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Application of the Gould–Jacobs reaction to 4-amino-2,1,3-benzoselenadiazole

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ABSTRACT

An effective method for the synthesis of 4-amino-2,1,3-benzoselenadiazole (**4**) has been described. Reduction of readily available 4-nitrobenzothiadiazole **6** with $SnCl_2 \cdot 2H_2O$ afforded 1,2,3-triaminobenzene dihydrochloride **2**. The latter upon treatment with aqueous SeO_2 solution provided desired amine **4**. Nucleophilic vinylic substitution of activated enol ethers **7** with amine **4** led to (benzoselenadiazol-4-yl-amino)methylene derivatives **8**. Thermal cyclization of derivatives **8a–c**, **e**, **f** under Gould–Jacobs reaction conditions gave angularly annelated 7-(non)substituted selenadiazolo[3,4-*h*]quinolones **9**. Acid hydrolysis of etyl ester **9c** afforded corresponding acid **10**. All prepared selenadiazoloquinolones were tested for antimicrobial activity.

1. Introduction

Organoselenium compounds are interesting from several points of view. They can be applied in electronics and in recent decades they have been frequently studied for their biological activity.[1,2] The well-known compound 2-phenylbenzisoselenazol-3(2*H*)-one (Ebselen) and its many analogues can be found in antiinflammatory agents, immunomodifiers, cytokine inducers, enzyme inhibitors, virucides[3,4] or bactericides.[5] There are also examples of selenaheterocycles possessing antitumor activity such as 1,2-selenazoles,[6] 1,3-selenazoles[7,8] and 1,2,5-selenadiazoles.[3] On the other hand, it should be strongly emphasized that organoselenium compounds are generally considered toxic and smelly. But the weakly volatile selenaheterocyclic compounds are odourless and their toxicity is expected to be low.[2,9] For example, the above-mentioned Ebselen is practically nontoxic (LD_{50}=6.8 g/kg).[3] However, inorganic selenium compounds represent higher danger because they are considered highly poisonous—a fact, that is, probably related to their higher volatility. Nevertheless, most organoselenium compounds can be handled with the same safety precautions as other potentially harmful chemicals occurring in chemical laboratory.[9]

Being encouraged by all these facts, we decided to prepare 7-substituted selenadiazoloquinolones **9** and **10**. This condensed heterocyclic system was expected to combine the antibacterial activity of quinolones[10,11] with the potential biological activity of selenium compounds. We therefore synthesized selenadiazolo/quinolones, which were tested for antimicrobial activity on four strains of bacteria, yeasts and filamentous fungi. Additionally, UVA photoexcitation of selenadiazoloquinolones in dimethylsulfoxide or acetonitrile resulted in the formation of paramagnetic species coupled with molecular oxygen activation generating a superoxide radical anion or singlet oxygen, as evidenced by EPR spectroscopy.[12]

2. Results and discussion

The most efficient and convenient route towards quinolones involves the Gould–Jacobs reaction. The key intermediate in a classical Gould–Jacobs synthesis is a (hetero)aromatic amine, frequently obtained after reduction of the corresponding nitro derivative. Next the amine enters into a reaction with an activated enol ether and the product of this reaction sequence is thermally (250 °C) cyclized to the appropriate quinolone derivatives.[13] Thus, the key intermediate for the synthesis of selenadiazoloquinolones **9** becomes the 4-amino-benzoselenadiazole **4**. However, when we attempted to prepare amine **4** by the procedures described in the literature starting from the readily accessible nitro derivative **5** using iron in acetic acid[14] or zinc in hydrochloric acid[15] as reducing agents, we did not obtain any satisfactory results. Therefore, we tried to develop our own way towards amine **4** utilizing 4-nitrobenzoselenadiazole **5**[15] as starting material. Attempts to take advantage of the selective reduction of the nitro group employing various reducing agents, such as $NaBH_4$, catalytic hydrogenation on Raney nickel or palladium on charcoal

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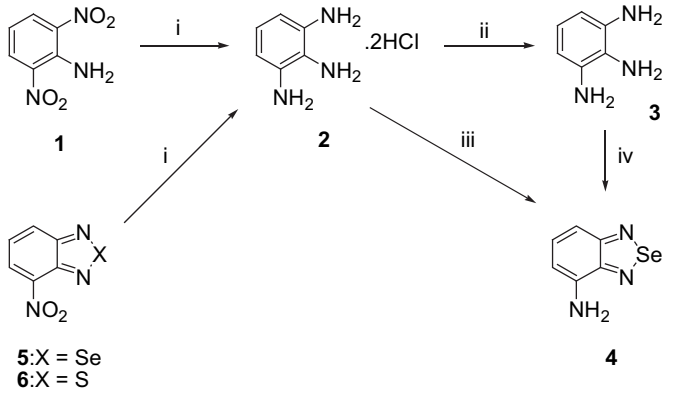
(various qualities) did not lead to success. Reduction on Raney nickel using hydrazine hydrate as the source of hydrogen failed also.

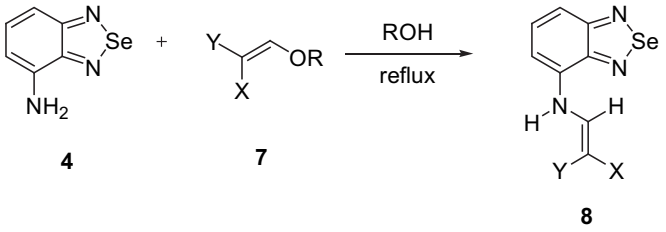
Another approach towards the key amine **4** involved the cyclization of 1,2,3-triaminobenzene (**3**) with aqueous selenium dioxide solution (Scheme 1).



Scheme 1. Reagents and conditions. (i) $SnCl_2{\cdot}2H_2O$, HCl, reflux, 5 h, 68–88%; (ii) $NaOH/H_2O$, 92%; (iii) SeO_2/H_2O, rt, 15 min., then $NaOH/H_2O$, 97%; (iv) SeO_2/H_2O, EtOH, rt, 15 min, 93%.

The only problem here is the possible oxidation and decomposition of the less stable triamine **3** either before or during the course of cyclization with selenium dioxide. First, triamine **3** was prepared by the reduction of not so easily available 2,6-dinitroaniline (**1**)[16,17] using catalytic hydrogenation on Raney nickel. The obtained triamine **3** was not isolated prior to cyclization with selenium dioxide. Nonetheless, yields of the final amine **4** were unsatisfactorily low (15–20%). One of the possible explanations is that the decay of triamine **3** occurred during the hydrogenation, which took more than 24 h.[18] Next dinitroaniline **1** was subjected to reduction with stannous chloride dihydrate in concentrated hydrochloric acid according to Avendano et al.[19] with slight a modification in the procedure to yield free triamine **3**. The triamine was liberated from its dihydrochloride **2** by sodium hydroxide solution (Scheme 1). Free triamine **3** was isolated in 81% yield (over two steps) as unstable white or brownish plates, which were instantly dissolved in ethanol and treated with aqueous selenium dioxide solution at room temperature to give the desired 4-aminobenzoselenadiazole **4** in excellent yield (93%) without any oxidation by-products (Scheme 1). Amine **4** obtained by this procedure is stable enough and can be stored under air for a long time without any decomposition ((hetero)aromatic amines are generally unstable in air).[18] A disadvantage of this synthesis is the use of not so readily available 2,6-dinitroaniline (**1**), which was replaced by the already mentioned easily available 4-nitrobenzoselenadiazole **5**. The latter was reduced with stannous chloride dihydrate under the same reaction conditions as described for dinitroaniline **1** to afford dihydrochloride **2** in 68% yield. Subsequent neutralization with sodium hydroxide solution furnished free amine **3**, which was once again immediately converted to amine **4** by using one equivalent of selenium dioxide. This reaction sequence starting from nitro derivative **5** has a few drawbacks. First, elementary selenium precipitates from the reaction mixture as a black solid, so the problem with hazardous selenium waste arises. Second, from the synthetic point of view, selenium is removed from the molecule only to be built-in in the next step (wasting selenium). And third, the yields of triamine **3** are slightly lower. To avoid all these handicaps, we tried to exploit 4-nitrobenzothiadiazole **6**, readily accessible in large quantities by nitration of benzothiadiazole[20] as starting material for preparation of the key amine **4** (Scheme 1). Nitrobenzothiadiazole **6** underwent the same reduction with $SnCl_2{\cdot}2H_2O$ as compounds **1** and **5**, respectively, followed by alkalization and cyclization with SeO_2 to produce the desired amine **4** in a good overall yield (74%). To shorten the whole procedure, we also tried direct cyclization of dihydrochloride **2** with selenium dioxide solution and finally after alkalization of the reaction mixture amine **4** was isolated in excellent yield (97%) (Scheme 1).

Furthermore, amine **4** entered into the nucleophilic vinylic substitution[21] with the appropriate alkoxymethylene derivatives **7a**–**j** (activated enol ethers) to provide (benzoselenadiazol-4-ylamino)ethenes **8a**–**i** (Scheme 2).



7, 8	R	X	Y	Yield (%)
a	Me	CO-O-CMe$_2$-O-CO		90
b	Me	COOMe	COOMe	74
c	Et	COOEt	COOEt	75
d	Et	COMe	COMe	82
e	Et	COMe	COOEt	84
f	Et	CN	COOEt	65
g	Me	COMe	COOMe	90
h	Me	CN	COOMe	60
i	Et	CN	COMe	85
j	Et	CN	CN	0

Scheme 2. Nucleophilic vinylic substitution.

Nucleophilic vinylic substitutions proceeded smoothly with good yields (60–90%) in refluxing methanol or ethanol. Surprisingly, 2-ethoxymethylenemalononitrile **7j** did not undergo nucleophilic vinylic substitution with amine **4**, however it reacted easily with various heteroaromatic amines such as aminoquinoxalines,[22] aminobenzotriazoles and aminobenzimidazoles.[23] Even employing toluene or dimethylformamide as solvent led to no reaction and only unreacted amine **4** or products of decomposition were identified. Quantum-chemical calculations indicate the highest nucleophilic character of the N(amine) atom of amine **4** based on FED HOMO reactivity indices (Table 1) whereas the analogous reactivity of C(7) atom is lower and moreover reduced by its positive atomic charge. The highest electrophilic character of the C (2) atom of **7j** as indicated by FED LUMO reactivity indices (Table 2) is supported by its high positive atomic charge. These findings are in agreement with the preferred **8j** product formation. Nevertheless, the interaction of the HOMO of **4** nucleophile with LUMO of **7j** electrophile in the reaction is conditioned by the close energies of both orbitals. In our case, the poor reactivity may be explained by their relatively large energy difference (ca. 3.5 eV).

Table 1
Selected electronic structure data of 4-amino-2,1,3-benzoselenadiazole (**4**)

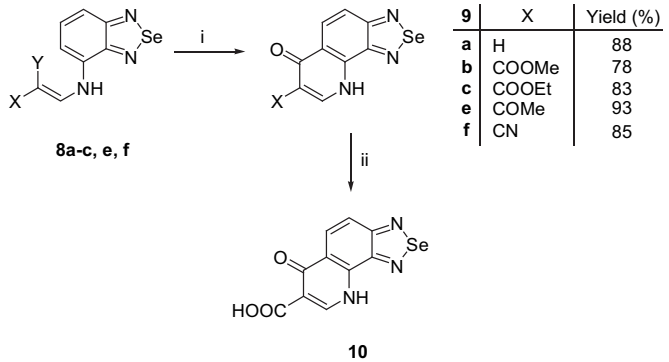
FED of atom	Atomic charge	HOMO	LUMO
C(7)	+0.022	0.309	0.086
C(6)	+0.064	0.056	0.141
C(5)	+0.041	0.262	0.067
N(amine)	−0.075	0.361	0.055
Orbital energy [eV]	—	−5.473	−2.261

Table 2
Selected electronic structure data of 2-ethoxymethylenemalononitrile **7j**

FED of atom	Atomic charge	HOMO	LUMO
C(1)	−0.068	0.379	0.340
C(2)	+0.268	0.167	0.984
C(cyano)	−0.067/−0.054	0.034/0.036	0.037/0.051
N(cyano)	−0.068/−0.081	0.198/0.170	0.130/0.173
Orbital energy [eV]	—	−7.238	−2.014

The structure of compounds **8** was established on the basis of their NMR spectroscopic characteristics. Higher values of the proton shifts of olefinic protons in the ^{1}H NMR spectra as expected are caused by their interaction with the nitrogen atom of the selenadiazole ring, similar to the case of 4-substituted benzazoles.[24] Because of the high vicinal coupling constant $^3J_{NH\text{-}CH}$ (12–14 Hz), an antiperiplanar conformation of the NH–CH moiety can be assigned. Compounds **8a**–**d** have identical substituents X and Y, whereas compounds **8e**–**i** with different X and Y can exist as mixtures of *E* and *Z* isomers. The relative ratio of the individual geometric isomers could be estimated from their NMR spectral data considering intensities of signals. Although in all cases both isomers were formed, the *E*-isomers prevailed (**8e**, **g**). That means that the configuration with the acetyl group hydrogen bonded to the amino group is preferred over a hydrogen bond with the alkoxycarbonyl group.[25] The cyanoacetic acid derivatives **8f**, **h** also gave predominantly the *E*-isomers, however this preference was based on steric factors only. The possible H-bond with alkoxycarbonyl group did not seem to play a major role.[25] The 3-oxobutanenitrile derivative **8i** confirmed these conclusions. These facts support the conclusion that nucleophilic vinylic substitution also runs on these systems with an inversion of configuration in accordance with the literature.[21] Chemical shifts of the carbon atoms on the double bond reflect its polarization, especially the carbons between the two electron-withdrawing groups: when cyano and ester groups are substituents, the chemical shifts of those atoms are 75.7 and 79.0 ppm, respectively, but in the case of two acetyl groups it is 114.5 ppm.[21–24] All these phenomena were evident regardless of the solvents and temperature used for recording the NMR spectra. The structure of the compounds **8a**–**h** has been checked by using EI mass spectrometry and their fragmentation was studied.[26]

Thermal cyclization of enamines **8a**–**c**, **e**, **f** under Gould–Jacobs reaction conditions led regioselectively to the angularly annelated 7-(non)substituted selenadiazolo[3,4-*h*]quinolones **9** (Scheme 3). Compounds **9e**, **f** can also be obtained from enamines **8g**, **h** in the same way. The most important factors for successful ring closure are (i) temperature above 250 °C, (ii) reaction time, (iii) purity of the starting substrate and (iv) appropriate dilution of the starting material in the solvent (diphenylether).



9	X	Yield (%)
a	H	88
b	COOMe	78
c	COOEt	83
e	COMe	93
f	CN	85

Scheme 3. Reagents and conditions: (i) Ph_2O, ~250 °C; (ii) for **9c**: 20% HCl, reflux, 2 h, 98%.

Derivatives **8a**–**c**, **e** required a dilution of 1:20 (1 g of starting aminomethylene derivative to 20 mL of diphenylether) and relatively short reaction times (15–30 min.), while cyclization of **8f** occurred successfully only after 6 h of reflux using a dilution of 1:100. The cyclization was carefully monitored by TLC (chloroform: methanol 100:1) to detect its accurate termination. Angular annelation of the pyridone and selenadiazole rings was confirmed by the coupling constants (about 9 Hz) resulting from the *ortho* interaction $^3J_{HH}$ of the benzene ring protons. 6,9-Dihydro-6-oxo-[1,2,5]selenadiazolo[3,4-*h*]quinoline-7-carboxylic acid (**10**) was obtained by acid hydrolysis of the corresponding ethyl ester **9c** in almost quantitative yield (Scheme 3). Hydrolysis of methyl ester **9b** provides similar results.

3. Antimicrobial evaluation

Six of the newly synthesized selenadiazoloquinolones were tested for their antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* as examples of Gram negative bacteria and *Staphylococcus aureus* and *Bacillus subtilis* as examples of Gram positive bacteria. They were also tested against yeasts *Candida utilis*, *Candida albicans*, *Candida parapsilosis* and *Saccharomyces cerevisiae* and filamentous fungi *Aspergilus niger*, *Rhizopus oryzae*, *Mucor racemosus* and *Penicillium cyaneum*. Selenadiazoloquinolones were tested at concentration range of 0.25–399.81 μmol L^{-1}. None of the tested compounds reached a higher IC_{50} value than the highest tested concentration in the case of all tested microorganisms. Thus these tested selenadiazoloquinolones demonstrate compounds with very weak antimicrobial activity.

4. Conclusions

In conclusion, six selenadiazoloquinolones **9** and **10** were prepared in good yields by application of Gould–Jacobs reaction to 4-aminobenzoselenadiazole **4**, and tested for antimicrobial activity. Antimicrobial evaluation revealed that the tested compounds exhibited only very weak antimicrobial activity. Currently, work is in progress on the more promising 9-ethylselenadiazoloquinolones.

5. Experimental section

5.1. General methods

Melting points (uncorrected) were measured on Koffler block using a digital thermometer DT012C. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 300-MHz spectrometer at 25 °C. Compounds **8f**, **h**, **i** were measured at 80 °C due to their lower solubility. Chemical shifts are reported in parts per million (δ-scale) relative to internal standard TMS (0.00 ppm). The operation frequency was 300 MHz for ^{1}H and 75.5 MHz for ^{13}C NMR. Coupling constants (J) are given in hertz. Infrared spectra were recorded on a Nicolet model NEXUS 470 FT-IR spectrometer in KBr with absorption in cm^{-1}. The reaction monitoring and purity of products were accomplished by TLC on silica-gel plates (Fluka) and stains were visualized by UV light (254 nm and 366 nm) or by using iodine vapors. Elemental analyses were determined with a Thermo Fingion CHNS(O) 1112 instrument.

5.2. Solvents and materials

The alkoxymethylene derivatives **7b**, **c**, **j** are commercially available, while compounds **7a**, **d**–**i** were synthesized by condensation of methyl or ethyl orthoformate (according to alkyl in ester group from second reactant to avoid transesterification) with corresponding methylene compound.[22a,27]

5.3. Quantum-chemical calculations

Standard geometry optimizations of the compounds under study at DFT level of theory using hybrid B3LYP functional[28] and cc-pVDZ basis sets[29,30] have been performed using Gaussian03 program package.[31] Their stability (i.e., that the geometry corresponds to a minimum of a potential energy surface) has been confirmed by vibrational frequencies calculations (no imaginary vibrations). Frontier electron densities (FED) of individual atoms in the highest occupied molecular orbital (HOMO) and in the lowest unoccupied molecular orbital (LUMO) have been evaluated as the corresponding net electron populations and used as reactivity indices of these atoms in nucleophilic and electrophilic reagents, respectively.

5.4. 4-Amino-2,1,3-benzoselenadiazole (4)

Method A (via 1,2,3-triaminobenzene (**3**)). Tin(II) chloride dihydrate (22.2 g, 98.4 mmol) was suspended in concentrated hydrochloric acid (33 mL) and to this suspension 2,6-dinitroaniline (**1**) (2.0 g, 11.0 mmol), 4-nitro-2,1,3-benzoselenadiazole (**5**) (2.5 g, 11.0 mmol) or 4-nitro-2,1,3-benzothiadiazole (**6**) (1.99 g, 11.0 mmol) was added portionwise. After the addition was completed, the reaction mixture was refluxed for 5 h. In case of using nitro derivative **5**, elementary selenium was filtered off while hot. The reaction mixture or filtrate was cooled to room temperature. Yellowish crystals were collected by suction, washed with ethanol and dried. Dihydrochloride **2** was dissolved in 10% NaOH solution (5 mL g^{-1}) and the resulting brown colored solution was extracted with ethyl acetate (4×25 mL). Combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to afford free triamine **3**. This triamine was immediately dissolved in ethanol (30 mL g^{-1}) and a stoichiometric amount of SeO_2 in water (1 g/20 mL) was added dropwise at room temperature while orange crystals were formed. After the addition was completed, the reaction mixture was stirred for an additional 10 min. Then water (50 mL) was added to the reaction mixture and orange needles were separated by suction, washed with water and dried. Yield 1.63 g (75%) starting from **1**; 1.26 g (58%) starting from **5**; 1.61 g (74%) starting from **6**.

Method B (via 1,2,3-triaminobenzene dihydrochloride (**2**)). Dihydrochloride **2** (4.26 g, 21.7 mmol) was dissolved in water (42 mL). To this solution, an aqueous solution of SeO_2 (2.41 g, 21.7 mmol) was added dropwise at room temperature while orange crystals were formed. After the addition was finished, reaction mixture was stirred for 15 min and then alkalized with 30% NaOH solution while cooling in an ice bath. Orange needles were separated by suction, washed with water and dried to give compound **4** (4.17 g (97%)).

Compound **4**: orange needles; mp 162–164 °C (Ref. 15 mp 160–161 °C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.87 (br s, 2H, NH_2), 6.38 (dd, ^3J=7.2 Hz, ^4J=0.7 Hz, 1H, 5-H), 6.96 (dd, ^3J=8.9 Hz, ^4J=0.8 Hz, 1H, 7-H), 7.27 (dd, ^3J=8.9 Hz, 7.2 Hz, 1H, 6-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 102.8, 109.0, 131.6, 141.3, 153.6, 160.5.

5.5. General procedure for the preparation of (benzoselenadiazol-4-ylamino)methylene derivatives (8)

A mixture of 4-aminobenzoselenadiazole **4** (1.98 g, 10 mmol) and corresponding alkoxymethylene derivative **7a**–**i** (11 mmol) was refluxed in methanol or ethanol (60 mL) until all amine **4** was consumed (TLC monitoring, eluent $CHCl_3$/MeOH, 100:1). Product **8c** crystallized from the cooled solution, while compounds **8a**, **b** and **8d**–**i** precipitated during reflux. After cooling, the precipitate was collected by suction, washed with methanol or ethanol and dried. Dry products were recrystallized from dimethylsulfoxide without addition of charcoal and filtration.

*5.5.1. 5-[(2,1,3-Benzoselenadiazol-4-ylamino)methylene]-2,2-dimethyl-1,3-dioxane-4,6-dione (**8a**).* Yellow needles (90%); mp 239–244 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.79 (s, 6H, 2×CH_3), 7.30 (d, ^3J=7.0 Hz, 1H, 5-H), 7.55 (dd, ^3J=9.1 Hz, 7.1 Hz, 1H, 6-H), 7.72 (dd, ^3J=9.1 Hz, ^4J=0.8 Hz, 1H, H-7), 9.09 (d, ^3J=14.1 Hz, 1H, =CH–), 12.11 (br d, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.1, 89.2, 105.4, 111.4, 120.5, 129.4, 130.9, 151.5, 153.5, 160.5, 163.4, 165.1. IR (KBr): 3448, 1730, 994, 925 cm^{-1}. Anal. Calcd for $C_{13}H_{11}N_3O_4Se$ (352.21): C, 44.33; H, 3.15; N, 11.93. Found: C, 44.42; H, 3.17; N, 11.90.

*5.5.2. Dimethyl 2-[(2,1,3-benzoselenadiazol-4-ylamino)methylene]-propanedioate (**8b**).* Yellow needles (74%); mp 213–215 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 7.14 (d, ^3J=7.1 Hz, 1H, 5-H), 7.49 (dd, ^3J=9.0 Hz, 7.1 Hz, 1H, 6-H), 7.56 (dd, ^3J=9.0 Hz, ^4J=0.7 Hz, 1H, 7-H), 8.76 (d, ^3J=13.7 Hz, 1H, =CH–), 12.0 (br d, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 51.7, 52.1, 95.6, 108.4, 118.3, 129.9, 132.3, 149.7, 153.4, 160.5, 165.7, 168.8. IR (KBr): 3447, 1689, 1098, 995 cm^{-1}. Anal. Calcd for $C_{12}H_{11}N_3O_4Se$ (340.20): C, 42.37; H, 3.26; N, 12.35. Found: C, 42.34; H, 3.27; N, 12.38.

*5.5.3. Diethyl 2-[(2,1,3-benzoselenadiazol-4-ylamino)methylene]-propanedioate (**8c**).* Ochre needles (75%); mp 153–157 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (s, ^3J=7.1 Hz, 3H, OCH_2CH_3), 1.41 (t, ^3J=7.1 Hz, 3H, OCH_2CH_3), 4.30 (q, ^3J=7.1 Hz, 2H, OCH_2CH_3), 4.41 (q, ^3J=7.1, 2H, OCH_2CH_3), 7.12 (d, ^3J=7.1 Hz, 1H, 5-H), 7.49 (dd, 1H, ^3J=9.0 Hz, 7.1 Hz, 6-H), 7.55 (dd, ^3J=9.0 Hz, ^4J=0.7 Hz, 1H, 7-H), 8.73 (d, ^3J=13.6 Hz, 1H, =CH–), 11.92 (br d, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 14.4, 60.4, 60.8, 96.4, 108.2, 118.1, 130.0, 132.4, 149.2, 153.5, 160.5, 165.5, 168.4. IR (KBr): 3442, 1683, 1013, 977 cm^{-1}. Anal. Calcd for $C_{14}H_{15}N_3O_4Se$ (368.25): C, 45.66; H, 4.11; N, 11.57. Found: C, 45.52; H, 4. 09; N, 11.62.

*5.5.4. 3-[(2,1,3-Benzoselenadiazol-4-ylamino)methylene]pentane-2,4-dione (**8d**).* Brownish solid (82%); mp 215–218 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.46 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 7.16 (d, ^3J=7.2 Hz, 1H, 5-H), 7.51 (dd, ^3J=9.0 Hz, 7.2 Hz, 1H, 6-H), 7.64 (dd, ^3J=9.0 Hz, ^4J=0.7 Hz, 1H, 7-H), 8.82 (d, ^3J=12.6, 1H, =CH–), 13.45 (br d, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.4, 32.2, 111.0, 114.5, 119.3, 129.6, 132.2, 150.6, 153.5, 160.7, 195.2, 201.5. IR (KBr): 3445, 1623, 989, 969 cm^{-1}. Anal. Calcd for $C_{12}H_{11}N_3O_2Se$ (308.20): C, 46.77; H, 3.60; N, 13.63. Found: C, 46.74; H, 3.67; N, 13.57.

*5.5.5. (E/Z)-Ethyl 2-[(2,1,3-benzoselenadiazol-4-ylamino)methylene]-3-oxobutanoate (**8e**).* Yellow-orange crystals (84%,); mp 215–218 °C; *E*/*Z*=4:1; ^{1}H NMR (300 MHz, $CDCl_3$,): *E*-isomer: δ 1.38 (t, ^3J=7.1 Hz, 3H, OCH_2CH_3), 2.61 (s, 3H, CH_3), 4.31 (q, ^3J=7.1 Hz, 2H, OCH_2CH_3), 7.19 (d, ^3J=7.2 Hz, 1H, 5-H), 7.50 (dd, ^3J=9.0 Hz, 7.2 Hz, 1H, 6-H), 7.60 (d, ^3J=9.0 Hz, 1H, 7-H), 8.77 (d, ^3J=13.1 Hz, 1H, =CH–), 13.48 (br d, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): *E*-isomer: δ 14.5, 31.6, 60.3, 104.9, 109.8, 119.0, 129.8, 132.4, 149.3, 153.5, 160.5, 166.6, 200.5. IR (KBr): 3444, 1703, 1629, 1027, 979 cm^{-1}. Anal. Calcd for $C_{13}H_{13}N_3O_3Se$ (338.23): C, 46.16; H, 3.89; N, 12.42. Found: C, 46.28; H, 3.91; N, 12.38.

*5.5.6. (E/Z)-Ethyl 3-(2,1,3-benzoselenadiazol-4-ylamino)-2-cyano-prop-2-enoate (**8f**).* Yellow powder (65%); mp 239–243 °C; *E*/*Z*=4:1; ^{1}H NMR (300 MHz, DMSO-d_6): *E*-isomer: δ=1.33 (t, ^3J=7.1 Hz, 3H, OCH_2CH_3), 4.32 (q, ^3J=7.1 Hz, 2H, OCH_2CH_3), 7.65–7.45 (m, 3H, Ar–H), 8.78 (d, ^3J=13.6 Hz, 1H, =CH–) 11.51 (br d, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): *E*-isomer: δ 13.6, 60.4, 76.0, 109.9, 116.7, 118.2, 129.2, 130.6, 151.7, 152.1, 159.2, 165.8. IR (KBr): 3451, 2214, 1672, 1032, 985 cm^{-1}. Anal. Calcd for

$C_{12}H_{10}N_4O_2Se$ (321.20): C, 44.87; H, 3.14; N, 17.44. Found: C, 45.03; H, 3.15; N, 17.43.

*5.5.7. (E/Z)-Methyl 2-[(2,1,3-benzoselenadiazol-4-ylamino)methylene]-3-oxobutanoate (**8g**).* Orange powder (90%); mp 215–218 °C; *E*/*Z*=5:1, ^{1}H NMR (300 MHz, $CDCl_3$): *E*-isomer: δ 2.61 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.21 (d, ^3J=7.2 Hz, 1H, 5-H), 7.50 (dd, ^3J=9.0 Hz, 7.2 Hz, 1H, 6-H), 7.61 (dd, ^3J=9.0 Hz, ^4J=0.7 Hz, 1H, 7-H), 8.78 (d, ^3J=13.1 Hz, 1H, =CH–), 13.51 (br d, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): *E*-isomer: δ 31.5, 51.5, 104.2, 109.9, 119.1, 129.8, 132.4, 149.8, 153.5, 160.5, 167.0, 200.5. IR (KBr): 3457, 1712, 1037, 981 cm^{-1}. Anal. Calcd for $C_{12}H_{11}N_3O_3Se$ (324.20): C, 44.46; H, 3.42; N, 12.96. Found: C, 44.27; H, 3.45; N, 12.71.

*5.5.8. (E/Z)-Methyl 3-(2,1,3-benzoselenadiazol-4-ylamino)-2-cyanoprop-2-enoate (**8h**).* Yellow powder (60%); mp 257–263 °C; *E*/*Z*=4:1; ^{1}H NMR (300 MHz, DMSO-d_6): *E*-isomer: δ 3.84 (s, 3H, OCH_3), 7.65–7.45 (m, 3H, Ar–H), 8.77 (d, ^3J=13.5 Hz, 1H, =CH–), 11.48 (br d, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): *E*-isomer: δ 51.2, 75.7, 110.0, 116.7, 118.3, 128.9, 130.6, 152.1, 152.7, 159.3, 164.2. IR (KBr): 3453, 2216, 1715, 1114, 1070 cm^{-1}. Anal. Calcd for $C_{11}H_8N_4O_2Se$ (307.17): C, 43.01; H, 2.63; N, 18.24. Found: C, 43.11; H, 2.66; N, 18.24.

*5.5.9. (E/Z)-2-[(2,1,3-Benzoselenadiazol-4-ylamino)methylene]-3-oxobutanenitrile (**8i**).* Yellow-orange powder, (85%); mp 232–237 °C; *E*/*Z*=1:8; ^{1}H NMR (300 MHz, DMSO-d_6): *Z*-isomer: δ 2.35 (s, 3H, CH_3), 7.70–7.50 (m, 3H, Ar–H), 8.81 (d, ^3J=13.2 Hz, 1H, =CH–), 12.82 (br d, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): *Z*-isomer: δ 27.8, 85.4, 111.1, 118.9, 119.1, 129.1, 130.6, 151.1, 152.2, 159.3, 195.9. IR (KBr): 3449, 2204, 1648, 1026, 983 cm^{-1}. Anal. Calcd for $C_{11}H_8N_4OSe$ (291.17) C, 45.38; H, 2.77; N, 19.24. Found: C, 45.42; H, 2.75; N, 19.31.

5.6. General procedure for preparation of [1,2,5]selenadiazolo [3,4-*h*]quinoline-6(9*H*)-ones (9)

A mixture of (benzoselenadiazol-4-ylamino)methylene derivatives **8a**–**c**, **e**, **f** (1 g) and diphenylether (20 mL, 100 mL for **8f**) was heated at 250–255 °C. After cooling the reaction mixture, the precipitated solid was collected by suction, washed with dichloromethane several times and dried in a vacuum oven at 80 °C for 6 h to remove diphenylether.

*5.6.1. [1,2,5]Selenadiazolo[3,4-h]quinoline-6(9H)-one (**9a**).* Brown solid (88%); mp 306–308 °C; ^{1}H NMR (300 MHz, TFA-*d*): δ 7.78 (d, ^3J=6.7 Hz, 1H, 7-H), 8.22 (d, ^3J=8.4 Hz, 1H, 4-H), 8.57 (d, ^3J=8.4 Hz, 1H, 5-H), 8.88 (d, ^3J=6.7 Hz, 1H, 8-H); ^{13}C NMR (75 MHz, TFA-*d*): δ 113.2, 122.0, 125.3, 126.4, 137.9, 145.0, 153.0, 161.3 172.2. IR (KBr): 3179, 1615, 1603, 961 cm^{-1}. Anal. Calcd for $C_9H_5N_3OSe$ (250.12): C, 43.22; H, 2.01; N, 16.80. Found: C, 43.28; H, 1.99; N, 16.86.

*5.6.2. Methyl 6,9-dihydro-6-oxo-[1,2,5]selenadiazolo[3,4-h]quinoline-7-carboxylate (**9b**).* Yellow powder (78%); mp 256–260 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 7.65 (d, ^3J=9.9 Hz, 1H, 4-H), 8.18 (d, ^3J=9.9 Hz, 1H, 5-H), 8.43 (s, 1H, 8-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 50.8, 114.7, 118.6, 123.8, 125.5, 129.4, 142.9, 152.0, 159.9, 164.5, 171.8. IR (KBr): 3168, 1684, 1624, 958 cm^{-1}. Anal. Calcd for $C_{11}H_7N_3O_3Se$ (308.16): C, 42.87; H, 2.28; N, 13.64. Found: C, 42.82; H, 2.26; N, 13.70.

*5.6.3. Ethyl 6,9-dihydro-6-oxo-[1,2,5]selenadiazolo[3,4-h]quinoline-7-carboxylate (**9c**).* Yellow powder (83%); mp 275–280 °C; ^{1}H NMR (300 MHz, TFA-*d*): δ 1.63 (t, ^3J=7.2 Hz, 3H, OCH_2CH_3), 4.78 (q, ^3J=7.2 Hz, 2H, OCH_2CH_3), 8.28 (d, ^3J=9.7 Hz, 1H, 4-H), 8.62 (d, ^3J=9.7 Hz, 1H, 5-H), 9.53 (s, 1H, 8-H); ^{13}C NMR (75 MHz, TFA-*d*): δ 14.4, 67.5, 112.0, 122.5, 125.5, 126.5, 138.3, 146.5, 153.2, 162.1, 169.3, 174.8. IR (KBr): 3166, 1716, 1624, 977 cm^{-1}. Anal. Calcd for $C_{12}H_9N_3O_3Se$ (322.18): C, 44.74; H, 2.82; N, 13.04. Found: C, 44.78; H, 2.81; N, 13.09.

*5.6.4. 7-Acetyl-[1,2,5]selenadiazolo[3,4-h]quinoline-6(9H)-one (**9e**).* Brown powder (93%); mp>410 °C (decay); ^{1}H NMR (300 MHz, TFA-*d*): δ 4.00 (s, 3H, CH_3), 9.20 (d, ^3J=9.6 Hz, 1H, 4-H), 9.61 (d, ^3J=9.6 Hz, 1H, 5-H), 10.62 (s, 1H, 8-H); ^{13}C NMR (75 MHz, TFA-*d*): δ 27.1, 116.8, 122.9, 125.3, 126.5, 138.1, 147.3, 152.8, 161.6, 175.6, 205.9. IR (KBr): 3164, 1664, 1621, 978 cm^{-1}. Anal. Calcd for $C_{11}H_7N_3O_2Se$ (292.16): C, 45.22; H, 2.42; N, 14.38. Found: C, 45.18; H, 2.46; N, 14.32.

*5.6.5. 6,9-Dihydro-6-oxo-[1,2,5]selenadiazolo[3,4-h]quinoline-7-carbonitrile (**9f**).* Brown crystals, (85%); mp 403–407 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.67 (d, ^3J=9.3 Hz, 1H, 4-H), 8.04 (d, ^3J=9.3 Hz, 1H, 5-H), 8.61 (s, 1H, 8-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 99.0, 116.1, 120.1, 122.3, 124.7, 133.7, 145.9, 152.0, 160.1, 173.4. IR (KBr): 3222, 2226, 1620, 995 cm^{-1}. Anal. Calcd for $C_{10}H_4N_4OSe$ (275.13): C, 43.66; H, 1.47; N, 20.36. Found: C, 43.60; H, 1.49; N, 20.39.

*5.6.6. 6,9-Dihydro-6-oxo-[1,2,5]selenadiazolo[3,4-h]quinoline-7-carboxylic acid (**10**).* A mixture of ester **9c** (1 g, 3.1 mmol) and 20% hydrochloric acid (50 mL) was refluxed for 2 h. After cooling the reaction mixture, water (30 mL) was added and the solid was collected by suction, washed with water and dried to afford acid **10** (0.89 g, 98%) as yellow solid.

Compound **10**: yellow solid; mp 321–324 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.69 (d, ^3J=9.4 Hz, 1H, 4-H), 8.11 (d, ^3J=9.4 Hz, 1H, 5-H), 8.53 (s, 1H, 8-H), 15.65 (br s, 1H, COOH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 112.3, 121.0, 122.2, 124.4, 134.7, 143.4, 151.7, 160.1, 165.9, 177.3. IR (KBr): 3552, 1716, 1622, 988 cm^{-1}. Anal. Calcd for $C_{10}H_5N_3O_3Se$ (294.13): C, 40.84; H, 1.71; N, 14.29. Found: C, 40.79; H, 1.73; N, 14.31.

5.7. Antimicrobial evaluation

5.7.1. Materials. The bacterial strains *E. coli* CCM 3988, *P. aeruginosa* CCM 3955, *S. aureus* CCM 3953, *B. subtilis* CCM 1718, the yeasts *C. utilis* CCY 29-38-86, *C. parapsilosis* CCY 29-20-19, *S. cerevisiae* CCY 21-4-19, *C. albicans* OBM UBVOZ and the filamentous fungi *A. niger* CCM F-237, *R. oryzae* OBM UBVOZ, *M. racemosus* CCM F-8190, *P. cyaneum* CCM F-376 were used. Tested selenadiazoloquinolones were used at concentrations in the range of 0.25–399.81 μmol L^{-1}. Pure selenadiazoloquinolone derivatives were dissolved in dimethylsulfoxide; their final concentration never exceeded 1% (v/v) in either control or treated samples.

5.7.2. Antibacterial assay. The antibacterial effect of the selenadiazoloquinolone derivatives was assayed by a microdilution method in 96-well microtitration plates.[32] The bacteria were cultured on Müller–Hinton medium at 30 °C. An overnight inoculum was prepared 12–16 h before the test. The growing inoculum was filtered and a 1.5% suspension of bacteria was prepared for the experiments. This suspension (180 μL) was added to 20 μL of the tested complex solution and cultured for 8 h on a reciprocal shaker in a thermostat at 30 °C. The time course of absorbance (A_{630}) was then determined in three parallels. After 8 h culturing with the tested selenadiazoloquinolones the bacteria were inoculated to a solid culture medium and cultured statically for 1 day at 37 °C.

5.7.3. Determination of the effect on yeasts. The yeasts were cultured on Sabourand-glucose medium at 28 °C. Seven mL of culture medium was inoculated with 0.5 mL of overnight growing culture and 75 μL solution of the tested selenadiazoloquinolones. The

cultures of yeasts were then cultured for 8 h on a reciprocal shaker in a thermostat at 28 °C. The A_{630} of triplicate sets of tubes were measured at 2 h intervals.

5.7.4. Antifungal assay. The filamentous fungi were cultured in Petri dishes on yeast extract-dextrose medium at 28 °C. Selenadiazoloquinolones (50 μL) was added to Petri dishes (diameter 50 mm) immediately before pouring 5 mL Saborad-glucose agar. The solidified plates were then inoculated in the center with 5 μL of the spore suspension. Triplicate sets of agar plates were incubated at 25 °C and the diameter of growing colonies was measured at intervals.

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Supplementary data

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References and notes

1. Nogueira, C. W.; Zeni, G.; Rocha, J. B. T. *Chem. Rev.* **2004**, *104*, 6255–6285.
2. Młochowski, J.; Kloc, K.; Lisiak, R.; Potaczek, P.; Wójtowicz, H. *Arkivoc* **2007**, *vi*, 14–46.
3. Mugesh, G.; du Mont, W.-W.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125–2179.
4. Osajda, M.; Kloc, K.; Młochowski, J.; Piasecki, E.; Rybka, K. *Pol. J. Chem.* **2001**, *75*, 823–830.
5. Nozawa, R. T.; Yokota, R.; Fujimoto, R. *Antimicrob. Agents Chemother.* **1989**, *33*, 1388–1390.
6. Ito, H.; Wang, J.-Z.; Shimura, K.; Sakakibara, J.; Ueda, T. *Anticancer Res.* **1990**, *10*, 891–895.
7. Kumar, Y.; Green, R.; Borysko, K. Z.; Wise, D. S.; Wotring, L. L.; Townsed, L. B. *J. Med. Chem.* **1993**, *36*, 3843–3845.
8. Kumar, Y.; Green, R.; Wise, D. S.; Wotring, L. L.; Townsed, L. B. *J. Med. Chem.* **1993**, *36*, 3849–3852.
9. Murphy, P. J. In *Science of Synthesis*; Thomas, J., Ed.; Thieme: Stutgart-New York, NY, 2003; Vol. 14, pp 817–854.
10. Albrecht, R. *Prog. Drug Res.* **1977**, *21*, 9–104.
11. Milata, V.; Claramunt, R. M.; Elguero, J.; Zálupský, P. *Targets Heterocycl. Syst.* **2000**, *4*, 167–203.
12. Barbieriková, Z.; Bella, M.; Kučerák, J.; Milata, V.; Jantová, S.; Dvoranová, D.; Veselá, M.; Staško, A.; Brezová, V. *Photochem. Photobiol.* **2010**, submitted for publication.
13. Curran, T. T. Gould–Jacobs reaction. In *Name Reactions in Heterocyclic Chemistry*, 1st ed.; Li, J. J., Ed.; John Wiley: Hoboken, 2005; pp 423–436.
14. Efros, L. S.; Todres-Selektor, Z. V. *Zh. Obshch. Khim.* **1957**, *27*, 983–989.
15. Sawicki, E.; Carr, A. *J. Org. Chem.* **1957**, *22*, 503–506.
16. Schultz, H. P. 2,6-Dinitroaniline In. *Organic Syntheses*; Rabjohn, N., Ed.; John Wiley: New York, NY, 1963; Coll. Vol. 4, pp 364–366.
17. Bella, M.; Milata, V. *Org. Prep. Proced. Int.* **2006**, *38*, 344–346.
18. Lythgoe, D. J.; McClenaghan, I.; Ramsden, C. A. *J. Heterocycl. Chem.* **1993**, *30*, 113–117.
19. Marcos, A.; Pedregal, C.; Avendano, C. *Tetrahedron* **1991**, *47*, 7459–7464.
20. (a) Komin, A. P.; Carmack, M. *J. Heterocycl. Chem.* **1975**, *12*, 829–833; (b) Neto, B. A. D.; Lopes, A. S. A.; Ebeling, G.; Gonçalves, R. S.; Costa, V. E. U.; Quina, F. H.; Dupont, J. *Tetrahedron* **2005**, *61*, 10975–10982.
21. Saloň, J.; Milata, V.; Gatial, A.; Prónayová, N.; Leško, J.; Černuchová, P.; Rappoport, Z.; Vo-Thanh, G.; Loupy, A. *Eur. J. Org. Chem.* **2005**, 4870–4878.
22. (a) Saloň, J.; Milata, V.; Prónayová, N.; Leško, J. *Monatsh. Chem.* **2000**, *131*, 293–299; (b) Saloň, J.; Milata, V.; Prónayová, N.; Leško, J. *Collect. Czech. Chem. Commun.* **2001**, *66*, 1691–1697; (c) Saloň, J.; Milata, V.; Chudík, M.; Prónayová, N.; Leško, J.; Seman, M.; Belicová, A. *Monatsh. Chem.* **2004**, *135*, 283–291.
23. (a) Milata, V.; Ilavský, D. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2918–2925; (b) Milata, V.; Ilavský, D.; Goljer, I. *Chem. Pap.* **1988**, *6*, 801–802.
24. Milata, V.; Ilavský, D.; Goljer, I.; Leško, J.; Chahinian, M.; Henry-Basch, E. *Collect. Czech. Chem. Commun.* **1990**, *55*, 1038–1048.
25. Couchouron, B.; Le Saint, J.; Courtot, P. *Bull. Soc. Chim. Fr., Part 2* **1983**, *3*, 66–72.
26. Leško, J.; Milata, V.; Schultz, M. *Molecules* **2000**, *5*, 937–940.
27. Ohmori, J.; Sakamoto, S.; Kubota, H.; Shimizu-Sasamata, M.; Okada, M.; Kawasaki, S.; Hidaka, K.; Togami, J.; Furuya, T.; Murase, K. *J. Med. Chem.* **1994**, *37*, 467–475.
28. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
29. Woon, D. E.; Dunning, T. H., Jr. *J. Chem. Phys.* **1993**, *98*, 1358–1371.
30. Wilson, A. K.; Woon, D. E.; Peterson, K. A.; Dunning, T. H., Jr. *J. Chem. Phys.* **1999**, *110*, 7667–7676.
31. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R. *Gaussian 03 (Revision C1)*; Gaussian,: Pittsburgh, PA, 2003.
32. Jantová, S.; Labuda, J.; Vollek, V.; Zastková, M. *Folia Microbiol.* **1997**, *42*, 324–326.