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Original article

Synthesis, structure and cytotoxicity of 3-*C*, *N*, *S*, *Se* substituted benzo[*b*] selenophene derivatives

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ABSTRACT

Synthesis, molecular structure and cytotoxic activity of a series of 3-*C*, *N*, *S*, *Se* substituted benzo[*b*] selenophene derivatives on human fibrosarcoma HT-1080, mouse hepatoma MG-22A, and mouse fibroblasts 3T3 cell lines are described. The correlation between compound LD_{50} 3T3 fibroblast cell line and HT-1080 morphology was shown.

1. Introduction

The interest of benzothiophene derivatives stems from the fact that this molecule is found as a chromophore core for drugs. *Raloxifene* and *Arzoxifene* are benzothiophene selective estrogen receptor modulators (SERMs) of clinical use in postmenopausal osteoporosis and treatment of breast cancer and potentially in hormone replacement therapy [1–6]. *Zileuton* is an orally active inhibitor of 5-lipoxygenase, and thus inhibits leukotrienes formation. It is used for the maintenance treatment of asthma. Benzothiophene piperazines and piperidines are fatty acid amide hydrolase (FAAH) inhibitors [7]. Also, it has been shown that 3-thiosubstituted benzothiophenes are serotonin receptors modulators [8]. On the other hand, selenium has attracted great interest as an essential element and certain diseases have been eradicated by dietary supplementation of this element. Selenium is essential for cell metabolism as a component of glutathione peroxidase and other enzyme systems. Current interest lies in the prevention of certain cancers by supplementation with selenium [9–11]. Selenium appears to operate by several mechanisms depending on the chemical form of selenium, the nature of the carcinogenic process, and its dosage. There was no significant difference in the potency of selenate, selenite, selenium dioxide, selenomethionine and selenocysteine to inhibit the development of mammary tumors, drug-resistant and drug non-resistant human ovarian tumor cells [12]. Taking into account the importance of the selenium as a trace element [13–17]in the organism our present investigation is connected with the elaboration of synthetic protocols and cytotoxic activity studies in a series of 3-*C*, *N*, *S*, *Se* substituted benzo[*b*]selenophene derivatives.

2. Results and discussion

2.1. Chemistry

Our synthetic strategy for the insertion of aryl and hetaryl groups in position 3 of benzo[*b*]selenophene **1** was based on *Suzuki*, *Stille*, and *Sonogashira* type coupling reactions. Interactions of **1** with various aryl(hetaryl)boronic acids, hetaryl stannanes and terminal acetylenes are presented in Scheme 1. After the optimization of the reaction conditions it has been found that arylboronic acids readily react with 3-bromobenzo[*b*]selenophene-2-carboxylic acid ethyl ester (**1**) in the presence of *in situ* prepared

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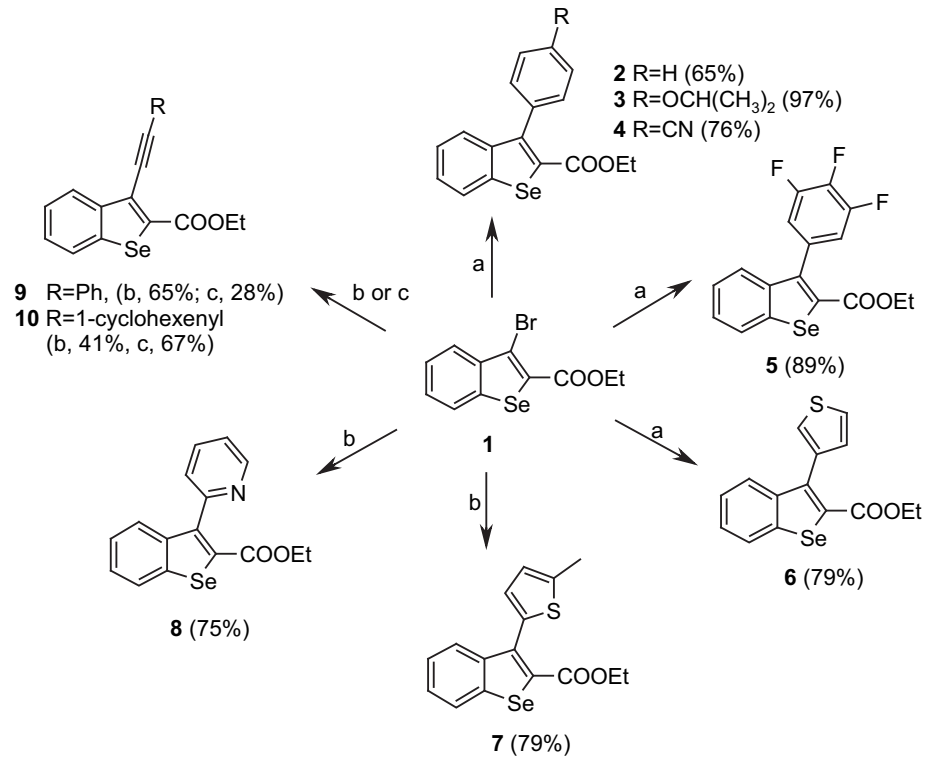


Scheme 1. Synthesis of benzo[*b*]selenophenes **2**–**10**. Reagents and reaction conditions: (a) aryl(hetaryl)boronic acid (2 eq.), $Pd(OAc)_2$ (10%), tri(*o*-tolyl)phosphine (20%), potassium phosphate (3.4 eq.), xylene/EtOH, 1–6 h; (b) hetaryl stannane (1.4 eq.), $(Ph_3P)_4Pd$ (4%), Ph_3As (7%), xylene, 110 °C, 2–24 h; (c) terminal acetylene (1.46 eq.), $PdCl_2$ (5%), Ph_3P (10%), CuI (10%), DMF/TEA, 100 °C, 20 h.

palladium catalyst ($Pd(OAc)_2$ and tri(*o*-tolyl)phosphine) using potassium phosphate as a base. According to GC MS data, reaction completed after heating in xylene/ethanol media (10:1) at 110 °C in half an hour and corresponding 3-aryl(hetaryl)benzo[*b*]selenophenes **2**–**6** were obtained in good yields (65–97%). It should be noted that almost quantitative yield was obtained in the case of 4-*iso*-propoxyphenyl boronic acid due to arylboronic acids with EWG groups are more stable in basic media [18]. Molecular structures of the compounds **3** and **4** are shown in Fig. 1A and B (Table 1). Both molecules in these crystal structures are characterized by two planar fragments: one of them is benzoselenophene system with ester group and the second is the phenyl ring. The dihedral angles between these fragments are equal to 70.8(4) and 65.7(5)° for **3** and **4**, respectively. Despite the similarity of molecular forms and sizes, the molecular packing in crystals of **3** and **4** is different: the crystal lattice of **3** is characterized by monoclinic space group $P2_1/n$, whilst for **4** there is triclinic lattice (space group $P\bar{1}$).

3-(5-Methylthienyl)- (**7**) and 3-(2-pyridyl)benzo[*b*]selenophenes (**8**) were prepared using *Stille* coupling protocol, because 2-thiophene and 2-pyridine boronic acids are expensive and not stable in basic media. Typically, the *Stille* coupling of aryl halides with aryl stannanes is performed under anhydrous conditions in aprotic solvents. Xylene is used as a solvent to increase the temperature of reaction. One day heating of corresponding stannanes with **1** in the presence of tetrakis(triphenylphosphino)palladium(0) in xylene at 130 °C leads to the formation of **7** and **8** in medium yields. With the aim of reducing the reaction time and to activate the catalyst, a catalytic amount (7%) of triphenylarsine was added [24] due to the donor-acceptor As–Pd bond being longer than P–Pd bond. As a result desired derivatives **7** and **8** were obtained in higher yields (75–79%) after only 2 h of heating. Then we examined introduction of terminal acetylenes using corresponding phenyletnynyl- and cyclohexenylethynyl trimethylstannanes under the *Stille* protocol. According to experimental data, it was found that corresponding 3-(phenylethylnyl)benzo[*b*]selenophene **9** forms in 65% yield after 1 h of heating, but cyclohexenyl analog **10** in moderate yield (28%). However, during interaction of terminal acetylenes with **1** under *Sonogashira* type conditions using classical $(Ph_3P)_2PdCl_2$ catalyst in DMF/TEA media 3-(cyclohexenylethynyl) benzo[*b*]selenophene (**10**) received in higher yield (67%) than phenylethynyl analog **9**.

With the purpose to introduce amino group in position 3 of benzo [*b*]selenophene **1** ring *Buchwald–Hartwig* type coupling has been examined. The benefits of 3-N-substituted benzo[*b*]selenophenes stems from the fact that these compounds could be more bioavailable over 3-C-substituted analogs. After reaction conditions optimization Pd_2dba_3 as a source of palladium(0) and xantphos as an appropriate ligand were chosen. According to our results aniline, 3- and 4-aminopyridines readily react with **1** performing reaction in xylene at 120 °C using cesium carbonate as a base. Desired 3-(phenylamino)- (**11**), 3-(3-pyridylamino)- (**12**), 3-(4-pyridylamino) benzo[*b*]selenophenes (**13**) were obtained in a very good yields, besides, in the case of 4-pyridylamino derivative in almost quantitative yield (96%) (Scheme 2). Molecular structure of compound **13** is illustrated in Fig. 1C. The intramolecular hydrogen bond of NH···O type between the amino group and carbonyl oxygen atom O11 was found in the structure of **13**. The hydrogen bond length is 2.825(2) Å (H15···O11 = 2.21 Å, N–H···O = 126°).

Notably, interaction of **1** with 2-aminopyridine under the same reaction conditions leads to the formation of a polycyclic derivative **14**. Seems that after the routine amination in position 3 of benzo[*b*]selenophene ring follows electrophilic attack of the ester group to the nitrogen atom of pyridine ring under the basic reaction conditions, as a result 10*H*-pyrido[1,2-*a*]benzo[*b*]

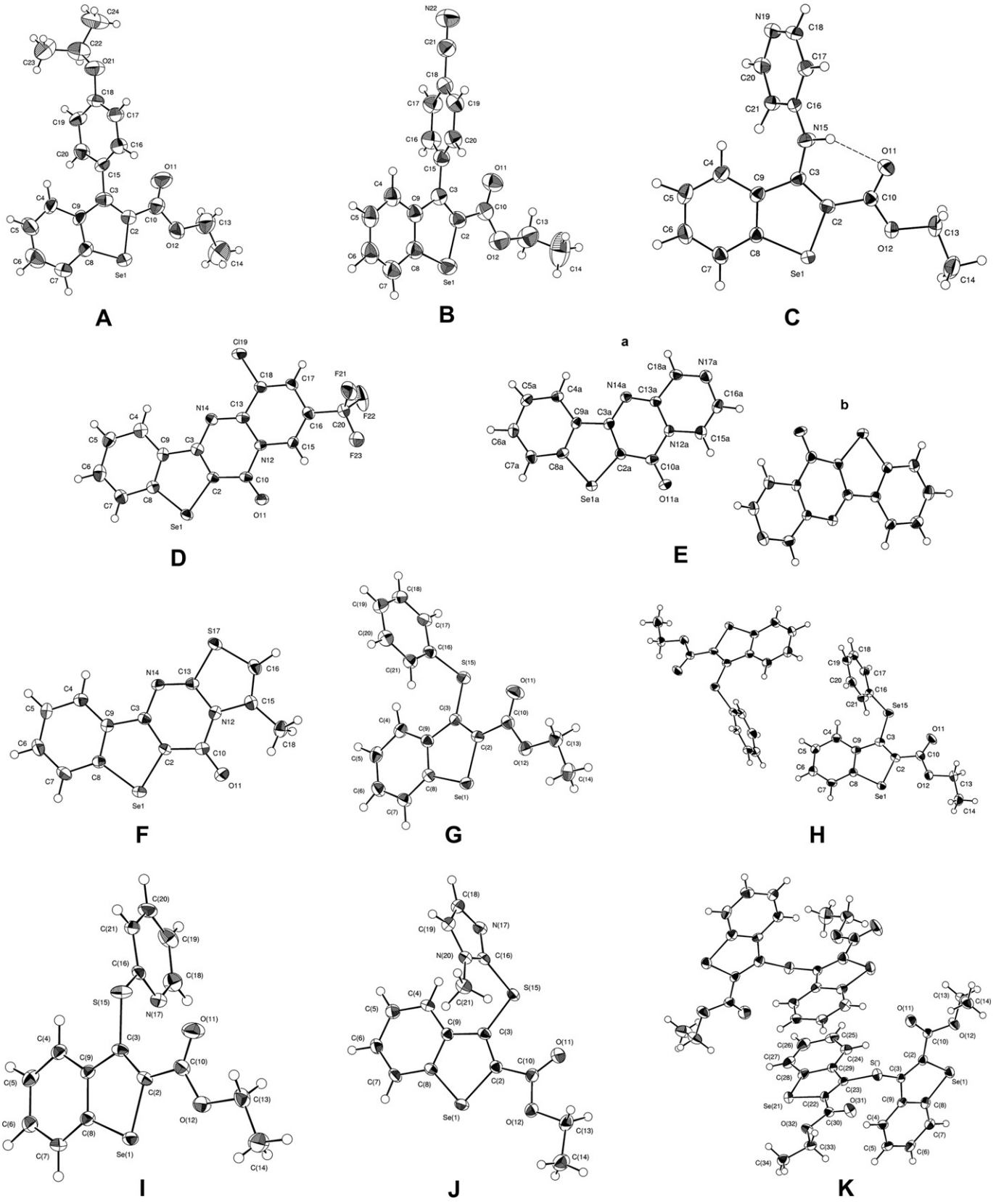


Fig. 1. ORTEP molecular structures of **3** (A), **4** (B), **13** (C), **17** (D), **18** (E), **20** (F), **22** (G), **23** (H), **24** (I), **26** (J), and **28** (K).

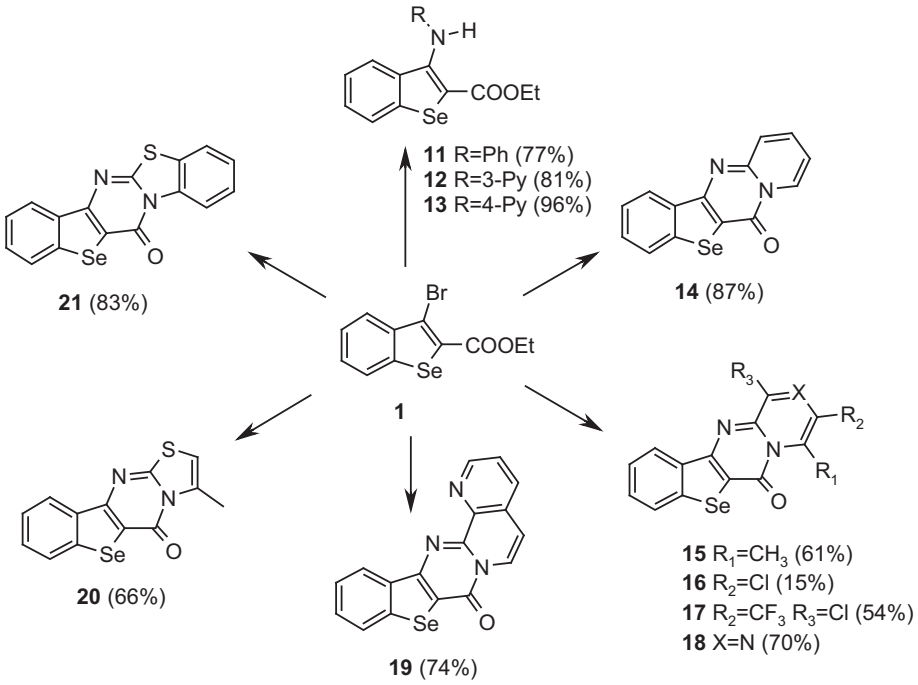
Table 1
Crystal data for the studied compounds.

	3	**4**	**13**	**17**	**18**	**20**	**22**
Brutto-formula	$C_{20}H_{20}O_3Se$	$C_{18}H_{13}NO_2Se$	$C_{16}H_{14}N_2O_2Se$	$C_{15}H_6ClF_3N_2OSe$	$C_{13}H_7N_3OSe$	$C_{13}H_8N_2OSSe$	$C_{17}H_{14}O_2SSe$
Molecular weight	387.32	354.27	345.26	401.63	300.18	319.24	361.32
Crystal system	Monoclinic	Triclinic	Orthorhombic	Tetragonal	Triclinic	Monoclinic	Triclinic
a, [Å]	11.3557(3)	6.4543(3)	7.5384(1)	14.1895(4)	7.4548(2)	7.3309(2)	7.3643(3)
b, [Å]	7.5538(3)	9.7467(4)	19.3170(3)	14.1895(4)	11.1499(4)	12.2720(4)	10.4661(4)
c, [Å]	21.4719(9)	12.8454(7)	20.5094(4)	27.2710(9)	13.9263(5)	13.3016(4)	10.9983(4)
α, [°]	90.00	79.760(2)	90.00	90.00	68.178(2)	90.00	64.099(2)
β, [°]	94.337(1)	78.427(2)	90.00	90.00	82.042(2)	103.607(2)	80.341(2)
γ, [°]	90.00	84.911(3)	90.00	90.00	80.139(2)	90.00	89.727(2)
V, [Å^3]	1836.6(1)	777.86(6)	2986.56(8)	5490.8(2)	1055.19(6)	1163.09(6)	749.49(5)
Space group	$P\,2_1/n$	$P\bar{1}$	*P cab*	$I\,4_1/a$	$P\bar{1}$	$P\,2_1/n$	$P\bar{1}$
Z	4	2	8	16	4	4	2
μ, [mm^{-1}]	2.058	2.420	2.520	2.969	3.550	3.391	2.640
Density (calc.) [g/cm^3]	1.401	1.513	1.536	1.943	1.890	1.823	1.601
$2\theta_{max}$ for data [°]	55.0	56.0	56.0	65.0	56.0	56.0	58.0
Reflection collected	6904	5818	13081	8506	7098	5043	5617
Independent reflections	4200 (R_{int} = 0.076)	3680 (R_{int} = 0.030)	3592(R_{int} = 0.029)	4982(R_{int} = 0.066)	4856(R_{int} = 0.034)	2764(R_{int} = 0.032)	3943(R_{int} = 0.030)
Reflections with $I > n\sigma(I)$	2678 (n = 2)	2891 (n = 3)	2806 (n = 3)	2908 (n = 2)	3332 (n = 3)	2180 (n = 3)	3127 (n = 3)
Final *R*-factor	0.078	0.037	0.028	0.049	0.034	0.061	0.034
*wR*2 index for all data	0.294	0.081	0.180	0.141	0.080	0.201	0.169
Temperature, [K]	293	293	173	173	193	193	173
Using programs	[19,20]	[20,21]	[19,20]	[22]	[19,20]	[20,23]	[20,23]
CCDC deposition number	CCDC 802935	CCDC 802936	CCDC 804882	CCDC 804883	CCDC 804884	CCDC 804885	CCDC 802440

	23	**24**	**26**	**28**
Brutto-formula	$C_{17}H_{14}O_2Se_2$	$C_{16}H_{13}NO_2SSe$	$C_{15}H_{14}N_2O_2SSe$	$C_{22}H_{18}O_4SSe_2$
Molecular weight	408.22	362.31	365.31	536.37
Crystal system	Triclinic	Triclinic	Monoclinic	Triclinic
a, [Å]	7.6940(2)	7.7695(2)	7.4986(1)	10.8102(3)
b, [Å]	10.3688(3)	9.8761(4)	24.4261(5)	11.5709(3)
c, [Å]	19.6867(7)	10.5483(4)	8.9567(2)	17.3054(6)
α, [°]	83.394(1)	76.160(2)	90.00	104.254(1)
β, [°]	79.378(1)	69.384(2)	112.780(1)	100.547(2)
γ, [°]	83.249(2)	77.368(2)	90.00	97.577(1)
V, [Å^3]	1525.91(8)	727.47(4)	1163.09(6)	2026.7(1)
Space group	$P\bar{1}$	$P\bar{1}$	$P\,2_1/a$	$P\bar{1}$
Z	4	2	4	4
μ, [mm^{-1}]	4.847	2.727	2.625	2.640
Density (calc.) [g/cm^3]	1.777	1.654	1.604	1.601
$2\theta_{max}$ for data [°]	57.0	55.0	58.0	55.0
Reflection collected	10342	4866	6716	13720
Independent reflections	7569(R_{int} = 0.031)	3307 (R_{int} = 0.035)	4007(R_{int} = 0.032)	9206(R_{int} = 0.046)
Reflections with $I > n\sigma(I)$	5294 (n = 3)	2385 (n = 3)	3056 (n = 3)	5090 (n = 3)
Final *R*-factor	0.036	0.029	0.031	0.046
*wR*2 index for all data	0.101	0.166	0.148	0.160
Temperature, [K]	153	153	153	183
Using programs	[19,20]	[19,20]	[20,23]	[19,20]
CCDC deposition number	CCDC 805117	CCDC 802441	CCDC 802442	CCDC 802443

selenopheno[3,2-*d*]pyrimidin-10-one **14** was formed in a very good yield (87%).in a very good yield (87%). To improve a method we made the same synthetic protocol using 6-methyl-, 4-chloro-, and 3-chloro-4-trifluoromethyl-2-aminopyridines, the corresponding fused benzo[*b*]selenophenes **15**–**17** were obtained. Notably, reaction of **1** with 2-amino-1,4-pyridazine leads to formation of 10*H*-8-pyrazino-[1,2-*a*]benzo[*b*]selenopheno[3,2-*d*] pyrimidin-10-one (**18**) in 70% yield. Also, fused 8-selena-1,6a,13-triaza-indeno[2,1-*b*]phenanthren-7-one (**19**) was prepared very successfully using 8-aminonaphtyridine-1,7 in impressive yield (74%). It should be noted that current protocol works well also in the case of five member heterocycles. Our attempts to utilize 2-amino-5-methylthiazole and 2-aminobenzothiazole led to the formation of the corresponding 7-methyl-9H-thiazolo[3,2-*a*] benzo[*b*]selenopheno[3,2-*d*]pyrimidin-9-one (**20**) and 9H-benzthiazolo[3,2-*a*]benzo[*b*]selenopheno[3,2-*d*]pyrimidin-9-one (**21**) in good yields (66% and 83%, correspondingly). Molecular structures of the compounds **17**, **18**, and **20** are given in Fig. 1D, E and F. The condensed heterocyclic system in molecules **17**, **18**, and **20** is characterized by the planar conformation. The crystal structure of these compounds is totally different. There is intermolecular stacking interaction between molecules in the crystal structure **20**; the distance between the centroid of phenyl ring C4, C5, C6, C7, C8, C9 and hydrogen atom H18A of methylgroup is 2.50 Å. By means of these interactions the centrosymmetrical dimers (space group $P\,2_1/n$) are formed in the crystal structure. In the crystal structure **18** (space group $P\bar{1}$) there are two independent molecules (**a** and **b**) in asymmetric unit; the intermolecular contact C7a···O11b [3.300(3) Å] can describe as a weak hydrogen bond of C···O type (H7a···O11b = 2.56 Å, C7a–H7a···O11b = 134°). In the high symmetrical (space group $I\,4_1/a$) crystal structure of **17** there is shortened contact (2.922(4) Å) between fluorine atoms F23 of the centrosymmetrically connected molecules.

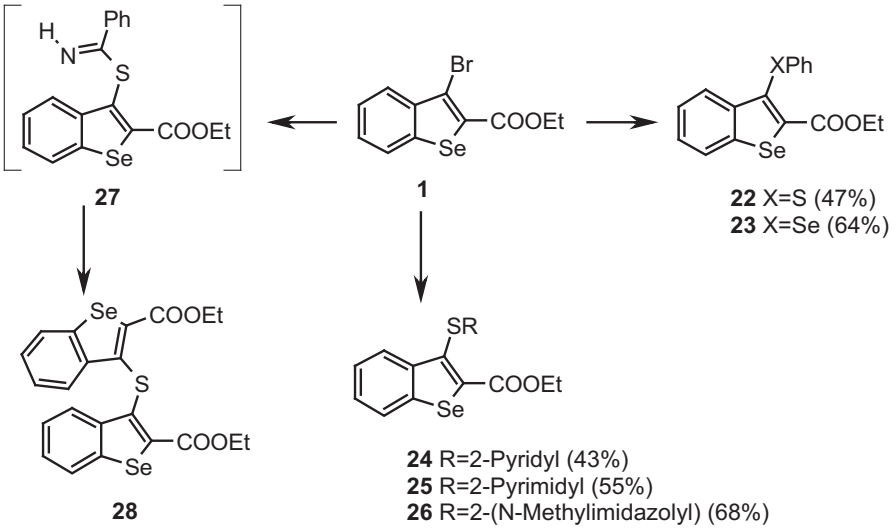
For the next step of our investigation we have focused on incorporation of sulfur and selenium substituent in position 3 of benzo[*b*]selenophene **1** ring (Scheme 3). As a model we chosen thiophenol and optimized reaction conditions. Unfortunately, according to experimental data well known copper iodide mediated



Scheme 2. Synthesis of benzo[*b*]selenophenes **11**–**21**. Reagents and reaction conditions: arylamine (1.3 eq.), Pd_2dba_3 (5%), xantphos (10%), Cs_2CO_3 (2.7 eq.), xylene, 120 °C, 20 h.

cross coupling leads to the formation of 3-phenylthiobenzo[*b*]selenophene-2-carboxylic acid ethyl ester (**22**) in a very poor yield. Better result was obtained using 2,2,6,6-tetramethylheptane-3,5-dione as a ligand (21%). However, it has been found that interaction of thiophenol with **1** in dry acetonitrile in the presence of freshly prepared potassium fluoride on Al_2O_3 and 18-crown-6 proceeds in moderate yield (47%) after overnight heating at 80 °C. The general advantage of this copper free method is an experimental simplicity and low price of a catalyst. 3-Phenylselenylbenzo[*b*]selenophene-2-carboxylic acid ethyl ester (**23**) was prepared under the same reaction conditions from selenolophenol in even better yield (64%) than corresponding sulfur analog **22**. The Fig. 1G and H illustrate the molecular structures of **22** and **23**. The geometrical parameters in the molecule **22** are near to corresponding values in **23**. In **22** the C3—S15—C16 valence angle value is 104.0(1)°, in **23** the mean value of C3—Se15—C16 angle is equal 100.3(1)°. However, the crystal structures of **22** and **23** are not isomorphous: for **22** there is one molecule in the asymmetric unit, while for **23** there are two molecules connected by pseudoinversion centre.

Heterocyclic thiols also interact with **1** in the presence of KF/Al_2O_3. We have been very satisfied when 3-(2-pyridylthio)benzo[*b*]selenophene-2-carboxylic acid ethyl ester (**24**) was successfully isolated from the reaction of **1** with 2-thiopyridine in 43% yield under the same synthetic protocol. Besides, 3-(2-pyrimidylthio)benzo[*b*]selenophene-2-carboxylic acid ethyl ester (**25**) and 3-[2-(*N*-methyl)imidazolylthio]benzo[*b*]selenophene-2-carboxylic acid



Scheme 3. Synthesis of benzo[*b*]selenophenes **22**–**28**. Reagents and reaction conditions: thiol, thioamide or selenol (1.3 eq.), KF/Al_2O_3 (3 eq.), 18-crown-6 (30%), MeCN, 80 °C, 20 h.

ethyl ester (**26**) were obtained in even higher yields (55% and 68%, correspondingly). Molecular structures of the compounds **24** and **26** are shown in Fig. 1I and J. These molecules considerably differ by conformations. The molecular conformation of these molecule can be characterized by the torsion angle of C3–S15–C16–N17; this angle is equal −18.0(3)° for **24** and 126.4(2)° for **24**. The crystal structure of **24** is isomorphous to one of **22**.

Finally, we tried to incorporate thiobenzamide moiety in the position 3 of benzo[*b*]selenophene **1** ring. After overnight heating of **1** with thiobenzamide in acetonitrile in the presence of potassium fluoride on Al_2O_3 diethyl 3,3′-thiodibenzo[*b*]selenophene-2-carboxylate (**28**) was isolated from the reaction mixture as a sole product. Seems, that the introduction of thiobenzamide in the position 3 of **1** proceeds very slowly, but interaction of unreacted **1** with intermediate **27** goes much faster; due to benzimido fragment could be a good leaving group under the reaction conditions. As a result, symmetrical sulfide **28** was obtained in 42% yield. The Fig. 1K shows the molecular structures of **28**. In this structure there are two independent molecules in the asymmetric unit; these molecules are connected by centre of pseudoinversion. The mean value of C3–S1–C23 valence angle is 105.1(1)°.

2.2. In vitro

The results of these experiments are summarized in Table 2. The initial 3-bromobenzo[*b*]selenophene **1** exhibits no cytotoxic activity on HT-1080 and slight activity on mouse hepatoma MG-22A tumor cell line. In a series of 3-*C*-substituted benzo[*b*]selenophenes **2**–**10** 3-(5-methylthienyl)benzo[*b*]selenophene (**7**) and 3-ethynyl derivatives **9** and **10** show medium *in vitro* activity on tumor cell lines. Analog **7** containing thiophene cycle shows the highest cytotoxicity (IC_{50} = 16 μg/mL on HT-1080 and IC_{50} = 19.5 μg/mL on MG-22A). According to our experiments polycyclic derivatives **14**–**21** exhibit more extended activity against HT-1080 and MG-22A tumor cell lines. Possibly, it stems from the fact that their structure recalls known antitumoral agent Batracylin [25,26]. In fact, 10*H*-pyrido[1,2-*a*]benzo[*b*]selenopheno[3,2-d]pyrimidin-10-one (**14**) as well as 4-chloro- (**16**) and 3-chloro-4-trifluoromethyl-2-aminopyridine (**17**) show a very good cytotoxic effect on tumor cell lines (IC_{50} = 2.75 ÷ 3.5 μg/mL on HT-1080 and MG-22A), however, insertion of the additional nitrogen atom in the molecule (compound **18**) leads to the complete activity disappearance. It should be noted that in the same concentrations benzo[*b*]selenophenes **14**–**17** selectively acts against tumor and normal mouse fibroblast (3T3) cells. Inspection of LD_{50} data shows that all derivatives studied exhibit medium or low acute toxicity (362–2216 mg/kg). The role of NO in biosystems has attracted considerable attention in the last decade. NO is formed by enzymatic and non-enzymatic mechanisms. Because of its molecular weight and high lipophilicity, NO has good diffusion properties. It may act not only in the cell where it is produced, but also in nearby tissues. Biologically produced NO originates from oxygen and L-arginine in the reaction catalyzed by NO synthase. NO, a long-lived radical with a wide range of actions, is known as a regulator of a variety of biological processes [27]. The NO level was determined according [28], NO release was defined using the Greyss reagent (by NO_2 concentration in the cultural medium). The yield of nitrite was expressed as NO_2 nmol/200 μL of cultural medium in testing plates for 100% alive cells after CV coloration assay (benzo[*b*]selenophenes concentration 100 μg/mL). It was shown (Table 2) that 3-(5-methylthienyl)- **7** and 3-(cyclohexen-1-yl) benzo[*b*]selenophene **10** readily increase NO concentration in the cultural medium (TG_{100} = 200–350%) on HT-1080 and MG-22A cell lines. However, series of cytotoxic 3-*N*-polycyclic benzo[*b*]selenophenes **14**–**17** and **21** exhibit very strong NO radical protector activity on both tumor cell lines (TG_{100} = 2–40%).

Table 2
In Vitro cytotoxicity in monolayer tumor cell lines [HT-1080 (human fibrosarcoma), MG-22A (mice hepatoma), NIH 3T3 (normal mouse fibroblasts) caused by benzo[*b*]selenophenes[a].

Compound	HT-TD_{50}[a]	1080 NO 100%[b]	MG-TD_{50}	22A NO 100%	NIH 3T3 TD_{50}	LD_{50}, mg/kg
1	[c]	14	40.5	40	896	2158
2	65	25	[c]	23	16	362
3	[c]	9	[c]	15	[c]	>2000
4	[c]	8	[c]	7	684	1984
5	[c]	6	81.5	12	100	881
6	[c]	13	[c]	14	31	503
7	16	350	19.5	350	15	349
9	76	15	87	10	614	1872
10	32.5	200	32	200	120	858
12	[c]	12	[c]	9	1000	2313
14	3	5	3	7	31	470
16	3	3	3	40	32	500
17	3.5	2	2.75	8	31	554
18	[c]	2	[c]	7	27	444
19	[c]	20	[c]	3	56	665
20	[c]	2	[c]	4	21	412
21	8.5	2	9.5	3	27	487
25	[c]	8	[c]	7	840	2216

[a] TD_{50} - Concentration (μg/mL) providing 50% cell killing effect [(CV + MTT)/2].
[b] NO Concentration (%) (CV: coloration).
[c] no cytotoxic effect.

2.3. Morphology

The influence of the studied benzo[*b*]selenophenes on the phenotype of mouse fibroblasts 3T3 and human fibrosarcoma HT-1080 was examined (Fig. 2). Figures in the table show the morphological changes after 72 h at 30 °C (visualization by acridine orange). Our experimental data shows that morphology of benzo[*b*]selenophenes on 3T3 cells correlates with cytotoxic data on 3T3 cell line. Fig. 2A and B shows the morphological structure of HT-1080 and 3T3 cells, correspondingly (control). The majority of cells were alive and the cell nucleus was a dark color. The inspection of benzo[*b*]selenophenes influence on 3T3 cell line morphology let us conclude that cytotoxic compounds **14** and **16** induce a slight apoptosis of normal cells (Fig. 2D and F). Besides, derivatives **14** and **16** changed the HT-1080 cell phenotype and powerful cell apoptosis with cell nucleus fragmentation was occurred (Fig. 2C and E). The insertion of trifluoromethyl substituent into molecule (**17**) significantly changed the type of action (Fig. 2G), tumor cells undergoing mitochondrial apoptosis with the change of HT-1080 cell phenotype. Surprisingly, benzothiazole fragment containing derivative **21** evoke gentle apoptosis without the change of cell morphology (Fig. 2I).

3. Experimental section

3.1. General

^1H, ^{13}C and ^{77}Se NMR spectra were recorded on a Varian 400 Mercury spectrometer at 400, 100.3 and 39.74 MHz correspondingly at 303 K in $CDCl_3$/TMS or DMSO-d_6 solution. The ^1H chemical shifts are given relative to TMS, ^{13}C – relative to chloroform or DMSO, and ^{77}Se – relative to dimethyl selenide. The melting points were determined on a “Digital melting point analyser” (Fisher), the results are given without correction. Diffraction data were collected on a Nonius KappaCCD diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). The crystal structures were solved by direct methods and refined by full-matrix least

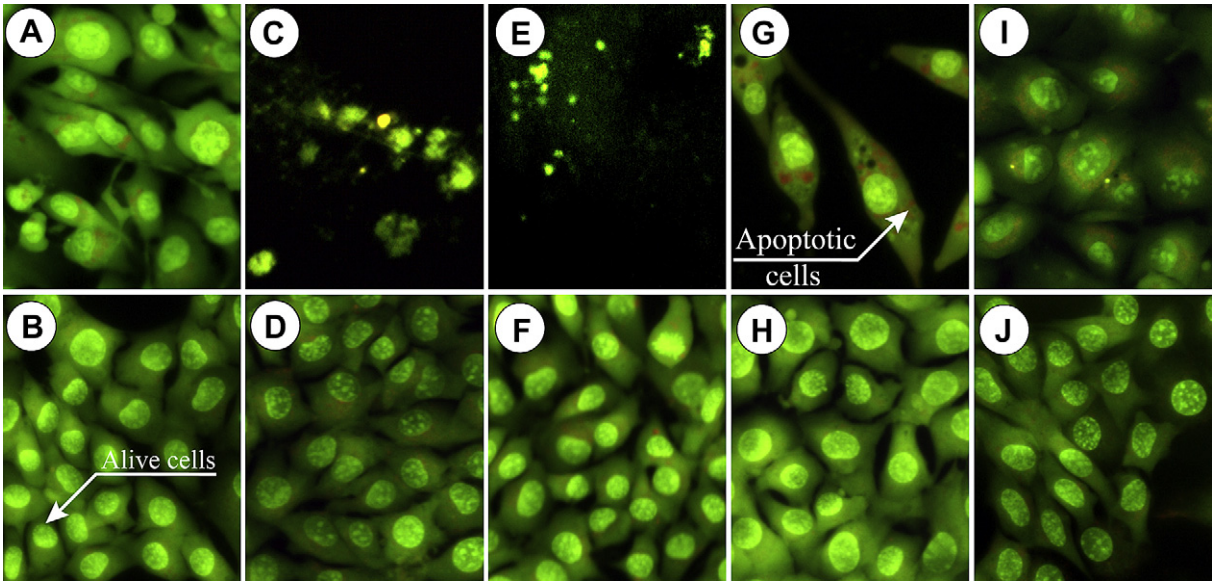


Fig. 2. View of HT-1080 (A) and 3T3 cells (B) visualized by staining the cellular DNA with the dye acridine orange. Living cells exhibit a green color, cells with orange or yellow color are apoptotic; (C) view of HT-1080 with **14**; (D) view of 3T3 with **14**; (E) view of HT-1080 with **16**; (F) view of 3T3 with **16**; (G) view of HT-1080 with **17**; (H) view of 3T3 with **17**; (I) view of HT-1080 with **21**; (J) view of 3T3 with **21**.

squares. The main crystallographic data and refinement parameters of the crystal structures are listed in Table 1. For further details, see crystallographic data for these compounds deposited with the Cambridge Crystallographic Data Centre as Supplementary Publications. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

3.2. General procedure for the preparation of benzo[b]selenophenes **2**–**6**

A vial charged with **1** (0.25 g, 0.753 mmol), palladium(II) acetate (17 mg, 0.0753 mmol), tri(*o*-tolyl)phosphine (69 mg, 0.226 mmol) and potassium phosphate (543 mg, 2.56 mmol) in 5 mL of xylene under argon atmosphere was stirred for 10 min at 40 °C. Then the corresponding aryl(hetaryl)boronic acid (1.28 mmol) in abs. ethanol (0.5 mL) was added and the resulting mixture was heated at 110 °C for 1–6 h. After usual workup crude product was purified by flash chromatography on silica gel using the mixture petroleum ether – ethylacetate as eluent.

3.2.1. 3-Phenylbenzo[b]selenophene-2-carboxylic acid ethyl ester (**2**)

Yield: 65%, mp = 68–69 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.16 (3H, t, CH_3, J = 7.6 Hz); 4.19 (2H, q, CH_2, J = 7.6 Hz); 7.28–7.52 (8H, m, 8 × ArH); 7.90–7.96 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 13.9; 61.2; 124.9; 125.5; 127.1; 127.6; 127.8; 128.0; 129.3; 131.7; 136.3; 142.0; 142.9; 147.0; 163.7. ^{77}Se NMR (39.74 MHz, $CDCl_3$), δ (ppm): 544.65. Anal. ($C_{17}H_{14}O_2Se$) C, H.

3.2.2. 3-(4-Isopropoxyphenyl)benzo[b]selenophene-2-carboxylic acid ethyl ester (**3**)

Yield: 97%, mp = 125–126 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.18 (3H, t, CH_3, J = 6.8 Hz); 1.38 (6H, d, 2 × CH_3, J = 6.0 Hz); 4.18 (2H, q, CH_2, J = 6.8 Hz); 4.61 (1H, sept, CH, J = 6.0 Hz); 6.93–7.00 (2H, m, 2 × ArH); 7.22–7.27 (2H, m, 2 × ArH); 7.28–7.34 (1H, m, ArH); 7.35–7.41 (1H, m, ArH); 7.47–7.51 (1H, m, ArH); 7.86–7.91 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.0; 22.1; 61.2; 69.8; 115.1; 124.8; 125.5; 127.0; 127.7; 127.9; 130.7; 131.2; 141.9; 143.1; 147.0; 157.7; 163.8. Anal. ($C_{20}H_{20}O_3Se$) C, H.

3.2.3. 3-(4-Cyanophenyl)benzo[b]selenophene-2-carboxylic acid ethyl ester (**4**)

Yield: 76%, mp = 147–148 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.20 (3H, t, CH_3, J = 7.2 Hz); 4.20 (2H, q, CH_2, J = 7.2 Hz); 7.31–7.52 (5H, m, 5 × ArH); 7.75–7.82 (2H, m, 2 × ArH); 7.92–7.99 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 13.9; 61.6; 111.7; 118.7; 125.2; 125.7; 126.8; 127.4; 130.3; 131.7; 132.5; 141.4; 141.9; 142.1; 144.5; 163.1. ^{77}Se NMR (39.74 MHz, $CDCl_3$), δ (ppm): 555.17. Anal. ($C_{18}H_{13}NO_2Se$) C, H, N.

3.2.4. 3-(3,4,5-Trifluorophenyl)benzo[b]selenophene-2-carboxylic acid ethyl ester (**5**)

Yield: 89%, mp = 113–115 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.25 (3H, t, CH_3, J = 7.0 Hz); 4.24 (2H, q, CH_2, J = 7.0 Hz); 6.91–7.08 (2H, m, 2 × ArH); 7.33–7.51 (3H, m, 3 × ArH); 7.91–7.99 (1H, m, ArH). Anal. ($C_{17}H_{11}F_3O_2Se$) C, H.

3.2.5. 3-(Thien-3-yl)benzo[b]selenophene-2-carboxylic acid ethyl ester (**6**)

Yield: 79%, mp = 74–76 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.21 (3H, t, CH_3, J = 7.2 Hz); 4.21 (2H, q, CH_2, J = 7.2 Hz); 7.14 (1H, dd, ArH, J = 0.8 Hz, J = 4.8 Hz); 7.31–7.43 (4H, m, 4 × ArH); 7.55–7.61 (1H, m, ArH), 7.89 (1H, d, ArH, J = 8.0 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.0; 61.3; 124.4; 124.5; 125.0; 125.5; 127.2; 127.5; 129.2; 132.1; 135.4; 141.7; 141.8; 142.8; 163.6. ^{77}Se NMR (39.74 MHz, $CDCl_3$), δ (ppm): 547.23. Anal. ($C_{15}H_{12}O_2SSe$) C, H.

3.3. General procedure for the preparation of benzo[b]selenophenes **7**–**10** (Method B)

A vial charged with **1** (0.25 g, 0.753 mmol), tetrakis(-triphenylphosphino)palladium(0) (35 mg, 0.03 mmol), triphenylarsine (16 mg, 0.053 mmol) and corresponding stannane (1.05 mmol) in 5 mL of xylene under argon atmosphere was stirred for 2–24 h at 110° C. After usual workup crude product was purified by flash chromatography on silica gel using the mixture petroleum ether – ethylacetate as eluent.

*3.3.1. 3-(5-Methylthien-2-yl)-benzo[b]selenophene-2-carboxylic acid ethyl ester (**7**)*

Yield: 79%, mp = 90–91 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.27 (3H, t, CH_3, J = 7.2 Hz); 2.56 (3H, d, CH_3, J = 0.8 Hz); 4.27 (2H, q, CH_2, J = 7.2 Hz); 6.81–6.84 (1H, m, ArH); 6.91 (1H, d, ArH, J = 3.6 Hz); 7.35–7.44 (2H, m, ArH); 7.72–7.75 (1H, m, ArH); 7.88–7.91 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.0; 15.4; 61.4; 125.0; 125.3; 125.4; 125.5; 127.2; 127.6; 128.2; 133.0; 133.5; 139.4; 141.0; 141.4; 142.9; 163.3. ^{77}Se NMR (39.74 MHz, $CDCl_3$), δ (ppm): 551.74. Anal. ($C_{16}H_{14}O_2SSe$) C, H.

*3.3.2. 3-(2-Pyridyl)-benzo[b]selenophene-2-carboxylic acid ethyl ester hydrochloride (**8**)*

Free base was converted to hydrochloride salt after purification using HCl solution in dry methanol. Yield: 75%, mp = 156–157 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.07 (3H, t, CH_3, J = 7.2 Hz); 4.14 (2H, q, CH_2, J = 7.2 Hz), 7.47 (2H, d, 2 × ArH, J = 3.6 Hz); 7.52–7.60 (1H, m, ArH); 7.90–8.01 (2H, m, 2 × ArH); 8.29 (1H, d, ArH, J = 8.0 Hz); 8.39–8.50 (1H, m, ArH); 8.94 (1H, d, ArH, J = 4.8 Hz). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 13.6; 61.6; 125.2; 125.8; 126.4; 126.5; 127.2; 127.7; 140.7; 141.7; 162.3. Anal. ($C_{16}H_{14}ClNO_2Se$) C, H, N.

*3.3.3. 3-Phenylethynylbenzo[b]selenophene-2-carboxylic acid ethyl ester (**9**)*

Yield: 65%, mp = 68–70 C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.45 (3H, t, CH_3, J = 7.2 Hz); 4.45 (2H, q, CH_2, J = 7.2 Hz); 7.38–7.56 (5H, m, 5 × ArH); 7.66–7.73 (2H, m, 2 × ArH); 7.87–7.91 (1H, m, ArH); 8.17–8.21 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.4; 61.7; 84.4; 97.7; 122.9; 125.4; 125.6; 126.4; 127.0; 127.6; 128.4; 128.9; 131.9; 137.7; 141.2; 142.0; 163.2. Anal. ($C_{19}H_{14}O_2Se$) C, H.

*3.3.4. 3-(Cyclohex-1-enylethynyl)benzo[b]selenophene-2-carboxylic acid ethyl ester (**10**)*

Yield: 41%, mp = 54–55 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.42 (3H, t, CH_3, J = 7.2 Hz); 1.62–1.78 (4H, m, 2 × CH_2); 2.16–2.24 (2H, m, CH_2); 2.34–2.40 (2H, m, CH_2); 4.41 (2H, q, CH_2, J = 7.2 Hz); 6.40–6.47 (1H, m, CH); 7.38–7.55 (2H, m, ArH); 7.82–7.89 (1H, m, ArH); 8.04–8.10 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.4; 21.5; 22.3; 26.0; 29.1; 61.6; 82.0; 100.1; 120.8; 125.2; 125.5; 127.0; 127.1; 127.5; 136.6; 137.1; 141.1; 142.1; 163.3. Anal. ($C_{19}H_{18}O_2Se$) C, H.

*3.4. General procedure for the preparation of benzo[b]selenophenes **9**–**10** (Method C)*

A vial charged with palladium(II) chloride (7 mg, 0.037 mmol), triphenylphosphine (20 mg, 0.075 mmol) and copper(I) iodide (14 mg, 0.075 mmol) in 5 mL of DMF was barbotated with Ar for 15 min at 40 °C. Then **1** (0.25 g, 0.753 mmol), terminal acethylene (1.1 mmol), and triethylamine (1 mL) was added to the reaction mixture. After 20 h of heating at 100° C and usual workup crude product was purified by flash chromatography on silica gel using the mixture petroleum ether – ethylacetate as eluent.

*3.4.1. 3-Phenylethynylbenzo[b]selenophene-2-carboxylic acid ethyl ester (**9**)*

Yield: 28%.

*3.4.2. 3-(Cyclohex-1-enylethynyl)benzo[b]selenophene-2-carboxylic acid ethyl ester (**10**)*

Yield: 67%.

*3.5. General procedure for the preparation of benzo[b]selenophenes **11**–**21***

A vial charged with **1** (0.25 g, 0.753 mmol), Pd_2dba_3 (34 mg, 0.037 mmol), xantphos (39 mg, 0.075 mmol) and arylamine (0.979 mmol) in 5 mL of xylene was barbotated with Ar for 15 min. Then cesium carbonate (0.663 g, 2.03 mmol) was added and reaction mixture was heated for 20 h at 120° C. After usual workup crude product was purified by flash chromatography on silica gel using the mixture petroleum ether – ethylacetate as eluent.

*3.5.1. 3-Phenylaminobenzo[b]selenophene-2-carboxylic acid ethyl ester (**11**)*

Yield: 77%, mp = 63–64 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.37 (3H, t, CH_3, J = 6.8 Hz); 4.33 (2H, q, CH_2, J = 6.8 Hz); 6.97–7.06 (3H, m, 3 × ArH); 7.11–7.17 (1H, m, ArH); 7.20–7.26 (2H, m, 2 × ArH); 7.30–7.36 (1H, m, ArH); 7.41–7.45 (1H, m, ArH); 7.77–7.82 (1H, m, ArH); 8.79 (1H, br s, NH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.4; 61.0; 108.3; 121.2; 123.0; 123.8; 126.3; 127.5; 127.6; 129.0; 135.1; 140.2; 143.0; 148.3; 166.4. Anal. ($C_{17}H_{15}NO_2Se$) C, H, N.

*3.5.2. 3-(Pyrid-3-ylamino)benzo[b]selenophene-2-carboxylic acid ethyl ester (**12**)*

Yield: 81%, mp = 174–175 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.39 (3H, t, CH_3, J = 7.2 Hz); 4.36 (2H, q, CH_2, J = 7.2 Hz); 7.15 (1H, dd, ArH, J = 4.4 Hz, J = 8.4 Hz); 7.18–7.23 (2H, m, 2 × ArH); 7.36–7.40 (2H, m, 2 × ArH); 7.84 (1H, dt, ArH, J = 1.4 Hz, J = 8.4 Hz); 8.29 (1H, dd, ArH, J = 1.4 Hz, J = 4.8 Hz); 8.38 (1H, d, ArH, J = 2.8 Hz); 8.72 (1H, br s, NH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.3; 61.3; 110.9; 123.4; 124.3; 126.5; 126.9; 127.0; 124.8; 127.9; 134.6; 139.6; 140.2; 142.5; 143.8; 146.7; 166.2. ^{77}Se NMR (39.74 MHz, $CDCl_3$), δ (ppm): 474.42. Anal. ($C_{16}H_{14}N_2O_2Se$) C, H, N.

*3.5.3. 3-(Pyrid-4-ylamino)benzo[b]selenophene-2-carboxylic acid ethyl ester (**13**)*

Yield: 96%, mp = 136–138 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.37 (3H, t, CH_3, J = 7.2 Hz); 4.34 (2H, q, CH_2, J = 7.2 Hz); 6.73 (2H, d, ArH, J = 5.2 Hz); 7.27–7.32 (1H, m, ArH); 7.39–7.44 (1H, m, ArH); 7.58 (1H, d, ArH, J = 4.0 Hz); 7.86 (1H, d, ArH, J = 4.0 Hz); 8.31 (1H, d, ArH, J = 5.2 Hz); 8.40 (1H, br s, NH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.3; 31.5; 112.6; 115.9; 124.5; 126.4; 127.0; 127.9; 135.3; 140.0; 144.2; 150.0; 150.3; 165.5. ESI-MS: 347.3. Anal. ($C_{16}H_{14}N_2O_2Se$) C, H, N.

*3.5.4. 11-Selena-5,9a-diazabenzo[b]fluoren-10-one (**14**)*

Yield: 87%, mp = 222–223 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 7.14–7.15 (1H, m, ArH), 7.55–7.57 (2H, m, ArH), 7.67–7.68 (1H, m, ArH), 7.69–7.78 (1H, m, ArH), 7.95–7.97 (1H, m, ArH), 8.46–8.49 (1H, m, ArH), 9.08–9.10 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 114.5; 125.3; 126.1; 126.4; 126.5; 129.8; 134.4; 137.0; 142.0; 149.6; 155.7; 156.9. ESI-MS: 300.9. Anal. ($C_{14}H_8N_2OSe$) C, H, N.

*3.5.5. 9-Methyl-11-selena-5,9a-diazabenzo[b]fluoren-10-one (**15**)*

Yield: 61%, mp = 203–205 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 3.13 (3H, s, CH_3); 6.62–6.63 (1H, m, ArH); 7.36–7.38 (1H, m, ArH); 7.48–7.53 (3H, m, ArH); 7.88–7.91 (1H, m, ArH); 8.35–8.38 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 25.1; 117.6; 125.2; 125.4; 125.9; 126.4; 129.5; 133.6; 136.9; 142.0; 143.1; 152.3; 155.5; 159.5. ESI-MS: 315.2. Anal. ($C_{15}H_{10}N_2OSe$) C, H, N.

*3.5.6. 11-Selena-5,9a-diaza-8-chlorobenzo[b]fluoren-10-one (**16**)*

Yield: 17%, mp>230 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 7.55–7.67 (3H, m, 3 × ArH), 7.72–7.76 (1H, m, ArH),

7.95–8.01 (1H, m, ArH), 8.45–8.51 (1H, m, ArH), 9.11–9.14 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 123.2; 124.2; 125.5; 126.2; 126.4; 127.4; 130.0; 135.8; 136.8; 142.1; 147.9; 154.8; 156.7. **ESI-MS**: **335.0**.

*3.5.7. 6-Chloro-8-trifluoromethyl-11-Selena-5,9a-diazabenzo[b] fluoren-10-one (**17**)*

Yield: 54%, mp = 215–217 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 7.56–7.60 (2H, m, ArH), 7.88 (1H, d, J = 2, 0 Hz, ArH), 7.94–7.97 (1H, m, ArH), 8.52–8.55 (1H, m, ArH), 9.30–9.31 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 116.9; 117.3; 117.4; 121.0; 123.7; 124.9; 125.7; 126.3; 126.7; 128.3; 130.4; 132.4; 136.6; 142.5; 146.0; 155.3; 156.2. ESI-MS: 403.0. Anal. ($C_{15}H_6ClF_3N_2OSe$) C, H, N.

*3.5.8. 11-Selena-5,7,9a-triazabenzo[b]fluoren-10-one (**18**)*

Yield: 70%, mp>230 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 7.61–7.65 (2H, m, ArH), 7.99–8.03 (1H, m, ArH), 8.13 (1H, d, J = 9.2 Hz, ArH), 8.53–8.57 (1H, m, ArH), 8.75 (1H, d, J = 12,4 Hz, ArH), 9.25 (1H, s, ArH). ESI-MS: 302.0. Anal. ($C_{13}H_7N_3OSe$) C, H, N.

*3.5.9. 8-Selena-1,6a,13-triaza-indeno [2,1-b]phenanthren-7-one (**19**)*

Yield: 74%, mp>230 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 7.28 (1H, d, J = 7.6 Hz, ArH), 7.57–7.64 (2H, m, 2 × ArH), 7.76 (1H, dd, J_1 = 4.4 Hz, J_2 = 8.0 Hz, ArH), 7.97–8.03 (1H, m, ArH), 8.13 (1H, dd, J_1 = 1.6 Hz, J_2 = 8.0 Hz, ArH), 8.80–8.86 (1H, m, ArH), 8.97 (1H, d, J = 8.0 Hz, ArH), 9.20 (1H, dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, ArH). ESI-MS: 352.0. Anal. ($C_{17}H_9N_3OSe$) C, H, N.

*3.5.10. 7-Methyl-9H-thiazolo [3,2-a]benzo[b]selenopheno [3,2-d] pyrimidin-9-one (**20**)*

Yield: 66%, mp>230 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 2.90 (3H, d, J = 1.2 Hz, CH_3), 6.46 (1H, d, J = 1.2 Hz, ArH), 7.49–7.56 (2H, m, 2 × ArH), 7.89–7.95 (1H, m, ArH), 8.30–8.36 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 18.7; 105.5; 125.3; 125.8; 126.4; 129.3; 135.8; 136.3; 141.9; 155.4; 159.0; 162.6. ESI-MS: 320.9. Anal. ($C_{13}H_8N_2OSSe$) C, H, N.

*3.5.11. 9H-Benzthiazolo [3,2-a]benzo[b]selenopheno [3,2-d] pyrimidin-9-one (**21**)*

Yield: 83%, mp>230 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 7.49–7.60 (4H, m, 4 × ArH), 7.70–7.75 (1H, m, ArH), 7.94–7.99 (1H, m, ArH), 8.37–8.43 (1H, m, ArH), 9.14–9.19 (1H, m, ArH). ESI-MS: 357.1. Anal. ($C_{16}H_8N_2OSSe$) C, H, N.

*3.6. General procedure for the preparation of benzo[b]selenophenes **22**–**28***

A vial charged with **1** (0.25 g, 0.753 mmol), KF/Al_2O_3 (0.7 g) thiol (1.1 mmol), and 18-crown-6 (0.05 g, 0.225 mmol) in 5 mL of acetonitrile was heated for 20 h at 80 °C. Then solvent was evaporated under reduced pressure and crude product was purified by flash chromatography on silica gel using the mixture petroleum ether – ethylacetate as eluent.

*3.6.1. 3-Phenylthiobenzo[b]selenophene-2-carboxylic acid ethyl ester (**22**)*

Yield: 47%, mp = 71–72 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.32 (3H, t, CH_3, J = 7.2 Hz); 4.33 (2H, q, CH_2, J = 7.2 Hz); 7.06–7.18 (5H, m, 8 × ArH); 7.32–7.7.42 (2H, m, ArH); 7.88–7.89 (1H, m, ArH); 7.96–7.99 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.2; 61.9; 125.3; 125.6; 125.7; 127.4; 127.6; 127.7; 128.9; 132.7; 136.7; 139.2; 141.4; 142.3; 162.8. Anal. ($C_{17}H_{14}O_2SSe$) C, H.

*3.6.2. 3-Phenylselenobenzo[b]selenophene-2-carboxylic acid ethyl ester (**23**)*

Yield: 64%, mp = 60–61 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.35 (3H, t, CH_3, J = 7.2 Hz); 4.36 (2H, q, CH_2, J = 7.2 Hz); 7.10–7.18 (3H, m, 3 × ArH); 7.25–7.31 (3H, m, 3 × ArH); 7.32–7.38 (1H, m, ArH); 7.84–7.89 (1H, m, ArH); 7.91–7.96 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.2; 61.9; 125.2; 125.5; 126.6; 127.3; 129.0; 129.2; 129.3; 130.8; 132.1; 137.2; 142.1; 143.0; 163.2. Anal. ($C_{17}H_{14}O_2Se_2$) C, H.

*3.6.3. 3-(Pyrid-2-ylthio)benzo[b]selenophene-2-carboxylic acid ethyl ester (**24**)*

Yield: 43%, mp = 124–125 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.28 (3H, t, CH_3, J = 7.2 Hz); 4.31 (2H, q, CH_2, J = 7.2 Hz); 6.76–6.80 (1H, m, ArH); 6.92–6.98 (1H, m, ArH); 7.33–7.46 (3H, m, 3 × ArH); 7.89–7.94 (1H, m, ArH); 7.99–8.04 (1H, m, ArH); 8.34–8.38 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.1; 61.9; 119.9; 121.1; 125.5; 125.6; 127.5; 127.6; 130.3; 136.6; 140.5; 141.5; 142.4; 149.5; 159.8; 162.8. Anal. ($C_{17}H_{13}NO_2SSe$) C, H, N.

*3.6.4. 3-(2-Pyrimidylthio)benzo[b]selenophene-2-carboxylic acid ethyl ester (**25**)*

Yield: 55%, mp = 95–97 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.28 (3H, t, CH_3, J = 7.2 Hz); 4.30 (2H, q, CH_2, J = 7.2 Hz); 6.95 (1H, m, ArH); 7.36–7.51 (2H, m, 2 × ArH); 7.89–7.98 (1H, m, ArH); 8.02–8.11 (1H, m, ArH); 8.43 (2H, d, 2 × ArH, J = 4.6 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.1; 61.7; 117.0; 125.3; 125.6; 127.0; 127.4; 129.1; 140.2; 141.3; 142.5; 157.4; 162.7; 171.5. ^{77}Se NMR (39.74 MHz, $CDCl_3$), δ (ppm): 588.14. Anal. ($C_{15}H_{12}N_2O_2SSe$) C, H, N.

*3.6.5. 3-[2-(N-Methylimidazolylthio)]benzo[b]selenophene-2-carboxylic acid ethyl ester (**26**)*

Yield: 68%, mp = 105–106 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.41 (3H, t, CH_3, J = 7.6 Hz); 3.56 (3H, s, CH_3); 4.40 (2H, q, CH_2, J = 7.6 Hz); 6.89 (1H, s, CH); 7.07 (1H, s, CH); 7.33–7.40 (2H, m, ArH); 7.83–7.85 (1H, m, ArH); 8.05–8.08 (1H, m, ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.3; 33.8; 61.9; 123.3; 125.5; 127.5; 127.6; 129.7; 132.0; 134.9; 138.1; 141.2; 141.8; 162.9. Anal. ($C_{15}H_{14}N_2O_2SSe$) C, H, N.

*3.6.6. Diethyl 3,3'-thiodibenzo[b]selenophene-2-carboxylate (**28**)*

Yield: 42%, mp = 128–129 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.29 (6H, t, 2 × CH_3, J = 7.2 Hz); 4.28 (4H, q, 2 × CH_2, J = 7.2 Hz); 7.18–7.25 (2H, m, 2 × ArH); 7.28–7.35 (2H, m, 2 × ArH); 7.73–7.77 (2H, m, 2 × ArH); 7.79–7.83 (2H, m, 2 × ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.2; 61.8; 125.2; 125.7; 126.6; 127.3; 133.9; 134.5; 140.8; 141.7; 162.9. Anal. ($C_{22}H_{18}O_4SSe_2$) C, H.

3.7. In vitro cytotoxicity assay

Monolayer tumor cell line: HT-1080 (human fibrosarcoma), MG-22A (mice hepatoma), and NIH 3T3 (normal mouse fibroblasts) were cultured in standard medium DMEM (Dulbecco's modified Eagle's medium) without an indicator ("Sigma") supplemented with 10% heat-inactivated fetal bovine serum ("Sigma"). After the ampoule was thawed the cells from 1 to 4 passages were used. About 2–5.10^4 cells/mL (depending on line nature) were placed in 96-well plates immediately after compounds were added to the wells. The control cells without test compounds were cultured on separate plate. The plates were incubated for 72 h, 37 °C, 5% CO_2. The number of surviving cells was determined using tri(4-dimethylaminophenyl)methyl chloride (Crystal Violet) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide (MTT),

and the concentration of nitric oxide (NO) was determined according to [28]. MTT-test: after incubating with preparations culture medium was removed and 200 μL fresh medium with 10 mM HEPES was added in each well of the plate, than 20 μL MTT (2 mg/mL in HBSS) was added. After incubation (3 h, 37 °C, 5% CO_2) the medium with MTT was removed and 200 μL DMSO and 25 μL glycine buffer pH 10.5 were added at once to each sample. The samples were tested at 540 nm on Anthos HT II photometer. CV-test: after incubating with preparations cell culture was removed and 100 ml 1% glutaraldehide in HBSS was added to each well. After incubation (15 min) the HBSS with glutaraldehide the samples washed off H_2O (1 time) and 0.05% crystal violet were added. After incubation with dye (15 min) the samples washed off H_2O (3 times) and citrate buffers pH 4.2 and ethanol (1:1) was added. The samples were tested at 540 nm.

3.8. Morphology assay

The change in cell morphology caused by benzo[*b*]selenophenes was investigated under inverted fluorescence microscope Nikon ECLIPSE TE 300. Acridine orange stain (Sigma) was used. Stain solution: 10 μg/mL acridin orange in phosphate buffered saline (PBS) pH 7.4. After incubating with benzo[*b*]selenophenes 72 h, 37 °C, 5% CO_2 the adherent cells were stained in 96-wells cell culture plate (Sarsted AG). Cell culture mediums were removed and 40 μL stain solution was added in each well. After 2 min the dye was removed and samples once washed with PBS. Then 40 μL PBS was added to the samples and they were investigated under microscope control. Chromatin condensation in apoptotic cells was visualized by staining the cellular DNA with the dye acridine orange [29]. Living cells stained green, apoptotic cells orange or yellow, and necrotic cells red (Fig. 2).

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