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EVALUATION OF HIGHLY ACTIVE CYTOTOXIC AGENTS IN THE SERIES OF NOVEL DERIVATIVES OF N-HYDROXY(AND N-ALKOXY)-ω-(BENZENESELANYL OR 2-BENZOSELENAZOLYLSULFANYL)-ALKANAMIDINES

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ABSTRACT:
Synthesis of novel derivatives of N-hydroxy (and N-alkoxy)-ω-(benzeneselanyl)alkaneamidines and 2-benzoselenazolylsulfanyl)alkaneamidines as potential cytotoxic agents was carried out in two or three steps. 6-(Benzoselenazol-2-ylsulfanyl)-N-hydroxyhexanamidine exhibit high activity *in vitro* on monolayer tumor cell lines: MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma).

Keywords: N-hydroxy (and N-alkoxy)-ω-(benzeneselanyl)alkaneamidines, N-hydroxy-ω-(2-benzoselenazolylsulfanyl)alkaneamidines, phase transfer catalysis, mouse hepatoma (MG-22A) cell line, human fibrosarcoma (HT-1080) cell line, cytotoxicity.

INTRODUCTION

Selenium containing oximes were widely investigated as valuable intermediates in organic synthesis [I]. Beside this selenium compounds were used in the cancer chemoprevention [II] and tumor cell invasion [III]. Selenium containing cobaloximes were proposed as B_{12} model compounds [IV]. 1,3-Selenazoles exhibit antibiotic and cancerostatic activity [V]. Antitumor and cytotoxic activity 4-methyl-1,2,3-selenadiazole-5-carboxylic acid amides [VI], 3-C, N, S, Se substituted benzo[b]selenophene [VII] and di(3-indole)selenides [VIII] also were described.

The pharmacological model of the studied type of anticancer agents consists of a aromatic [IX] cap group able to interact with the rim space at the entrance of the catalytic tunnel of the enzyme linked to a hydrophobic spacer (for example, C_3-C_5-alkyl) through a polar connection unit (amide group, etc.). At the end of the hydrophobic spacer a hydroxylamine or amide group (binding group, BG) assures inhibition of enzyme (for example, epidermal growth factor receptor tyrosine kinase [X]). However, the amidoxime moiety as BG is practically not described in literature. A number of reviews are devoted to the chemistry and biological activity of heterocyclic oximes and their derivatives [XI]. The aim of the research is to obtain and to investigate cytotoxicity of a novel class of oximes – derivatives of N-hydroxy (and N-alkoxy)-ω-(benzeneselanyl or 2-benzoselenazolylsulfanyl)alkaneamidines.

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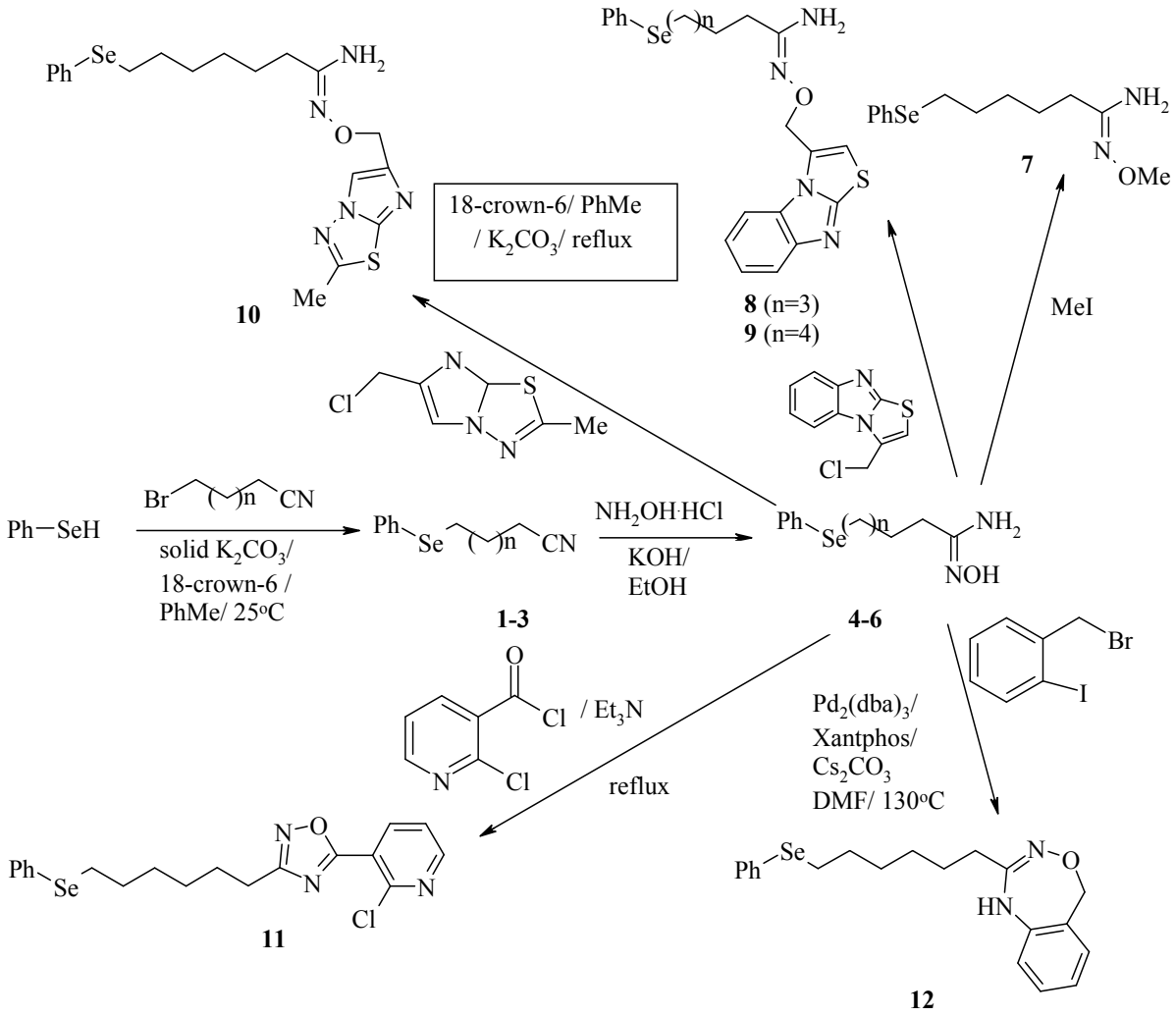
RESULTS AND DISCUSSION

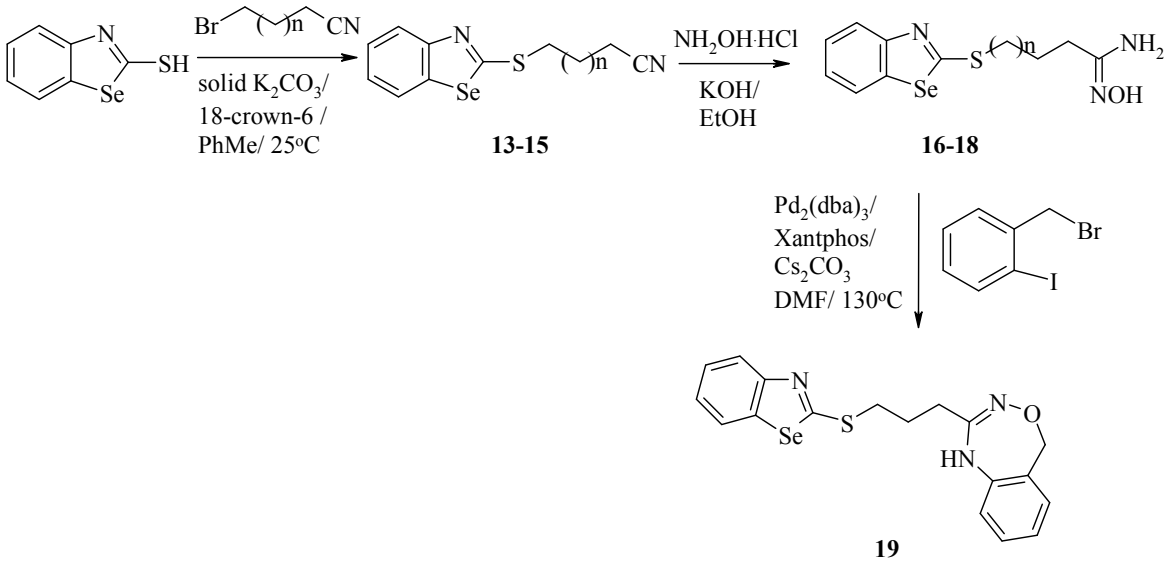
The general synthetic route chosen for preparation of N-hydroxy-ω-(benzeneselanyl)alkaneamidines **4-6** and N-hydroxy-ω-(2-benzoselenazolylsulfanyl)-alkaneamidines **16-18** included two steps. Thus, alkylation of benzeneselenol or 2-mercaptoselenazole with 1-bromo-ω-cyanoalkanes was successfully realized in the PTC (phase transfer catalytic) system solid K_2CO_3 / 18-crown-6 / toluene. Products **1-3, 13-15** were isolated in 72-99% yields (Scheme 1).



1, 3 (n=2); **2, 5** (n=3); **3, 6** (n=4)

Scheme 1

The reaction of nitriles **1-3, 13-15** with hydroxylamine hydrochloride in the presence of NaOH in refluxing aqueous ethanol afforded novel E-amidoximes **4-6, 16-18** in 44-59% yields (Scheme 1, See Experimental). Alkylation of amidoximes **5** and **6** was carried out in PTC system alkyl halides/ solid K_2CO_3 / 18-crown-6 in refluxing toluene. Oxime O-alkyl derivatives **7** and **10** were isolated as pure E-isomers in 21-59% yields.



13, 16 (n=1); **14, 17** (n=2); **15, 18** (n=3)

Scheme 2

Beside oxime and oxime O-ether derivatives some additional derivatives were prepared as masked oxime ether derivatives. At first, palladium-catalyzed one-flask method for the preparation of selenium containing 3-substituted 1,2,4-oxadiazepines **12** and **19** directly from corresponding *E*-amidoximes **6** and **16** and *o*-iodobenzyl bromide was elaborated. 2-Chloro-3-[3-(6-phenylselanyl-hexyl)-[1,2,4]oxadiazol-5-yl]-pyridine (**11**) were prepared in 78% yield from N-hydroxy-7-phenylselanylheptanamidine (**6**), 2-chloronicotinoyl chloride and Et_3N in refluxing toluene.

Cytotoxic activity of N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkaneamidines **1-18** was tested *in vitro* on monolayer tumor cell lines - MG-22A and HT-1080. The activity of high effective compounds was determined also on another cell lines (Table 1). Concentrations providing 50% of tumor death effect (IC_{50}) were calculated according to the known procedure using 96 well plates.

The experimental evaluation of cytotoxicity is presented in Table 1. The preliminary analysis of the structure-activity relationship for cytotoxic activity clearly indicated a strong influence of aromatic or heteroaromatic substituent (Ar) on toxic effects *in vitro*. In the 2-benzoselenazolylsulfanyl derivative **16-18** the IC_{50} values range from 2-3 μg/mL (compound **18** on human fibrosarcoma HT – 1080 cell line) to 18 μg/mL for oxime **16**. Beside this, N-hydroxy-7-phenylselanylheptanamidine **(6)** exhibit high activity on HT-1080 cancer line (IC_{50} 5μg/mL). It clearly indicates that the optimal length of the alkyl chain (hydrophobic spacer) between the aromatic ring and the oxime group is C_5 or C_6. Compound **18** also exhibit high activity on MG-22A cancer line (IC_{50} 2 μg/mL) (Table 1).

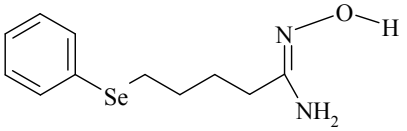
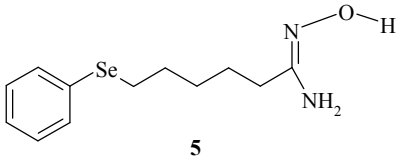
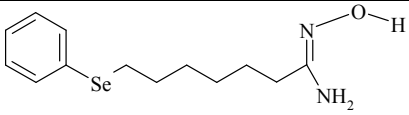
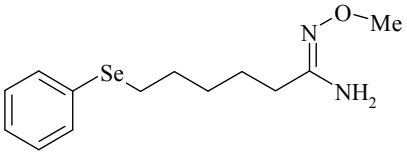
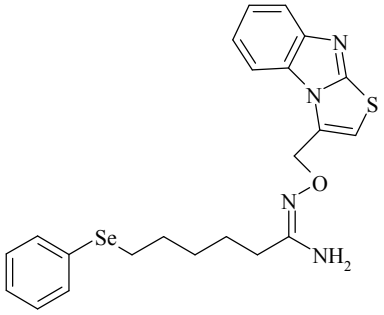
Interestingly, that 2-benzoselenazolyl derivative **18** exhibit higher cytotoxicity on the both above cancer cell lines than N-hydroxy-6-phenylselanylhexanamidine **(5)** containing similar length of the alkyl chain (hydrophobic spacer).

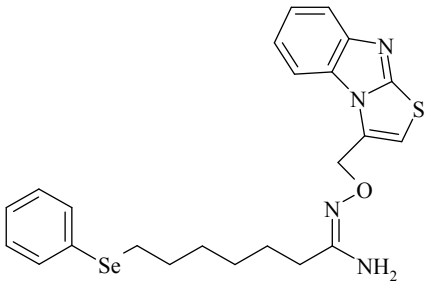
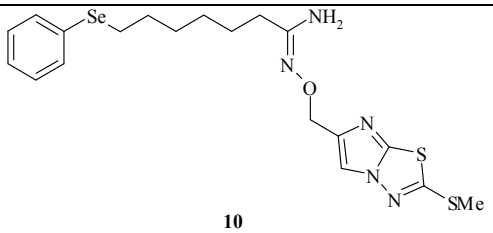
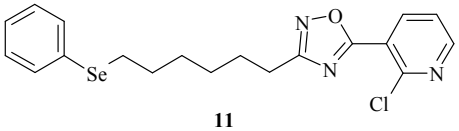
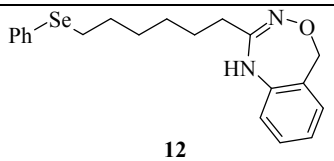
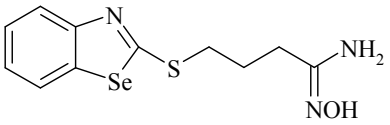
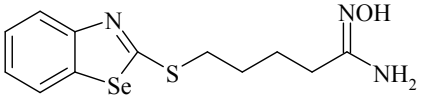
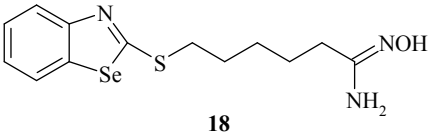
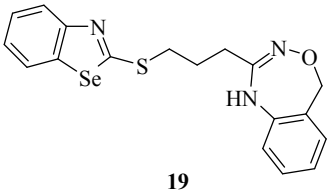
N-Methoxy-6-phenylselanylhexanamidine (**7**) exhibit high activity on the HT-1080 and MG-22A cancer cell lines (IC_{50} 1μg/mL) using tri(4-dimethylaminophenyl)methyl chloride (crystal violet: CV) test. It means that this compound influence oxidative-reducing activity of ferments in the mitochondria. Our previous results showed that introduction benzo[4,5]imidazo[2,1-b]thiazol-3-ylmethoxy substituent in oxime derivatives diminishes acute toxicity. Our investigations show that acute toxicity LD_{50} was diminished in most of the

cases. However, cytotoxicity of oxime ethers **8-10** in comparison with unsubstituted oxime **6** was considerably diminished. 2-Chloro-3-[3-(6-phenylselanyl-hexyl)-[1,2,4]oxadiazol-5-yl]-pyridine **(11)** was essentially unactive on the HT-1080 and MG-22A cancer cell lines.
Beside this, compound **19** formally is masked amidoxime O-benzyl ether. Thus, compound **19** exhibit high activity on the MG-22A cancer cell line (IC_{50} 4 μg/mL). Corresponding 4-(benzoselenazol-2-ylsulfanyl)-N-hydroxybutyramidine (**16**) exhibit considerably less cytotoxicity on this cancer line (IC_{50} 12 μg/mL).

Acute toxicity of synthesized compounds was tested on 3T3- Swiss Albino mice embrio fibroblasts**.** In general, compounds **4-12** and **16-19** exhibit middle toxicity in the range LD_{50} 443-2000 mg/kg (Table 1).

Table 1. ***In vitro*** **cell cytotoxicity of derivatives of N-hydroxy(or alkoxy)-ω-(benzeneselanyl or 2-benzoselenazolylsulfanyl)-alkanamidines (IC_{50} (μg/ml).**

Compound	3T3	HT-1080		MG-22A	
	LD_{50} mg/kg	IC_{50} CV	IC_{50} MTT	IC_{50} CV	IC_{50} MTT
 **4**	1715	23	18	21	18
 **5**	656	9	3	3	9
 **6**	539	4	6	4	7
 **7**	1078	1	32	1	22
 **8**	1369	16	8	12	18

 **9**	486	14	8	12	12
 **10**	820	15	17	14	59
 **11**	>2000	*	*	*	*
 **12**	581	31	43	26	36
 **16**	786	32	24	10	13
 **17**	571	24	21	10	10
 **18**	445	3	2	1	2
 **19**	443	11	16	4	3

* No cytotoxic effect

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were registered on Varian Mercury BB instrument (400 and 100 MHz, respectively) in $CDCl_3$. The residual proton signal of the solvent (δ=7.26 pm) was used as the reference. Electron impact ionization mass spectra were recorded on Agilent Technologies 5975C MSD detector at 70 eV. Melting points were detected on Boetius

aparatos equipped with visual detector PHMH 05. The progress of the reactions was monitored by TLC Silica gel 60 F_{254} aluminium sheets using hexane: ethyl acetate in the different mixtures as eluent. 2-Iodobenzyl bromide, 1-bromo-ω-cyanoalkanes (AlfaAesar), 18-crown-6 (Acros), $Pd_2(dba)_3$) (Acros), Xantphos (Acros), Cs_2CO_3 (Acros), benzeneselenol (Aldrich) and dioxane (extra dry over molecular sieves, Acros) were used without purification. 1-(Benzeneselanyl or 2-benzoselenazolylsulfanyl)-ω-cyanoalkanes **1-3** and **13-15** were prepared from benzeneselenol or 2-mercaptoselenazole in the system solid 1-bromo-ω-cyanoalkane (1 equivalent) / K_2CO_3 (3 equivalents)/18-crown-6 (5 mol. %) / PhMe at room temperature according literature [IXf].

General procedure for the synthesis of N-hydroxy-ω-(benzeneselanyl or 2-benzoselenazolylsulfanyl)-alkanamidines 4-6 and 16-18. A solution of 1-(benzeneselanyl or 2-benzoselenazolylsulfanyl)-ω-cyanoalkane **1-3, 13-15** (16.7 mmol), hydroxylamine hydrochloride (3.46 g, 50.2 mmol) and NaOH (2.01 g, 50.2 mmol) in mixture of EtOH (15 ml) and H_2O (10 ml) was refluxed for 24 h. Reaction mixture was evaporated to dryness under reduced pressure. Product was extracted with a mixture of $CHCl_3$: EtOH 10:1 (100ml), dried over anhydrous Na_2SO_4 and the solvents were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent $CHCl_3$: EtOH 20:1 or 10:1) to obtain desired products **4-6** and **16-18.** Compounds **4-6** and **16-18** were isolated as pure E-isomers.

N-Hydroxy-5-phenylselanylpentanamidine (4). Yield 59%. Yellow oil**.** ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):1.64-1.79 (m, 4H, $CH_2(CH_2)_2$); 2.12 (t, 2H, J = 7.0 Hz, CH_2), 2.91 (t, 2H, J = 6.0 Hz, $SeCH_2$), 4.48 (bs, 2H, NH_2), 7.23-7.27 un 7.44-7.49 (both m, 5H, Ph). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 26.77, 27.60, 29.60, 31.67, 127.60, 129.27, 130.51, 132.81, 152.92. Mass-spectrum, *m/z* (I_{rel}, %): 272 (M^++1, 70), 271 (M^+, 90), 213 (15), 115 (100). Found, %: C 48.55; H 5.71; N 10.11. $C_{11}H_{16}N_2OSe$. Calculated, %: C 48.71; H 5.95; N 10.33.

N-Hydroxy-6-phenylselanylhexanamidine (5). Yield 56%. White crystals, m.p. 76^{o}C**.** ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.41-1.71 (m, 6H, $CH_2(C\underline{H}_2)_3$); 2.08 (t, 2H, J = 8.0 Hz, CCH_2), 2.86 (t, 2H, J = 8.2 Hz, $SeCH_2$), 4.52 (bs, 2H, NH_2), 7.19-7.46 (m, 5H, Ph). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 26.07, 27.56, 29.11, 29.75, 31.07, 126.62, 128.96, 130.50, 130.55, 153.70. Mass-spectrum, *m/z* (I_{rel}, %): 286 (M^++1, 100), 285 (70), 227 (10), 113 (42). Found, %: C 50.42; H 6.21; N 9.67. $C_{12}H_{18}N_2OSe$. Calculated, %: C 50.53; H 6.36; N 9.82.

N-Hydroxy-7-phenylselanylheptanamidine (6). Yield 46%. White crystals, m.p. 76-78^{o}C**.** ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.32-1.72 (m, 8H, $(CH_2)_4$), 2.10 (t, 2H, J =6.1 Hz, CCH_2), 2.89 (t, 2H, J = 6.0 Hz, $SeCH_2$), 4.48 (bs, 2H, NH_2), 7.22-7.29 and 7.44-7.49 (abi m, 5H, Ph). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 26.40, 27.76, 28.40, 29.36, 29.89, 31.12, 126.58, 128.94, 130.52, 132.35, 153.82. Mass-spectrum, *m/z* (I_{rel}, %): 300 (M^++1, 75), 299 (100), 241 (10). Found, %: C 52.21; H 6.54; N 9.27. $C_{13}H_{20}N_2OSe$. Calculated, %: C 52.17; H 6.74; N 9.36.

4-(Benzoselenazol-2-ylsulfanyl)-N-hydroxybutyramidine (16). Yield 52%. White crystals, m.p. 108^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.66-1.77, 1.86-1.92 and 2.93- 2.99 (all m, 6H, $CH_2CH_2CH_2$), 4.64 (bs, 2H, NH_2), 6.82, 6.99 and 7.41 (all m, 4H, Ph), 8.41 (bs, 1H, NOH)**.** ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 26.39, 29.87, 33.07, 122.79, 124.30, 126.16, 138.11, 153.09, 153.20, 154.53, 168.26. Mass-spectrum, *m/z* (I_{rel}, %): 314 (M^+, 100). Found, %: C 42.30; H 4.00; N 13.24. $C_{11}H_{13}N_3OSSe$. Calculated, %: C 42.04; H 4.17; N 13.37.

5-(Benzoselenazol-2-ylsulfanyl)-N-hydroxypentanamidine (17). Yield 50%. White crystals, m.p. 85^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.60-1.81 and 1.97-2.05 (both m, 6H, $CH_2CH_2CH_2$), 5.36 (bs, 2H, NH_2), 7.27 and 7.43 (both t, 2H, J = 8.2 Hz, Ph), 7.83 and

8.04 (both d, 2H, *J* = 8.2 Hz, Ph), 8.72 (bs, 1H, NOH). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 25.57, 28.49, 30.59, 33.56, 122.83, 124.26, 126.13, 138.06, 153.51, 154,60, 154.63, 168.57. Mass-spectrum, *m/z* (I_{rel}, %): 328 (M^+, 100). Found, %: C 43.81; H 4.51; N 12.91. $C_{12}H_{15}N_3OSSe$. Calculated, %: C 43.90; H 4.61; N 12.80.

6-(Benzoselenazol-2-ylsulfanyl)-N-hydroxyhexanamidine (18). Yield 44%. White crystals, m.p. 85°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.58 and 1.85 (both m, 6H, $CH_2CH_2CH_2$), 2.16 and 3.32 (both t, 4H, *J* = 8.0 Hz, CH_2C and SCH_2), 4.50 (bs, 2H, NH_2), 7.20 and 7.38 (both t, 2H, *J* = 8.0 Hz, Ph), 7.75-7.88 (m, 2H, Ph). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 26.00, 28.04, 28.76, 31.01, 33.95, 122.83, 124.23, 126.11, 138.02, 153.69, 153.72, 154.69, 168.73. Mass-spectrum, *m/z* (I_{rel}, %): 342 (M^+, 100). Found, %: C 45.58; H 5.02; N 12.41. $C_{13}H_{17}N_3OSSe$. Calculated, %: C 45.61; H 5.01; N 12.28.

General procedure for the synthesis of N-alkoxy-ω-(benzeneselanyl)-alkanamidines 7-10 from N-hydroxy-ω-(benzeneselanyl)-alkanamidines 5-6 under phase transfer catalysis conditions. A suspension of N-hydroxy-ω-(benzeneselanyl)-alkanamidines **5** or **6** (2.2 mmol), alkyl halide (2.2 mmol), 18-crown-6 (0.058g, 0.22 mmol) and solid K_2CO_3 (1.21 g, 8.8 mmol) in toluene (30 ml) was refluxed for 18 h. Reaction mixture was filtered and filtrate was evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (eluent hexane: EtOAc from 1:1 to 0:1) to obtain desired products **7-10.** Compounds **7-10** were isolated as pure E-isomers.

N-Methoxy-6-phenylselanylhexanamidine (7). Yield 59%. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42-1.69 (m, 6H, $CH_2(C\underline{H}_2)_3$); 2.03 (t, 2H, *J* = 6.2 Hz, CH_2), 2.87 (t, 2H, *J* = 6.4 Hz, $SeCH_2$), 3.73 (s, 3H, Me), 7.20-7.25 and 7.42-7.47 (both m, 5H, Ph. ^{13}C NMR (100.58 MHz, $CDCl_3$) δ ppm: 24.83, 26.02, 27.56, 29.10, 29.73, 31.05, 126.63, 128.96, 130.40, 132.39, 153.79. Mass-spectrum, m/z (I_{rel}, %): 299(M^++1, <1), 254 (10), 157 (12), 143 (100), 101 (16), 88 (17), 58 (12). Found, %: C 52.06; H 6.59; N 9.32. $C_{13}H_{20}N_2OSe$. Calculated, %: C 52.17; H 6.74; N 9.36.

N-(Benzo[4,5]imidazo[2,1-b]thiazol-3-ylmethoxy)-6-phenylselanyl-hexanamidine (8).

(7). Yield 47%. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.41-1.73 (m, 6H, $CH_2(CH_2)_3$), 2.09 (t, 2H, *J* = 8.2 Hz, CH_2), 2.88 (t, 2H, *J* = 8.0 Hz, SCH_2), 4.39 (bs, 2H, NH_2), 5.24 (s, 2H, CH_2), 6.73 (s, 1H, thiazole proton), 7.22-7.47 and 7.76-7.85 (both m, 9H, Ph and C_6H_4). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ ppm: 26.24, 27.60, 28.97, 29.69, 31.03, 66.74, 109.48, 111.62, 119.08, 121.00, 123.34, 126.71, 128.99, 129.99, 130.46, 130.48, 132.47, 148.34, 154.70, 157.03. Mass-spectrum, m/z (I_{rel}, %): 472 (M^++1, 100),188 (70). Found, %: C 56.01; H 5.21; N 11.99. $C_{22}H_{24}N_4OSSe$. Calculated, %: C 56.04; H 5.13; N 11.88.

N-(Benzo[4,5]imidazo[2,1-b]thiazol-3-ylmethoxy)-7-phenylselanyl-heptanamidine (9). Yield 53%. White crystals, m.p. 65°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.24-1.72 (m, 6H, $CH_2(C\underline{H}_2)_3$), 2.09 (t, 2H, *J* = 8.0 Hz, CH_2), 2.89 (t, 2H, *J* = 8.0 Hz, SCH_2), 4.38 (bs, 2H, NH_2), 5.24 (s, 2H, CH_2), 6.73 (s, 1H, thiazole proton), 7.19-7.49 and 7.76-7.85 (both m, 9H, Ph and C_6H_4). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ ppm: 26.62, 27.78, 28.29, 29.32, 29.89, 31.08. 109.47, 111.62, 119.13, 120.99, 123.34, 126.66, 128.99, 130.46, 130.51, 132.42, 132.44, 148.40, 154.83, 157.06. Mass-spectrum, m/z (I_{rel}, %): 486 (M^++1, 100), 329 (50), 188 (70). Found, %: C 56.71; H 5.31; N 11.49. $C_{23}H_{26}N_4OSSe$. Calculated, %: C 56.90; H 5.40; N 11.54.

N-(2-Metylsulfanylimidazo[2,1-b][1,3,4]thiadiazol-6-ylmethoxy)-7-phenyllselanyl-heptanamidine (10). Yield 21%. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.21-1.67 (m, 8H, $CH_2(C\underline{H}_2)_4$), 2.04 (m, 2H, CCH_2), 2.71 (s, 2H, OCH_2), 2.87 (t, 2H, *J* = 8.0 Hz, $SeCH_2$), 4.50 (bs, 2H, NH_2), 7.21-7.48 (m, 5H, Ph). 7.67 (s, 1H, imidazole proton). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 26.80, 27.79, 28.20, 28.43, 28.81, 29.92, 31.25, 69.34,

126.64, 128.97, 128.98, 130.54, 132.41, 132.42, 132.43, 134.91, 141.02. Mass-spectrum, m/z (I_{rel}, %): 483 (M^++1, 100), 225 (30). Found, %: C 47.19; H 5.11; N 14.33. $C_{19}H_{25}N_5OS_2Se$. Calculated, %: C 47.29; H 5.22; N 14.51.

2-Chloro-3-[3-(6-phenylselanyl-hexyl)-[1,2,4]oxadiazol-5-yl]-pyridine (11). A suspension of N-hydroxy-7-phenylselanylheptanamidine (**6**) (0.095 g, 0.35 nnol), 2-chloronicotinoyl chloride (0.062 g, 0.35 mmol) and Et_3N (0.098 ml, 0.7 mmol) in toluene (3 ml) was refluxed for 18 h. Reaction mixture was filtered and filtrate was evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (eluent hexane: EtOAc 2:1) to obtain desired product **11.** Yield 78%. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.40-1.80 (m, 8H, $(CH_2)_4$), 2.82 (t, 2H, J = 6.2 Hz, CCH_2), 2.90 (t, 2H, J = 6.0 Hz, $SeCH_2$), 4.48 (bs, 2H, NH_2), 7.21-7.49 (m, 5H, Ph and 5-H), 8.37 (dd, 1H, J_1 = 8.0 Hz, J_2= 2.2 Hz, 4-H), 8.58 (dd, 1H, J_1 = 6.0 Hz, J_2= 4.0 Hz, 6-H). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 25.93, 26.79, 27.76, 28.48, 29.30, 29.89, 120.96, 122.36, 126.62, 128.95, 130.49, 132.39, 140.42, 149.80, 152.30, 171.18, 172.36. Mass-spectrum, m/z (I_{rel}, %): 421 (M^++1, 100), 266 (40). Found, %: C 54.17; H 4.69; N 9.80. $C_{19}H_{20}ClN_3OSe$. Calculated, %: C 54.23; H 4.79; N 9.99.

Synthesis of 6-(6-phenylselanylhexyl)-5,9-dihydro-8-oxa-5,7-diazabenzocycloheptene (12) and 6-[3-(benzoselenozol-2-ylsulfanyl)propyl]-5,9-dihydro-8-oxa-5,7-diazabenzocycloheptene (19). Mixture of oxime **6** or **16** (1 mmol), 2-iodobenzyl bromide (0.30 g, 1 mmol), $Pd_2(dba)_3$ (0.0366g, 0.04 mmol), Xantphos (0.0232 g, 0.04 mmol) and anhydrous Cs_2CO_3 (1.30 g, 4 mmol) in dry dioxane (3 ml) was heated at 60^{o}C for 12h then at 120^{o}C for 48h in glass reactor under argon. Reaction mixture was diluted with ethyl acetate (30 ml), filtered, solvent was removed under reduced pressure and crude residue was chromatographed on silica using ethyl acetate : hexane (1:2 to1:1) as eluent.

6-(6-Phenylselanylhexyl)-5,9-dihydro-8-oxa-5,7-diazabenzocycloheptene (12). Yield 34%. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.24-1.72 (m, 8H, $CH_2(C\underline{H}_2)_4$), 2.29 (t, 2H, J = 8.0 Hz, CCH_2), 2.88 (t, 2H, J = 7.2 Hz, $SeCH_2$), 4.88 (s, 2H, OCH_2), 6.24 (s, 1H, NH), 6.72 and 7.70 (both m, 9H, Ph and C_6H_4). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 22.96, 23.72, 27.33, 28.90, 29.58, 33.79, 77.11, 117.31, 121.24, 126.64, 128.22, 128.97, 130.06, 130.85, 132.40, 139.74, 157.06, 167.73. Mass-spectrum, m/z (I_{rel}, %): 388 (M^++1, 100). Found, %: C 61.91; H 6.11; N 7.00. $C_{20}H_{24}N_2OSe$. Calculated, %: C 62.01; H 6.24; N 7.03.

6-[3-Benzoselenazol-2-ylsulfanylpropyl)-5,9-dihydro-8-oxa-5,7-diazabenzocyclo-heptene (19). Yield 32%. White crystals, m.p. 82-83^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.26 (m, 2H, $CH_2C\underline{H}_2$), 2.54 (t, 2H, J = 8.0 Hz, CCH_2), 3.47 (t, 2H, J = 6.2 Hz, SCH_2), 4.93 (s, 2H, OCH_2), 6.76-7.39 and 7.72-7.78 (both m, 8H, both C_6H_4). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ (ppm): 27.91, 32.02, 32.89, 77.08, 117.59, 121.23, 122.70, 124.32, 124.42, 126.20, 127.55, 128.26, 130.10, 138.07, 139.86, 154.31, 156.69, 168.62. Mass-spectrum, m/z (I_{rel}, %): 403 (M^++1, 100), 189 (95). Found, %: C 53.54; H 4.14; N 10.32. $C_{18}H_{17}N_3OSSe$. Calculated, %: C 53.73; H 4.26; N 10.44.

In vitro cytotoxicity assay. Monolayer tumor cell lines –HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma), 3T3 (mouse Swiss Albino embryo fibroblasts), - were cultured in standard medium (Dulbecco`s modified Eagle`s medium; "Sigma") supplemented with 10% fetal bovine serum ("Sigma"). Tumor cell lines were obtained from the "ATCC". After the ampoule had thawed, cells from one to four passages were used in three concentrations test compound: 1, 10 and 100 μg ml $^{-1}$. About 10 x10^4 cells ml^{-1} were placed in 96-well plates immediately after compounds were added to the wells; the volume of each plate was 200 μl. The control cells without test compounds were cultured on separate plate. The plates were incubated for 72h, 37^{o}C, 5% CO_2. The number of surviving cells was determined using tri(4-dimethylaminophenyl)methyl chloride (crystal violet: CV) or 3-(4,5-dimethylthiazol-2-yl)-

2,5-diphenyltetrazolinium bromide (MTT) [XII, XIII]. The quantity on the control plate was taken in calculations for 100%. LD_{50} was tested according „Alternative Toxicological Methods" [XIV]. The program Graph Pad Prism® 3.0 was used for calculations ($r < 0.05$.).

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