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Il Farmaco 58 (2003) 1235–1242
IL FARMACO
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Functionalized alkyl and aryl diselenides as antimicrobial and antiviral agents: synthesis and properties

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Received 15 April 2003; accepted 8 August 2003

Abstract

The different dialkyl and diaryl diselenides with carbamoyl and sulfamoyl moieties **2**, **3**, **5** and other substituents in the *ortho* position of benzene ring **4**, **7**, **8** as well as derivatives of 1,2,4-benzoselenadiazine (**6**) were designed as antiviral and antimicrobial agents and synthesized. Some of them, particularly **8a** and **8b**, were found in the antiviral assay in vitro to be strong inhibitors of cytopathic activity encephalomyocarditis virus (EMCV). The compound **4a** and **8a** were found to have a broad spectrum of acivity against bacteria, yeasts and pathogenic fungi in vitro.

Keywords: Diselenides; Ebselen; Bacteriocides; Fungicides; Virucides

1. Introduction

Interest in the use of organoselenium compounds in biochemistry and medicinal biology started with findings that they are much less toxic compared with the inorganic species [1]. Studies of the anti-infective organoselenium compounds have been initiated about 50 years ago, when the selenium analogs of sulfonamides were tested until last decade but no particular interest has been paid for this problem [2,3]. Since 1984 when 2-phenyl-benzisoselenazol-3(2H)-one named ebselen (**1**) was found as an nontoxic glutathione peroxidase mimic [4,5] a lot of effort has been directed toward the development of stable organoselenium compounds that could be used as antioxidants, enzyme inhibitors, cytokine inducers, immunomodulators and antiradiation agents [6,7]. Although a broad spectrum of biological activity of organoselenium compounds has been presented, a little attention has been focused on their antimicrobial properties.

Most recently it has been found that ebselen (**1**), other benzisoselenazolones and related compounds exhibited inhibitory activity against some strains of bacteria and fungi [8]. Some of these compounds also exhibited cytopathic activity against RNA and DNA viruses: EMCV, HSV-1 and VSV [9,10]. In the present work fourteen different dialkyl and diaryl diselenides (**2**–**5**, **7**, **8**) are described. Some of them (compounds **5a**,**b**) have phenylsulfamoyl groups and other (**2**, **3a**–**d**) have 4-aminophenylsulfamoyl moiety potentially responsible for inhibition of dihydropteridine synthetase involved in the biosynthesis of folic acid [11]. All of them having Se–Se moiety should be able to interact covalently with thiol groups of peptides and enzymes in the similar way as selenenamide Se–N group [6,7]. For comparison of biological response of diselenides and selenenamides three their representatives—ebselen (**1**) and two other cyclic selenenamides **6d** and **6e** having new 1,2,4-benzoselenadiazine ring system [12] were obtained. All

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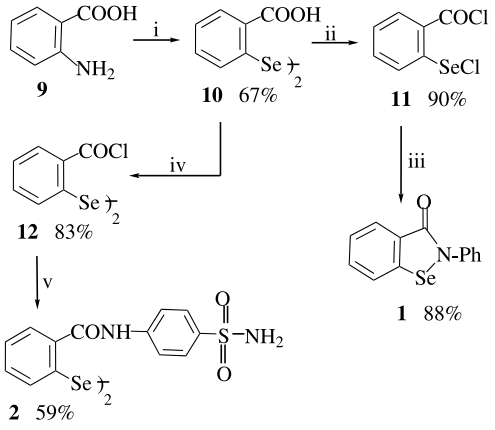
doi:10.1016/j.farmac.2003.08.003

these compounds were tested as antibacterial, antifungal and antiviral agents in vitro.

2. Chemistry

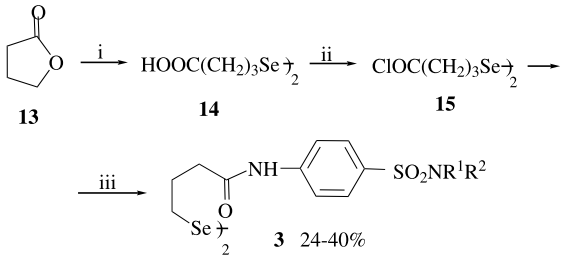
Ebselen was synthesized from anthranilic acid **9** which was diazotized and diazonium salt reacting with disodium diselenide produced 2,2′-diselenobisbenzoic acid (**10**). The acid **10** was converted to 2-(chloroseleno)benzoyl chloride (**11**) by treatment with thionyl chloride, used in large excess in the presence of DMF as a catalyst. The reaction of chloride **11** with aniline gave ebselen **1**. The same acid **10** treated with a stoichiometric amount of thionyl chloride in benzene solution gave 2,2′-diselenobisbenzoyl chloride (**12**) (Scheme 1). For this purpose the procedures elaborated earlier in our laboratory were applied [13,14]. The chloride **12** was used as the reagent for acylation of 4-aminobenzenesulfonamide and 2,2′diselenobis[benz(*p*-sulfonamido)anilide] (**2**) was obtained in this way.

4,4′-Diselenobisbutyramides (**3**) were synthesized according as it is shown in Scheme 2. The key substrate was γ-butyrolactone (**13**). In the first step it was treated with dilithium diselenide generated in situ from elemental lithium and selenium, in the manner similar to described earlier [15]. The procedure was slightly modified since 4,4′-di(*tert*-butyl)biphenyl was used as catalyst for selenenylation instead of diphenylacetylene and the reaction was carried out using the ultrasonic bath. It made the reaction more effective since dilithium diselenide was produced faster [10]. The acid **14**, thus obtained, was converted into chloride **15** by treatment with oxalyl chloride. This chlorinating reagent made the reaction highly chemoselective and possible chlorination of selenium was avoided. The last step of the synthesis



i = 1. $NaNO_2$, HCl, -5°C; 2. Na_2Se_2; ii = $SOCl_2$ (excess), DMF (cat.) reflux; iii = $PhNH_2$, MeCN; iv = $SOCl_2$, benzene, reflux; v = 4-$NH_2C_6H_4SO_2NH_2$, toluene, pyridine, reflux.

Scheme 1.



$R^1 = R^2 =$ H (**3a**); $R^1 =$ H, $R^2 =$ Bu (**3b**); $R^1 =$ H, $R^2 = COCH_3$ (**3c**)

$R^1, R^2 = -(CH_2)_5-$ (**3d**)

i = 1. Li_2Se_2, THF, 20°C; 2. HCl, H_2O; ii = ClCOCOCl, benzene, 20°C; iii = 4-$NH_2C_6H_4SO_2NR^1R^2$, NMM, THF, 20°C

Scheme 2.

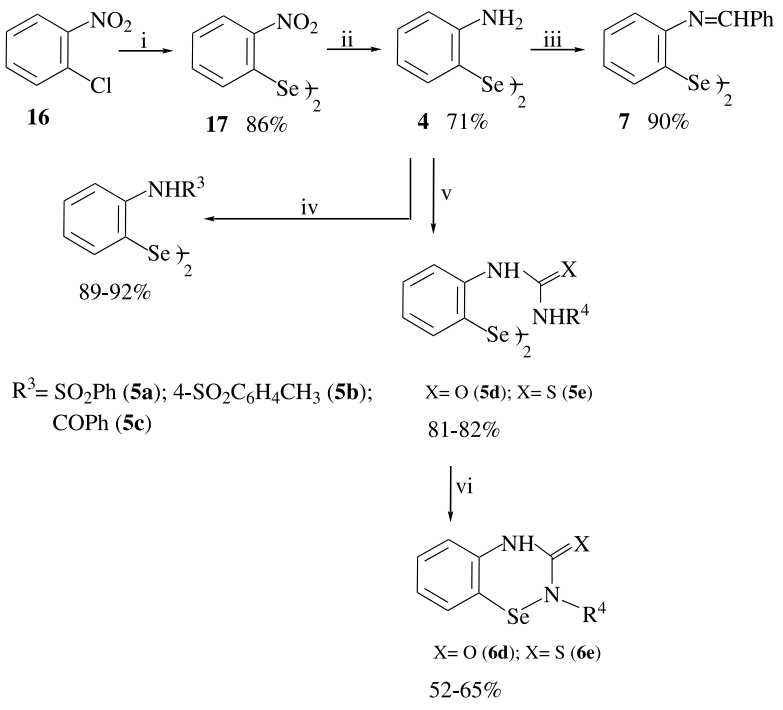
involved acylation of amino group of 4-aminobenzenesulfonamides, prepapared as described in Ref. [15], with acyl chloride **14** in the presence of 4-methylmorpholine (NMM) as a base.

The key substrate for synthesis of diselenides **5**, 1,2,4-benzoselenadiazin-3(4H)-one (**6d**) and related thione **6e** was bis(2-aminophenyl) diselenide (**4**). It was obtained by selenenylation of 1-chloro-2-nitrobenzene (**16**) with dilithium diselenide [15] and reduction of resulted bis(2-nitrophenyl) diselenide (**17**) with hydrazine in the presence of catalytic Ni (Raney) (Scheme 3). The amine **4** was sulfonylated and acylated to corresponding sulfonamides (**5a**,**b**) and carboxyamide (**5c**). Treatment of amine **4** with benzaldehyde gave imine **7** while its reaction with isocyanate or isothiocyanate resulted in bis[2-(carbamoylamino)phenyl] diselenide (**5d**) and bis[2-(thiocarbamoylamino)phenyl] diselenide (**5e**). The compounds **5d** and **5e** were converted to corresponding benzoselenadiazinone (**6d**) and benzoselenadiazinthione (**6e**) by oxidation with benzoyl peroxide.

Bis(2-hydroxyphenyl) diselenide (**8a**) was synthesized in the way shown in the Scheme 4. The methoxymethyloxybenzene (**18**) [19] was lithiated at the *ortho* position and formed **19**, which was selenenylated to compound **20**. It converted to selenol **21**, which finally was oxidized to diselenide **22**. The intermediates **19** and **20** were not isolated and selenol **21** was used in the crude form. The last step involved acid hydrolysis of methoxymethyloxy group and bis(2-hydroxyphenyl) diselenide (**8a**) was produced. The reaction of propylisocyanate with **8a** gave carbamate **8b** (Scheme 4) but all attempts for its oxidative cyclization to expected **8c** failed since no individual product could be isolated from the tarry mixtures.

3. Results and discussion

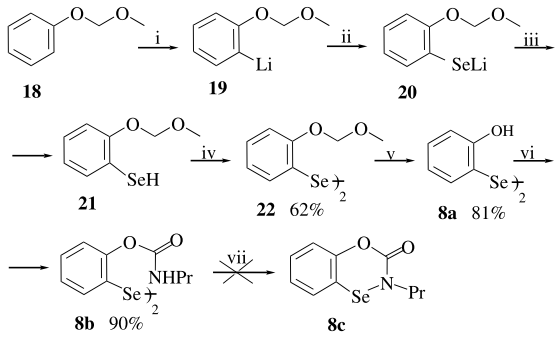
Compounds **2**, **3a**–**d**, **4**, **5d**–**e**, **6d**–**e**, **7** and **8a**–**b** were tested against bacteria, yeasts and pathogenic fungi. The



R^3= SO_2Ph (**5a**); 4-$SO_2C_6H_4CH_3$ (**5b**); COPh (**5c**)

i = Li_2Se_2, THF, HMPT, 20°C; ii = N_2H_4 H_2O, Ni (Raney), *n*-PrOH, 95°C;
iii = PhCHO, benzene, reflux; iv = $PhSO_2Cl$, 4-$CH_3C_6H_4SO_2Cl$ or PhCOCl, pyridine, -5°C;
v = R^4NCX (excess), 80°C; vi = $(PhCOO)_2$, benzene, 80°C

Scheme 3.



i = n-BuLi, THF, -10°C; ii = Se; iii = aq NH_4Cl; iv = MeOH, NaOH (cat.), O_2; v = MeOH, H_2O, HCl, 60°C; vi = PrNCO, 70°C; vii = $(PhCOO)_2$, benzene, 80°C

Scheme 4.

results of these tests, expressed as MIC (minimum inhibitory dose) values, are shown in Table 1, together with those obtained for the same strains for mentioned earlier ebselen (**1**)—one of the most active organoselenium compounds [7,8].

The broadest spectra of activity against tested microorganisms were observed for bis(2-aminophenyl) diselenide (**4**) and bis(2-hydroxyphenyl) diselenide (**8a**). Both of them have similar MIC values in the range 3.4–173 μg/ml. For the bacteria and yeasts the biological response observed for **4** and **8a** was similar to that established for ebselen (**1**). Moreover, both of these diselenides exhibited appreciable activity against fungal strains *R. oryzae*, *A. alternata* as well as for *M. gypsum* resistant toward ebselen. None of organoselenium compound tested was active against *B. cinarea*. Some other *ortho*-substituted bisaryl diselenides derived from amine **4** (compounds **5a**–**c**, **7**) and phenol **8a** (compound **8b**) were highly active against Gram-positive *S. aureus* remaining inactive toward Gram-negative *E. coli*. 4,4′-Diselenobutyramides (**3a**–**d**), bisaryl diselenides with urea or thiourea moiety (**5d**,**e**), 2-propyl-1,2,4-benzoselenadiazinone (**6d**) and 2-phenyl-1,2,4-benzoselenadiazin-3(4H)-thione (**6e**) were practically inactive against all tested microbial strains (MIC values above 500 μg/ml).

Antiviral effects of ebselen (**1**), diselenides **2**, **3a**–**d**, **4**, **5d**,**e**, **7**, **8a**,**b** and compounds **6d**,**e** were measured against encephalomyocarditis virus (EMCV, non-enveloped RNA virus), vesicular stomatitis virus (VSV, enveloped DNA virus) and herpes simplex virus type 1 (HSV-1, enveloped DNA virus). The results are presented in Table 2.

Strong anti-EMCV activitiy, similar or even higher that activity of ebselen (**1**) was found for bis(2-aminophenyl) diselenide (**4**), its derivatives **5d**, **7** and benzose-

Table 1
Antimicrobial activity of organoselenium compounds **1**–**8** against bacteria, yests and filamentous fungi characterized by values of MIC (μg/ml)

Compound	Bacteria		Yests	Filamentous fungi			
	S. aureus	*E. coli*	*C. parapsilosis*	*R. oryzae*	*A. alternata*	*B. cinaerea*	*M. gipsum*
1	13.7 [a]	137.0 [a]	13.7 [b]	> 500.00	> 500.00	> 500.00	274 [a]
2	35.4 [a]	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
3a	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
3b	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
3c	> 500.00	> 500	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
3d	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
4	17.3 [b]	173.0 [b]	17.3 [a]	17.3 [b]	17.3 [a]	> 500.00	3.5 [a]
5a	29.7 [a]	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
5b	124.8 [b]	> 500.00	321.0 [b]	312.0 [b]	> 500.00	> 500.00	156.0 [b]
5c	27.5 [b]	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
5d	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
5e	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
6d	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
6e	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00	> 500.00
7	25.9 [b]	259.0 [b]	2.6 [b]	51.8 [b]	51.8 [b]	> 500.00	5.2 [a]
8a	17.2 [b]	172.0 [a]	17.2 [b]	34.4 [b]	17.2 [a]	> 500.00	3.4 [a]
8b	25.7 [b]	> 500.00	51.4 [b]	> 500.00	> 500.00	> 500.00	51.4 [a]

[a] Microbicidal effect.
[b] Microbistatical effect.

lanadiazinone (**6d**) as well as for phenol **8a** and its carbamate **8b**. Contrary to highly active ebselen, among all organoselenium compounds tested, only **4** and **5a** were moderate and **6d** weak inhibitors of cytopathic activity of herpex simplex virus (HSV-1), other were inactive. None of the compounds tested had appreciable anti-VSV activity. Only phenol **8a** and its derivative **8b** exhibited weak activity against this virus.

Cytotoxicity of the compounds reported in this work, determined on human A 549 cells, presented in Table 2 falls in a broad range from 2.5 μg/ml for **6d** to 275 μg/ml for low toxic compound **3d**. Because of cytotoxicity only a few compounds—ebselen (**1**) and diselenides **3c**, **7**, **8a**,**b** have appreciable indices > 1 (inhibiting virus dose is lower than cytotoxic dose) for EMCV and only ebselen for HSV-1.

Table 2
Virucidal activity of organoselenium compounds **1**–**8**

Compound	Cytotoxicity [a]	EMCV		HSV-1		VSV	
		MIC [b]	Index [c]	MIC [b]	Index [c]	MIC [b]	Index [c]
1	15.0	10.0	1.50	2.0	7.50	> 1000.0	< 0.02
2	60.0	600.0	0.10	> 1000.0	< 0.06	> 1000.0	< 0.06
3a	20.0	> 1000.0	< 0.02	> 1000.0	< 0.02	> 1000.0	< 0.02
3b	40.0	> 1000.0	0.04	> 1000.0	< 0.04	> 1000.0	< 0.04
3c	234.0	100.0	2.34	> 1000.0	< 0.23	> 1000.0	< 0.23
3d	275.0	> 1000.0	< 027	> 1000.0	< 0.27	> 1000.0	< 0.77
4	7.0	10.0	0.70	40.0	0.18	> 1000.0	< 0.01
5a	30.0	200.0	0.15	100.0	0.30	> 1000.0	< 0.03
5b	10.0	60.0	0.17	600.0	< 002	> 1000.0	< 0.01
5c	50.0	> 1000.0	< 0.05	> 1000.0	< 0.05	> 1000.0	< 0.05
5d	3.0	10.0	0.30	> 1000.0	< 0.01	> 1000.0	< 0.01
5e	117.0	> 1000.0	< 0.12	> 1000.0	< 0.12	> 1000.0	< 0.12
6d	2.5	4.0	0.62	400.0	< 0.01	> 1000.0	< 0.01
6e	117.0	100.0	< 0.12	> 1000.0	< 0.12	> 1000.0	< 0.12
7	10.0	8.0	1.25	> 1000.0	< 0.01	> 1000.0	< 0.01
8a	7.5	4.0	1.88	> 1000.0	< 0.01	400.0	< 0.02
8b	15.0	4.0	3.75	> 1000.0	< 0.02	400.0	< 0.04

[a] Cytotoxicity determined on human A549 cells (μg/ml).
[b] Minimal inhibiting virus concentration (μg/ml).
[c] Index = cytotoxicity/MIC.

It may be concluded that the structural modifications of organodiselenides induce their improvement in the antimicrobial and antiviral activity. Some of them particularly *ortho*-substituted bisphenyl diselenides were found to be highly active inhibitors of cytopathic activity encephalomyocarditis virus EMCV (compound **8a** and **8b**) and to have activity against broad spectrum of microorganisms (compounds **4**, **8a**). It make evidence that introduction of amino- or hydroxyl-groups in *ortho* positions of phenyl rings of bisaryl diselenides plays an important role in enhancement of the antimicrobial and antiviral activity. On the contrary, introduction of sulfonyl group into diselenide molecule or selenenamide moiety into heterocyclic ring, another than benzisoselenazonyl one, had no influence on the antimicrobial or antiviral activity such modified organoselenium compounds.

4. Experimental

4.1. Chemistry

All reagents and solvents were purchased from Aldrich or 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 NMR spectra were recorded in DMSO-d_6 on a Bruker DRX spectrometer 300 MHz. Chemical shifts δ are reported in ppm relative to TMS. Reaction progress was monitored by thin layer chromatography (TLC) on silica gel $60F_{254}$ coated aluminium TLC plates from Merck. Elemental analyses were performed in our analytical laboratory and agreed with theoretical values to within 0.3%.

4.1.1. Ebselen (*1*)

This compound was prepared from anthranilic acid (**9**) following the synthetic route described previously [12].

4.1.2. 2,2′-Diselenobis[benz(4-sulfonamido)]anilide (*2*)

The solution of acid chloride **12**, obtained in the way reported in Ref. [13], (0.47 g, 2.3 mmol) sulfanilamide (0.86 g, 5 mmol) and pyridine (1 ml) in toluene (40 ml) was refluxed for 4 h until chloride was consumed. The solvent was evaporated in vacuo and the residue was washed with 5% HCl and then with water. The crude product was recrystallized from dimethylformamide; yield 62%, m.p. 316–318 °C, ref. [10] 315–318 °C.

4.1.3. 4,4′-Diselenobisbutyramides (*3a–d*)

These compounds were prepared from γ-butyrolactone (**13**) following the procedures described for **3a**,**b**,**d** in our earlier paper [10]. Compound **3c** (34%): m.p. 172–173 °C (acetonitrile); IR ν 3272 cm^{-1} (N–H), 1667 cm^{-1} (C–O), 1153 cm^{-1} (S–O): ^{1}H NMR δ 0.89 (t, 6H, $J = 6.6$ Hz, CH_3), 1.21–1.40 (m, 24H, CH_2), 2.08 (quintet, 4H, $J = 7.2$ Hz, CH_2), 2.54 (t, 4H, $J = 7.3$ Hz, CH_2), 2.75 (q, 4H, $J = 6.5$ Hz, CH_2), 3.04 (t, 4H, $J = 7.2$ Hz, CH_2), 7.44 (t, 2H, $J = 6.0$ Hz, SO_2NH), 7.79 (dd, 8H, $J = 13.8$ and 9.0 Hz, ArH), 10.36 (s, 2H, CONH).

4.1.4. bis(2-Nitrophenyl) diselenide (*17*)

Compound **17** was obtained by selenenylation of 1-chloro-2-nitrobenzene (**16**) with dilithium diselenide in the way reported in Ref. [15]. Yield 86%, m.p. 219–220 °C, Ref. [16] 220 °C.

4.1.5. bis(2-Aminophenyl) diselenide (*4*)

Raney nickel (ca. 10 g) was added to a solution of diselenide **17** (18.0 g, 45 mmol) in propanol (150 ml), heated to 95 °C and then hydrazine (15 ml, 300 mmol) was added dropwise and the mixture was stirred for 3 h. After additional 2 h the hot reaction mixture was filtered, the solvent was evaporated in vacuo and the residue was recrystallized from ethanol; yield 70%, m.p. 83.2 °C, Ref. [17] 83.5 °C.

4.1.6. bis[(2-Phenylsulfonylamino)phenyl] diselenide (*5a*) and bis[2-(4-methylphenyl-sulfonylamino)phenyl] diselenide (*5b*)

A solution of benzenesulfonyl chloride (3.51 g, 20 mmol) or *p*-toluenesulfonyl chloride (3.81g, 20 mmol) in pyridine (5 ml) was added dropwise, to the vigorously stirred solution of bis(2-aminophenyl) diselenide (**4**, 3.42 g, 10 mmol) in pyridine (10 ml) and cooled on the ice/salt bath for 20 min. Then mixture was stirred additionally for 20 h at r.t. After, pyridine was evaporated in vacuo and the residue was stirred with 5% solution of hydrochloric acid (15 ml) until the bright-yellow precipitate was formed. It was filtered and recrystallized from ethanol. Compound **5a** (89%): m.p. 146–147 °C: IR ν 3261–3237 cm^{-1} (N–H), 3062 cm^{-1} (C–H), 1581, 1471–1447 cm^{-1} (=C–H), 1399–1388, 1332 cm^{-1} (SO_2, sulfonamide), 1168, 1157 cm^{-1} (SO_2, sulfonamide): ^{1}H NMR δ 6.61 (d, 2H, $J = 7.56$ Hz, ArH), 7.11 (t, 2H, $J = 7{,}50$ Hz, ArH), 7.18 (t, 2H, $J = 7.2$ Hz, ArH), 7.49 (d, 2H, $J = 7.62$ Hz, ArH), 7.59 (t, 4H, $J = 7.47$ Hz, ArH), 7.67 (t, 6H, $J = 6.84$ Hz, ArH), 10.10 (s, 2H, ArNH): Compound **5b** (92%): m.p. 144–145 °C: IR ν 3293–3243 cm^{-1} (N–H), 3060 cm^{-1} (C–H_{ar}), 2956 cm^{-1} (C–H_{aliph}), 1582, 1472–1442 cm^{-1} (=C–H_{ar}), 1398–1385, 1337 cm^{-1} (SO_2, sulfonamide), 1156 cm^{-1} (SO_2, sulfonamide): ^{1}H NMR δ 2.37 (s, 6H, CH_3), 6.63 (d, 2H, $J = 7.32$ Hz, ArH), 7.00–7.20 (q, 4H, $J = 8.30$ Hz, ArH), 7.38 (d, 2H, $J = 8.13$ Hz, ArH), 7.50 (d, 2H, $J = 7.41$ Hz, ArH), 7.56 (d, 2H, $J = 8.01$ Hz, ArH), 9.99 (s, 2H, ArNH).

*4.1.7. bis[(2-Benzoylamino)phenyl] diselenide (**5c**)*

A solution of benzoyl chloride (2.84 g, 20 mmol) in pyridine (5 ml) was added dropwise to the vigorously stirred solution of bis(2-aminophenyl) diselenide (**4**; 3.42 g, 10 mmol) in pyridine (10 ml) and cooled on the ice/salt bath for 20 min. Then the mixture was stirred additionally for 20 h at r.t. After, pyridine was evaporated in vacuo and the residue was stirred with 5% solution of hydrochloric acid (15 ml) until the bright-yellow precipitate was formed. It was filtered and recrystallized from ethanol. Compound **5c** (90%): m.p. 168–169 °C: IR ν 3365–3319 cm^{-1} (N–H), 3057 cm^{-1} (C–H), 1677 cm − 1 (C=O), 1576–1519 cm^{-1} (N–H, C–N, amide) 1305–1252 cm^{-1} (amide): ^{1}H NMR δ 7.16–7.21 (m, 2H, ArH), 7.31 (t, 4H, $J = 7.30$ Hz, ArH), 7.54 (t, 4H, $J = 7.50$ Hz, ArH), 7.62 (d, 2H, $J = 6.72$ Hz, ArH), 7.72 (d, 2H, $J = 7.56$ Hz, ArH), 7.97 (d, 4H, $J = 7.65$ Hz, ArH), 10.15 (s, 2H, ArNH).

*4.1.8. bis[[2-(N-Propylcarbamoyl)amino]phenyl] diselenide (**5d**) and bis[[2-(N-phenyl-thiocarbamoyl)amino]phenyl] diselenide (**5e**)*

A mixture of amine **4** (3.42 g, 10 mmol) with propyl isocyanate (2.55 g, 30 mmol) or with phenyl isothiocyanate (4.06 g, 30 mmol) was heated at 80 °C until it solidified (2 h). The excess of reagent was evaporated in vacuo and the residue was recrystallized from toluene. **5d** (80%): m.p. 174–175 °C: IR ν 3306 cm^{-1} (N–H amide), 3097, 2958, 2931, 2872 cm^{-1} (C–H), 1639 cm^{-1} (C=O, urea): ^{1}H NMR δ 0.88 (t, 6H, $J = 7.3$ Hz, CH_3), 1.40–1.47 (m, 4H, CH_2), 2.99–3.31 (m, 4H, CH_2N), 6.76 (t, 2H, $J = 7.4$ Hz, NH) 6.89 (t, 2H, $J = 7.5$ Hz, ArH), 7.21 (t, 2H, $J = 7.5$ Hz, ArH), 7.49 (d, 2H, $J = 8.0$ Hz, ArH), 7.59 (d, 2H, $J = 8.0$ Hz, ArH), 8.01 (s, 2H, ArNH); Compound **5e** (82%): m.p. 121–122 °C: IR ν 3179 cm^{-1} (N–H, thioamide), 3047 cm^{-1} (C–H_{ar}), 1624 cm^{-1} (C=S, thiourea), 1450–1445 cm^{-1} (N–CS–N, thiourea), 1241 (C=S): ^{1}H NMR δ 6.94–6.99 (m, 4H, ArH), 7.23–7.30 (m, 6H, ArH), 7.45 (d, 4H, $J = 7.7$ Hz, ArH), 7.55 (d, 2H, $J = 7.7$ Hz, ArH), 7.70 (d, 2H, $J = 8.1$ Hz, ArH), 8.33 (s, 2H, ArNH), 9.23 (s, 2H, ArNH).

*4.1.9. 2-Propyl-1,2,4-benzoselenadiazin-3(4H)-one (**6d**) and 2-Phenyl-1,2,4-benzoselenadiazin-3(4H)-thione (**6e**)*

A magnetically stirred solution of **5d** (0.441 g, 1 mmol) or **5e** (0.495 g, 1 mmol) and benzoyl peroxide (0.27 g, 1.1 mmol) in dry benzene (30 ml) was heated to 80 °C for 20 h. After this period the solvent was evaporated in vacuo and product **6d** or **6e** was isolated from the residue by column chromatography on silica gel using hexane–ethyl acetate (4:1) as eluent and recrystallized from toluene. Compound **6d** (65%): m.p. 106–107 °C: IR ν 3305 cm^{-1} (N–H, amide), 3097, 2957, 2933, 2871 cm^{-1} (C–H), 1664 cm^{-1} (C=O, urea): ^{1}H NMR δ 0.85 (t, 3H, $J = 7.3$ Hz, CH_3), 1.42–1.55 (m, 2H, CH_2), 3.25 (t, 2H, $J = 7.3$, CH_2N), 6.97 (t, 2H, $J =$ 7.6 Hz, ArH), 7.15 (t, 1H, $J = 7.3$ Hz, ArH), 7.49 (d, 1H, $J = 7.6$ Hz, ArH), 7.29 (s, 1H, ArNH); Compound **6e** (52%): m.p. 128–129 °C: IR ν 3179 cm^{-1} (N–H), 3061 cm^{-1} (C–H), 1625 cm^{-1} (C=S, thiourea), 1466–1428 (N–CS–N, thiourea), 1241 (C=S): ^{1}H NMR δ 6.99–7.05 (m, 2H, ArH), 7.27–7.38 (m, 3H, ArH), 7.57 (d, 1H, $J = 8.1$ Hz, ArH), 7.82 (t, 3H, $J = 8.4$ Hz, ArH), 10.43 (s, 1H, ArNH).

*4.1.10. bis[(2-Benzylimino)phenyl] diselenide (**7**)*

A solution of bis(2-aminophenyl) diselenide (**4**; 3.42 g, 10 mmol) and benzaldehyde (2.12 g, 20 mmol) in benzene (50ml) was refluxed under azeotropic adapter, until the appropriate amount of water was formed. The solvent was evaporated in vacuo and the solid residue was recrystallized from ethanol. Compound **7** (90%): m.p. 169–170 °C: IR ν 3057 cm^{-1}, 2879 cm^{-1} (C–H), 1621 cm^{-1} (Ar–N=CH–Ar), 1565, 1458–1437 cm^{-1} (= C–H): ^{1}H NMR δ 7.18 (t, 2H, $J = 7.53$ Hz, ArH), 7.31 (t, 2H, $J = 7.14$ Hz, ArH), 7.38 (d, 2H, $J = 7.77$ Hz, ArH), 7.51 (d, 2H, $J = 7.80$ Hz, ArH), 7.57 (m, 6H, ArH), 8.02 (t, 4H, $J = 4.62$ Hz, ArH), 8.80 (s, 2H, ArNH).

*4.1.11. bis[(2-Methoxymethyloxy)phenyl] diselenide (**22**)*

A solution of *n*-buthyllithium (10 ml, 2.7 M) in hexane was added dropwise to a vigorously stirred solution of methoxymethyloxybenzene (**18**) [18] (2.76 g, 20 mmol) in tetrahydrofuran (50 ml) and cooled on the ice/salt bath under nitrogen for 10 min. The mixture was stirred additionally for 2 h while the white solid **19** was precipitated. Finally powdered gray selenium (2.0 g, 25 mmol) was added in three portions to the mixture and the reaction was continued on the ice/salt bath for 1 h and then at r.t. for additional 1 h. Then, saturated aqueous ammonium chloride (50 ml) was added and after stirring for 10 min the layers were separated and the water layer was shaken with ether. The organic layers were combined, washed with saturated aqueous sodium chloride, dried with magnesium sulfate and the solvent was evaporated in vacuo. The oily crude selenol **21** was dissolved in methanol (25 ml), sodium hydroxide (one pellet) was added to the stirred solution and stream of air was passed over the reaction mixture for 3 h. Then water was added and the mixture was shaken with three portions of dichloromethane (30, 20 and 20 ml). The combined extracts were dried with magnesium sulfate, solvent was evaporated in vacuo and pure compound **22** was isolated from the residue on silica gel column, using hexane–ethyl acetate (9:1) as an eluent. Compound **22** (62%): m.p. 34–35 °C: IR ν 3060 cm^{-1}, 2954 cm^{-1} (C–H), 1575, 1468–1439 cm^{-1} (=C–H), 1123, 1083, 987 cm^{-1} (C–O–C): ^{1}H NMR 3.39 (s, 6H, CH_3), 5.30 (s, 4H, CH_2), 6.97 (t, 2H, $J = 7.50$ Hz, ArH), 7.10 (d, 2H,

$J = 8.04$ Hz, ArH), 7.22 (t, 2H, $J = 7.17$ Hz, ArH), 7.45 (d, 2H, $J = 7.77$ Hz, ArH).

4.1.12. bis(2-Hydroxyphenyl) diselenide (**8a**)

A stirred mixture of diselenide **22** (2.72 g, 6.3 mmol), methanol (25 ml) and concentrated hydrochloric acid was heated to 60 °C for 3 h. Water (50 ml) was added to the cooled mixture and compound **8a** was isolated in the some manner as described for **22**. Finally it was recrystallized from cyclohexane–toluene. Compound **8a** (81%): m.p. 65.0–66.5 °C: IR ν 3419 cm^{-1} (O–H), 3063 cm^{-1} (C–H), 1589–1573, 1463–1450 cm^{-1} (=C–H), 1332, 1182, 1022 cm^{-1} (C–O): ^{1}H NMR δ 6.75 (t, 1H, $J = 7.53$ Hz, ArH), 6.79 (d, 1H, $J = 7.95$ Hz, ArH), 7.07 (t, 1H, $J = 7.83$ Hz, ArH), 10.31 (s, 1H, OH).

4.1.13. bis[2-(N-Propylcarbamate)phenyl] diselenide (**8b**)

A mixture of bis(2-hydroxyphenyl) diselenide (**8a**, 3.44 g, 10 mmol) with *n*-propyl isocyanate (2.55 g, 30 mmol) was heated at 70 °C until it solidified. The excess of reagent was evaporated in vacuo and the solid residue was recrystallized from ethanol. Compound **8b** (90%): m.p. 110–111 °C: IR ν 3318 cm^{-1} (N–H), 3061 cm^{-1}, 2971, 2932 cm^{-1} (C–H), 1716 cm^{-1}(C=O), 1546, 1462–1444 cm^{-1} (=C–H), 1246, 1206 cm^{-1} (COO): ^{1}H NMR 0.88 (t, 6H, $J = 7.3$ Hz, CH_3), 1.44–1.56 (m, 4*H*, CH_2CH_3), 3.07–3.13 (m, 4H, NCH_2), 5.08 (s, 2H, $NHCH_2$), 6.09–7.09 (m, 4H, ArH), 7.18–7.23 (q, 2H, $J = 8$, 1 Hz, ArH), 7.58 (d, 2H, $J = 7.1$ Hz, ArH).

4.2. Biological activity

4.2.1. Antimicrobial assay

Bacterial strains: *Staphylococcus aureus* CCM 3953, *Escherichia coli* CCM 3988, *Candida parapsilosis* (received from the laboratory of Medical Mycology, postgraduate Medical Institute, Bratislava), filamentous fungi *Rhizopus oryzae*, *Alternaria alternata* (obtained from the Collection of Microorganisms of the Slovak University of Technology), *Botrytis cinaerea* CCMF 16, *Microsporum gypseum* 1/94 (isolated from patient) were used.

Tested compounds **1**–**8** were dissolved in dimethyl sulfoxide, its final concentration in broth medium never exceeded 1% (V/V) and the same volume of solvent was present in both control and treated samples. The samples were used at concentration 1–500 μg/ml.

The effects of the tested compounds on the bacteria and yeasts were determined by the macrodilution method in L-shape tubes. The cultures of bacteria (in meat liquid medium) and yeasts (in Sabouraud's liquid medium) were incubated under vigourus shaking at 30 °C and absorbance was measured at 650 nm.

The effects of **1**–**8** compounds on filamentous fungi were tested by macrodilution technique on Sabouraud's agar (dermatophytes) and malt agar (other filamentous fungi) during cultivation at 25 °C and the diameter of groving colonies was measured at intervals [19].

The microbial activity was characterized by MIC values (minimal concentration of the compounds which inhibits growth by 100%). The MIC values were read from toxicity curves. MIC experiments on subculture dishes were used to assess of the minimal microbicidal concentration. Subcultures were prepared separately in Petri dishes containing an appropriate agar medium and icubated at 30 °C for 2 days (bacteria, yeasts) and at 25 °C for 4 days (filamentous fungi). The minimal microbicidal concentration value was the lowest concentration which completely suppressed growth of microbial colonies in the subculture dishes.

4.2.2. Cytotoxicity

Cytotoxicity of the compounds was determined in human lung adenocarcinoma cell line A549 (ATCC 185) and mouse fibroblast-like cells L929 (ATCC CCL 1). The experiment was performed in 96-cells microplates. The cells were treated with various doses of the compounds for 48 h at 37 °C in the atmosphere of 5% CO_2 in air. Then, the cultures were examined under microscope and next stained with MTT. The minimal concentration of a compound which was toxic to approximately 50% of the cells was taken as $TCCD_{50}$ [20].

4.2.3. Antiviral assay

The compounds in various concentrations were incubated with the following viruses: VSV (vesicular stomatis virus, *Rhabdoviridae*, enveloped virus), EMCV (encephalomyocarditis virus, *Picornaviridae*, naked virus) and HSV-1 (herpes simplex virus type 1, *Herpesviridae*, enveloped virus). Viruses VSV and EMCV were used at dose of 10^5 $TCID_{50}$/ml, HSV-1 at dose of 10^4 $TCID_{50}$/ml. After 2 h incubation at r.t., the virus titer was measured in human A549 cells and minimal virus-inhibiting concentration (MIC) was determined.

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