (CH), 126.37 (CH), 128.01 (C), 128.62 (CH), 128.93 (CH), 130.14 (CH), 131.99 (C), 132.07 (CH), 133.45 (C), 135.54 (C), 137.48 (C), 166.26 (C=O). HRMS (TOF MS ESI): *m/z* for (C₂₈H₂₁Cl₂N₂O₂Se₂ + Na)⁺ calculated: 668.9297, 670.9284, 672.9272; found: 668.9341, 670.9288, 672.9265.

Bis[2-(5-chloro-2-methoxyphenylcarbamoyl)]phenyl diselenide (6e). Grey powder, yield 85%, mp 194–195 °C (from methylene chloride); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 3.86 (s, 6H, OCH₃), 7.15 (d, *J* 8.9 Hz, 2H, ArH-3), 7.29 (dd, *J* 2.6 Hz, *J* 8.8 Hz, 2H, ArH-4), 7.38-7.51 (m, 4H, PhH-4,5), 7.78 (d, *J* 7.8 Hz, 2H, PhH-6), 7.84 (d, *J* 2.6 Hz, 2H, ArH-6), 7.99 (d, *J* 7.5 Hz, 2H, PhH-3), 9.93 (s, 2H, NH). HRMS (TOF MS ESI): *m/z* for (C₂₈H₂₂Cl₂N₂O₄Se₂ + H)⁺ calculated: 680.9363; found: 680.9400.

Bis[2-(2-chloro-5-methylphenylcarbamoyl)]phenyl diselenide (6g). White solid, yield 75%, mp 203–204 °C (from methylene chloride); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 2.35 (s, 6H, CH₃), 7.18 (dd, *J* 8.1 Hz, *J* 1.1 Hz, 2H, ArH-4), 7.43-7.46 (m, 4H, PhH-5, ArH-6), 7.47 (d, *J* 8.2 Hz, 2H, ArH-3), 7.50 (dd, *J* 7.4 Hz, *J* 1.0 Hz, 2H, PhH-4), 7.80 (d, *J* 8.0 Hz, 2H, PhH-6), 8.06 (d, *J* 7.5 Hz, 2H, PhH-3), 10.36 (s, 2H, NH). ¹³C NMR (75 MHz, DMSO-*d*₆): δ_c 20.4, 126.5, 126.7, 128.6, 128.7, 129.0, 129.3, 130.2, 132.3, 132.5, 132.6, 134.2, 137.3, 166.5. HRMS (TOF MS ESI): *m/z* for (C₂₈H₂₁Cl₂N₂O₂Se₂ + Na)⁺ calculated: 670.9284; found: 670.9287.

Bis[2-(2-chloro-4-methylphenylcarbamoyl)]phenyl diselenide (6h). White powder, yield 62%, mp 260–261 °C (from methylene chloride); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 2.35 (s, 6H, CH₃), 7.23 (d, *J* 8.4 Hz, 2H, ArH-5), 7.40-7.51 (m, 8H, PhH-4,5, ArH-3,6), 7.77 (d, *J* 7.4 Hz, 2H, PhH-6), 8.05 (d, *J* 6.9 Hz, 2H, PhH-3), 10.35 (s, 2H, NH). ¹³C NMR (151 MHz, DMSO-*d*₆): δ_c 20.8, 126.9, 128.7, 129.0, 129.1, 130.1, 130.3, 130.6, 132.3, 132.7, 133.0, 133.1, 138.3, 167.0. HRMS (TOF MS ESI): *m/z* for (C₂₈H₂₂Cl₂N₂O₂Se₂ + H)⁺ calculated: 648.9465; found: 648.9327.

Bis[2-(5-chloro-2-methylphenylcarbamoyl)]phenyl diselenide (6j). White powder, yield 86%, mp 247–248 °C (from 1,2-dichloroethane); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 2.28 (s, 6H, CH₃), 7.28 (dd, *J* 8.3 Hz, *J* 2.2 Hz, 2H, ArH-4), 7.34 (d, *J* 8.3 Hz, 2H, ArH-3), 7.40-7.52 (m, 4H, PhH-4,5), 7.53 (d, *J* 2.2 Hz, 2H, ArH-6), 7.80 (dd, *J* 7.8 Hz, *J* 1.3 Hz, 2H, PhH-6), 8.02 (dd, *J* 7.4 Hz, *J* 1.4 Hz, 2H, PhH-3), 10.28 (s, 2H, NH). ¹³C NMR (75 MHz, DMSO-*d*₆): δ_c 17.4, 125.85, 125.9, 126.4, 128.6, 129.8, 130.1, 131.9, 132.1, 132.3, 132.5, 132.9, 137.2, 166.5. HRMS (TOF MS ESI): *m/z* for (C₂₈H₂₁Cl₂N₂O₂Se₂ + Na)⁺ calculated: 670.9284; found: 670.9291.

Bis[2-(3-methoxyphenylcarbamoyl)]phenyl diselenide (6k). White powder, yield 74%, mp 221–223 °C (from methylene chloride); ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 3.78 (s, 6H, OCH₃), 6.74 (dd, *J* 8.2 Hz, *J* 1.7 Hz, 2H, ArH-4), 7.30 (dd, *J* 8.2 Hz, *J* 8.1 Hz, 2H, ArH-5), 7.40 (dd, *J* 8.1 Hz, *J* 1.2 Hz, 2H, ArH-6), 7.42 (ddd, *J* 8.0 Hz, *J* 7.0 Hz, *J* 1.2 Hz, 2H, PhH-5), 7.45-7.48 (m, 4H, PhH-4, ArH-2), 7.81 (dd, *J* 8.0 Hz, *J* 1.1 Hz, 2H, PhH-6), 7.96 (dd, *J* 7.6 Hz, *J* 1.3 Hz, 2H, PhH-3), 10.54 (s, 2H, NH). ¹³C NMR (151 MHz, DMSO-*d*₆): δ_c 55.0, 106.2, 109.5, 112.7, 126.4, 128.6, 129.5, 130.1, 131.9, 132.0, 133.7, 139.8, 159.4, 166.3. HRMS (TOF MS ESI): *m/z* for (C₂₈H₂₄N₂O₄Se₂ + H)⁺ calculated: 612.0189; found: 612.0232.

Bis[2-(4-methoxyphenylcarbamoyl)]phenyl diselenide (6l). White powder, yield 62%, mp 285.5–287.5 °C (from methylene chloride) (lit.³⁵ 290–292 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ_H 3.76 (s, 6H, OCH₃), 6.97 (d, *J* 9.0 Hz, 4H, ArH-3,5), 7.40 (ddd, *J* 7.1 Hz, *J* 6.9 Hz, *J* 0.9 Hz, 2H, PhH-4), 7.44 (ddd, *J* 8.0 Hz, *J* 7.1 Hz, *J* 1.3 Hz, 2H, PhH-5), 7.68 (d, *J* 9.0 Hz, 4H, ArH-2,6), 7.79 (dd, *J* 8.0 Hz, *J* 0.8 Hz, 2H, PhH-6), 7.95 (d, *J* 6.8 Hz, 2H, PhH-3), 10.44 (s, 2H, NH). ¹³C NMR (151 MHz, DMSO-*d*₆): δ_c 55.7, 114.3, 122.7, 126.9, 129.0, 130.6, 132.2, 132.4, 132.5, 134.3, 156.4, 166.4. HRMS (TOF MS ESI): *m/z* for (C₂₈H₂₄N₂O₄Se₂ + H)⁺ calculated: 612.0189; found: 612.01823.

Evaluation of antiviral activity and cytotoxicity. The compounds **4** and **6** at various concentrations were incubated with following viruses: HHV-1 (human herpes virus type 1), EMCV (encephalomyocarditis virus) and VSV (vesicular stomatitis virus) for 1 hr at RT. The HHV-1 was used at the dose of 10^6 TCID₅₀/mL, whereas EMCV and VSV at the dose of 10^8 TCID₅₀/mL. The TCID₅₀ (tissue culture infectious dose) determines the cytopathic effect caused by the virus in about 50% of infected cells. The virus titer was measured in human cell line A549 (human lung adenocarcinoma cell line, ATCC 185) which was observed for cytopathic effect after 48 h. The minimal concentration that caused 1000-fold decrease of virus titer was taken as minimal inhibitory concentration, MIC ($\mu\text{g}/\text{mL}$).

In parallel, the cytotoxicity of the compounds was determined in A549 cell line (cell line used in antiviral activity test) by incubation at various concentrations for 48 h at 37 °C in the atmosphere of 5% CO₂ in air. The minimal concentration which was toxic to approximately 50% of the cells was taken as TCCD₅₀.

Both, cytotoxicity and virus titers were determined using MTT assay. To ensure that colourful compounds did not interfere with MTT reagents, extent of cell damage was evaluated by microscopic examination prior to MTT assay.

Evaluation of antimicrobial activity. Antimicrobial activity of compounds **4** and **6** was determined by serial dilution method using gram-positive bacterial strains: Staphylococcus aureus PCM 1944, Bacillus subtilis PCM 1949, gram-negative bacterial strains: Escherichia coli PCM 2057, Pseudomonas aeruginosa PCM 2058 and yeast Candida albicans ATCC 90028 obtained from the Polish Collection of Microorganisms, following procedure previously described.²⁶ The antimicrobial activity was characterized by MIC value which is minimal concentration of the compound in $\mu\text{g}/\text{ml}$ that totally inhibits growth of the microorganisms.

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