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Novel potent organoselenium compounds as cytotoxic agents in prostate cancer cells

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Abstract—A series of 17 symmetrical substituted imidothiocarbamate and imidoselenocarbamate derivatives has been synthesized by reacting appropriately substituted acyl chlorides with alkyl imidothiocarbamates and alkyl imidoselenocarbamates. The antitumoral activities of the compounds were evaluated in vitro by examining their cytotoxic effects against human prostate cancer cells (PC-3). Five compounds showed interesting activity levels and **3p** (IC_{50} = 1.85 μM) was 7.3 times more active than the standard etoposide used in the treatment of prostate cancer and emerges as the most interesting compound.

Numerous observations in the epidemiological literature have linked various dietary, lifestyle, genetic, and non-traditional factors with the risk of developing prostate cancer.[1] Prostate cancer is the most common cancer in men and the second highest cause of male cancer deaths in the United States and the United Kingdom. In recent years, many epidemiological studies[2–5] have suggested that an essential trace element, such as selenium (Se), acts to protect against cancer, particularly prostate cancer.[6] There is a marked geographic variability of Se in food and this is related to local soil content. Se is also widely available in over-the-counter supplements and multivitamins. There is increasing evidence to suggest that Se works by inhibiting important molecular pathways of the early carcinogenesis in a variety of experimental models and that the anticancer activity is dependent on its chemical form. Se occurs in both organic and inorganic forms. The organic form is found predominantly in grains, fish, meat, poultry, eggs, and dairy products and enters the food chain through plant consumption.[7,8] A number of potential mechanisms have been proposed for its antitumorigenic effects and these include antiandrogen activity.[9,10] It has also been suggested that selenium may exert growth inhibitory effects by regulation of p53,[11,12] by antioxidant function,[13] DNA damage,[14] and numerous pathways involve apoptosis[15–19] as a critical event. The regulatory mechanisms of apoptosis are extremely complex and for selenium compounds they mainly involve mitochondrial pathway,[15,16] protein kinases,[17] tumor necrosis factor,[18] and reactive oxygen species.[19] A survey of the diverse literature in this field shows that very few organoselenium compounds have been described, but those that have do show promising activities. Among them are the selenoproteins,[20] such as selenomethionine and methylselenocysteine, and a number of synthetic derivatives such as *p*-xylylbismethylselenide, sodium selenite,[21] and methylseleninic acid.[22] In addition, the combination of some of these derivatives with chemotherapeutic agents shows synergistic activity in prostate cancer. For instance, methylselenocysteine enhances the effect of docetaxel,[23] whereas methylseleninic acid improves substantially the therapeutic effect of etoposide in vivo.[24]

In recent reports, we have described[25–28] the synthesis, cytotoxicity, and apoptotic evaluation of a series of symmetrical diaryl derivatives. We observed that symmetry is a structural property that is frequently present in cytotoxic and proapoptotic drugs.[29] In addition, some

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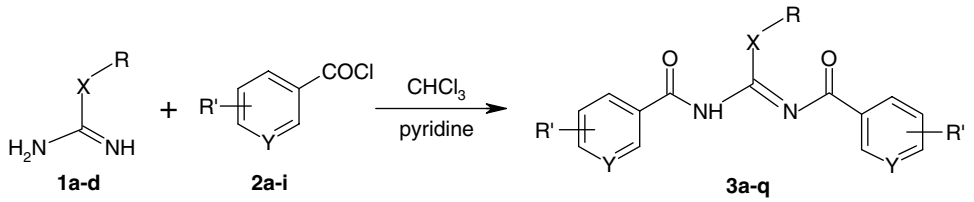


1a: X = S; R = CH_3
1b: X = Se; R = CH_3
1c: X = Se; R = CH_2-CH_3
1d: X = Se; R = $CH(CH_3)_2$

2a: R' = 2-Cl; Y = N
2b: R' = H; Y = C
2c: R' = 3,5-diOCH_3; Y = C
2d: R' = 4-Cl; Y = C
2e: R' = 4-NO_2; Y = C
2f: R' = 4-CF_3; Y = C
2g: R' = 4-CN; Y = C
2h: R' = 4-CH_3; Y = C
2i: R' = 4-$C(CH_3)_3$; Y = C

Scheme 1. Synthesis of bisacylimidocarbamates **3a–q**.

reported selenium derivatives have this property: 1, 4-phenylenebis(methylene)selenocyanate,[30] 1,2-[bis(1, 2-benzoiso selenazolone)-3(2*H*)-ketone]ethane,[31] and alkyl[32] and biscyclodextrin[33] diselenides.

Based on these findings we planned to undertake the synthesis of new symmetrical compounds that contain selenium. The rationale behind the design of these compounds was to maintain the general structural pattern described in our previous studies,[25–28] which in this paper corresponds to molecules with a central nucleus consisting of an alkyl imidothiocarbamate (alkyl isothiourea) or alkyl imidoselenocarbamate (alkyl isoselenourea) connected by a carbonyl group onto two identical lateral aromatic or heteroaromatic rings. The sulfur and selenium substituents were varied between methyl, ethyl, and isopropyl in order to determine the effect of the alkyl chain length and its ramifications on the activity.[34] In addition, the differences in the anticarcinogenic activity between two similar chemical elements, selenium and sulfur,[35] were studied. The lateral rings in these systems are aromatic rings bearing one or more electron-donating (methoxy, methyl, *tert*-butyl) or electron-withdrawing (nitro, trifluoromethyl, cyano, chloro) groups. In some examples the ring system is a heteroaromatic unit such as pyridine.

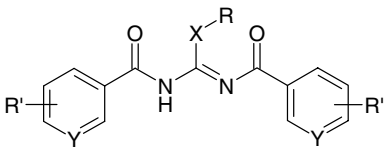
The synthesis of the bisacylimidocarbamates **3a–q**[36] was carried out according to Scheme 1, starting from the appropriate *S*-alkyl imidothiocarbamate (**1a**) or *Se*-alkyl imidoselenocarbamate (**1b–d**) hydroiodides and the corresponding acyl chloride (**2a–i**) in a 1:2.1 molar ratio, respectively, in chloroform in the presence of pyridine as a catalyst at room temperature. The compounds were obtained in yields ranging from 26% to 89%. The scope and generality of this procedure are illustrated in Table 1. The purity of the compounds was assessed by TLC and elemental analyses and their structures were identified from spectroscopic data.[36]

New compounds (**3a–q**) were evaluated for their in vitro cytotoxic activity against a human prostate cancer cell line (PC-3, ATCC, Manassas, VA) using the MTT assay.[37] Results are tabulated as IC_{50} values. All experiments were independently performed at least three times and the values were calculated after 72 h exposure (compound concentrations of 2, 5, 7, and 10 μM). The results are shown in Table 2 and in Figure 1. High cyto-

Table 1. Synthesis of title compounds **3a–q**

Compound	From	Time (h)	Yield (%)	Mp (°C)	Recrystallization solvent
3a	(**1a** + **2a**)	38	38	191–192	EtOH/*N*,*N*-DMF
3b	(**1b** + **2a**)	30	35	176–177	EtOH/*N*,*N*-DMF
3c	(**1a** + **2b**)	48	67	147–148	EtOH
3d	(**1b** + **2b**)	48	86	137–138	EtOH
3e	(**1c** + **2b**)	48	50	104–105	EtOH
3f	(**1d** + **2b**)	48	56	97–98	EtOH
3g	(**1b** + **2c**)	52	45	160–162	EtOH/*N*,*N*-DMF
3h	(**1d** + **2c**)	48	40	149–151	EtOH/*N*,*N*-DMF
3i	(**1a** + **2d**)	15	60	174–175	EtOH
3j	(**1b** + **2d**)	40	35	177–178	EtOH
3k	(**1d** + **2d**)	36	89	155–156	EtOH
3l	(**1b** + **2e**)	15	33	214–215	EtOH/*N*,*N*-DMF
3m	(**1b** + **2f**)	33	26	164–165	EtOH
3n	(**1b** + **2g**)	24	26	179–180	EtOH
3o	(**1a** + **2h**)	48	35	152–153	EtOH/*N*,*N*-DMF
3p	(**1b** + **2h**)	48	77	159–160	EtOH
3q	(**1b** + **2i**)	60	65	141–142	EtOH

Table 2. Cytotoxic activity of the compounds **3a**–**q**



Compound	X	Y	R	R′	IC_{50}[a] (μM)	LD_{50}[b] (μM)
3a	S	N	Methyl	2-Cl	>10	nd[c]
3b	Se	N	Methyl	2-Cl	9.14	>10
3c	S	C	Methyl	H	>10	nd
3d	Se	C	Methyl	H	2.50	4.1
3e	Se	C	Ethyl	H	>10	nd
3f	Se	C	Isopropyl	H	>10	nd
3g	Se	C	Methyl	3,5-diOCH_3	6.50	nd
3h	Se	C	Isopropyl	3,5-diOCH_3	>10	nd
3i	S	C	Methyl	4-Cl	>10	nd
3j	Se	C	Methyl	4-Cl	7.60	nd
3k	Se	C	Isopropyl	4-Cl	>10	nd
3l	Se	C	Methyl	4-NO_2	>10	nd
3m	Se	C	Methyl	4-CF_3	>10	nd
3n	Se	C	Methyl	4-CN	>10	nd
3o	S	C	Methyl	4-CH_3	>10	nd
3p	Se	C	Methyl	4-CH_3	1.85	>10
3q	Se	C	Methyl	4-$C(CH_3)_3$	>10	nd
MSA[d]	—	—	—	—	8.38[37]	
Etoposide	—	—	—	—	13.6 ± 2.2[38]	

[a] Cell line PC-3.
[b] Cell line RWPE-1.
[c] nd, no data.
[d] MSA, methylseleninic acid.

toxic activity values were found for five compounds (**3b**, **3d**, **3g**, **3j**, and **3p**) and these ranged from 1.85 to 10 μM. Comparison of these results with the standards used showed that five of the compounds are more active than methylseleninic acid and all of them have IC_{50} values lower than that of etoposide. Compound **3p** was the most potent (IC_{50}: 1.85 μM) and was 4.5 times more active than standard methylseleninic acid (IC_{50} = 8.38 μM[38]) and 7.3 times more active than etoposide (IC_{50} = 13.6 μM[39]), a drug currently used in the treatment of prostate cancer.

In the present study a precise structure–activity relationship cannot be defined, although it is possible to highlight some general trends. A relationship seems to exist between the presence of substituents with strongly deactivating electron-withdrawing groups (e.g., nitro and trifluoromethyl) in the phenyl ring and reduced activity (**3l**, **3m**) and this is followed closely by the cyano group (**3n**). However, electron-donating groups like methoxy and methyl appear to increase the activity (**3g**, **3p**). On the other hand, the biological results for the *tert*-butyl derivative (**3q**), an electron-donating but voluminous substituent, showed this to be inactive and this result suggests that the size of R′ is also important for activity.

The effect of changing the alkyl chain length was also investigated and the order of activity was found to be methyl > ethyl > isopropyl (**3d** > **3e** > **3f**, **3g** > **3h**, and **3j** > **3k**). The cytotoxic activity assay for four pairs of sulfur and selenium analogs (**3a**–**3b**, **3c**–**3d**, **3i**–**3j**, and **3o**–**3p**) was also investigated. Replacement of sulfur with selenium in **3a**, **3c**, **3i**, and **3o** had a positive effect on the activity. S and Se are similar in some aspects but different in others. In essence, the trends for oxidized and reduced Se and S species are similar, but the proportions differ quite significantly, suggesting important differences in the biochemistry of S and Se.[40] Ip[35] reported comparative studies between analogous sulfur and selenium compounds and demonstrated that selenium is much more active than sulfur in inhibiting cancer cell growth. Furthermore, selenium may have a multi-modal mechanism in preventing cellular transformation. El-Bayoumi[41] later found evidence to support this idea by extending the studies to other analogous sulfur and selenium derivatives.

Some of the compounds (**3b**, **3d**, and **3p**) that showed good in vitro activity were examined in more detail for toxicity with regard to selectivity and, as an orientative measure, in a cell culture of one non-tumoral prostate line (RWPE-1). Drug concentrations studied ranged from 2 to 10 μM. The results are reported in Table 2 and in Figure 2 and expressed as DL_{50}. All the compounds showed low toxicity with DL_{50} > 10 μM except for **3d**, whose DL_{50} was only 1.6 times higher than its IC_{50}. For this reason, and for its low solubility it has

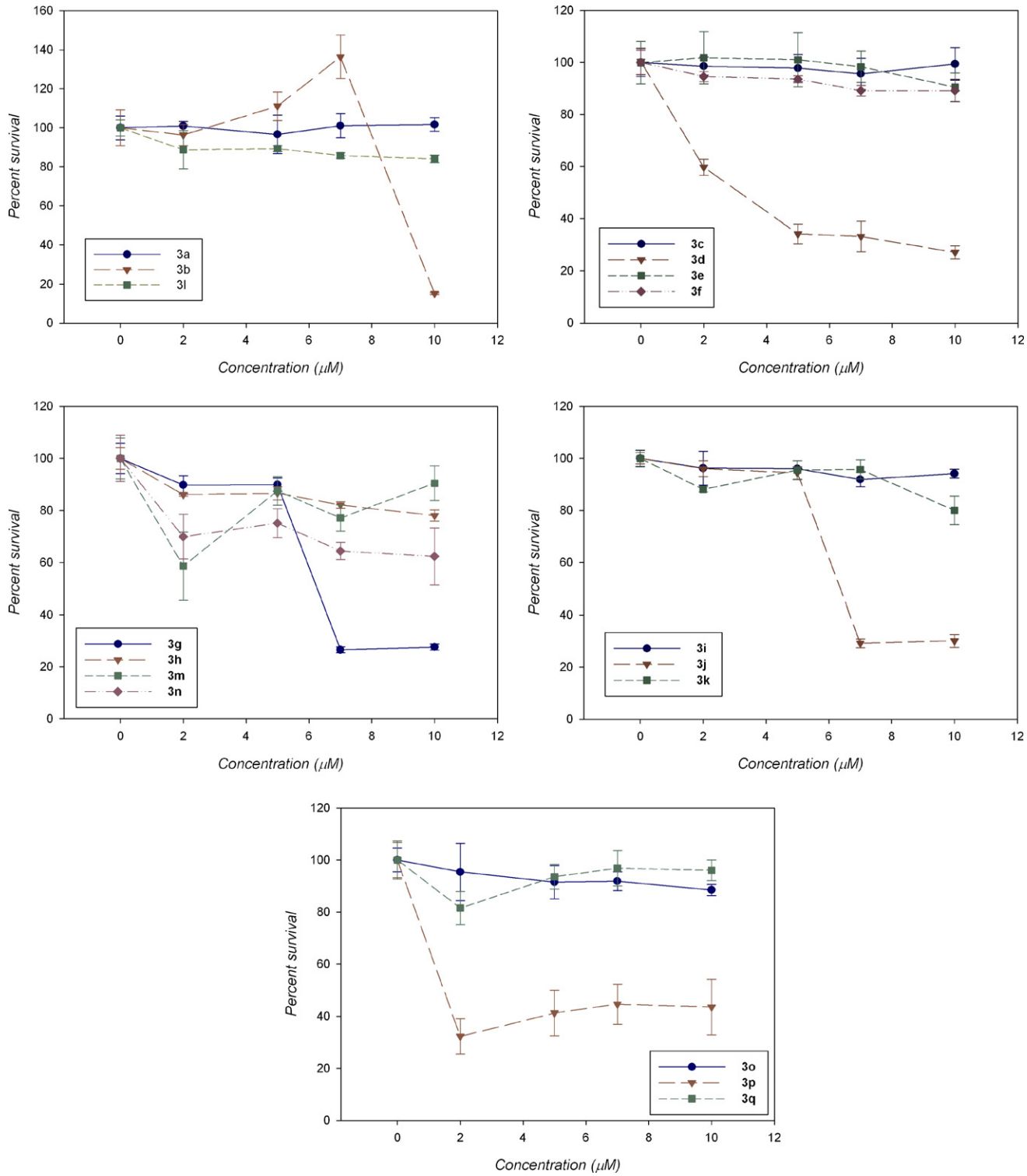


Figure 1. Effects of compounds **3a**–**q** on growth of cells. Dose-dependent effect on PC-3 cells. Data are expressed as percent survival and error bars show SD ($n = 4$).

not been selected for studying the mechanism of action.

In conclusion, it has been shown that the potency and selectivity of these compounds make them valid leads for the synthesis of new compounds that possess improved activity. Compound **3p** can be highlighted as a candidate as an antitumoral agent for prostate cancer. However, further biological work is urgently needed to elucidate the mechanism of action. Our efforts are now focused on evaluating its effects on apoptosis, oxidative stress, and cell cycle. These results have encouraged us

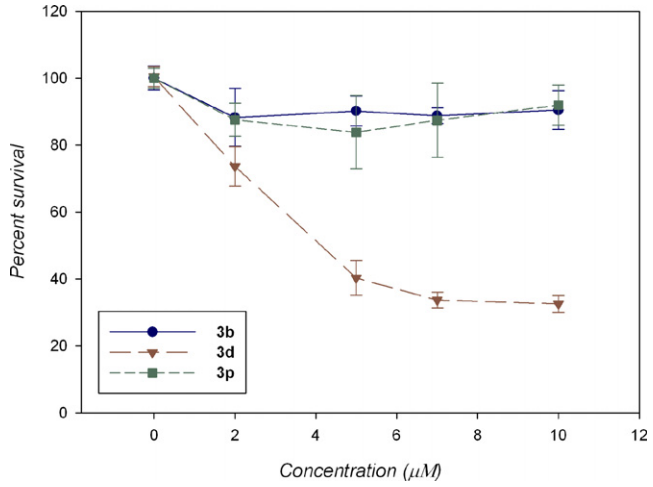


Figure 2. Effects of compounds **3b**, **3d**, and **3p** on non-tumoral prostate cells (RWPE-1). Data are expressed as percent survival and error bars show SD ($n = 4$).

to carry out further work in the general area of selenocompounds.

Acknowledgments

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36. General procedure for the synthesis of compounds (**1a**–**d**):To a cooled (0 °C), stirred mixture of thiourea (2.47 g, 32.5 mmol) or selenourea (4.0 g, 32.5 mmol) in dry ethanol (25 mL) was added dropwise the respective alkyl iodide (45.0 mmol). The mixture was heated under reflux for 90 min. The solvent was removed in vacuo and the product recrystallized from ethanol.
Methyl imidothiocarbamate hydroiodide, **1a**:IR (KBr):3311–3102, 1638 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ):2.57 (s, 3H, S–CH_3), 8.97 (br s, 4H, NH·HI, NH_2). Anal. Calcd for $C_2H_6N_2S$·HI (%):C, 15.52; H, 3.88; N, 12.07. Found:C, 15.65; H, 3.86; N, 12.05.
Methyl imidoselenocarbamate hydroiodide, **1b**:IR (KBr):3319–3102, 1635 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ):2.47 (s, 3H, Se–CH_3); 6.57 (br s, 1H, NH); 9.00 (br s, 2H, NH_2). Anal. Calcd for $C_2H_6N_2Se$·0.8-HI·0.3NH_3 (%):C, 9.81; H, 3.14; N, 13.16. Found:C, 10.04; H, 2.66; N, 13.44.

Ethyl imidoselenocarbamate hydroiodide, **1c**:IR (KBr):3266–3101, 1636 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ):1.42 (t, 3H, Se–CH_2–CH_3); 3.18 (q, 2H, Se–CH_2-CH_3); 6.58 (br s, 1H, NH); 9.06 (br s, 2H, NH_2). Anal. Calcd for $C_3H_8N_2Se{\cdot}0.85HI{\cdot}0.55NH_3$ (%):C, 13.37; H, 3.90; N, 13.26. Found:C. 13.61; H, 3.59; N, 13.00.

Isopropyl imidoselenocarbamate hydroiodide, **1d**:IR (KBr):3262–3108, 1643 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ):1.46 [d, 6H, Se–CH–(CH_3)$_2$]; 4.07 [m, 1H, Se–CH–(CH_3)$_2$]; 6.58 (br s, 1H, NH); 9.14 (br s, 2H, NH_2). Anal. Calcd for $C_4H_{10}N_2Se{\cdot}0.9HI{\cdot}0.5NH_3$ (%):C, 16.62; H, 3.94; N, 12.12. Found:C, 16.84; H, 3.80; N, 12.36. General procedure for the synthesis of compounds (**3a**–**q**):A solution of the corresponding acyl chloride **2a**–**i** (6.87 mmol) in chloroform (10 mL) was slowly added dropwise to a stirred solution of compounds **1a**–**d** (3.27 mmol) in dry chloroform (15 mL) and pyridine (5 mL). The mixture was stirred for 15–60 h at room temperature. Solvents were removed under vacuum by rotatory evaporation and the residue was treated with water (50 mL) and purified as indicated in Table 1.

Methyl N,N′-bis(2-chloropyridine-3-carbonyl)-imidothiocarbamate, **3a**:IR (KBr):3426, 1697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ):2.61 (s, 3H, S–CH_3); 7.37 (dd, 1H, J_{5-4} = 6.9 Hz, J_{5-6} = 4 Hz, H_5); 7.45 (dd, 1H, $J_{5'-4'}$ = 6.9 Hz, $J_{5'-6'}$ = 4 Hz, $H_{5'}$); 8.11 (d, 1H, H_4); 8.40 (d, 1H, $H_{4'}$); 8.52 (d, 1H, $H_{6'}$); 8.61 (d, 1H, H_6); 13.72 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{10}Cl_2N_4O_2S$ (%):C, 45.54; H, 2.73; N, 15.17. Found:C, 45.26; H, 2.78; N, 14.96.

Methyl N,N′-bis(2-chloropyridine-3-carbonyl)-imidoselenocarbamate, **3b**:IR (KBr):3419, 1688 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ):2.46 (s, 3H, Se–CH_3); 7.37 (dd, 1H, J_{5-4} = 7.4 Hz, J_{5-6} = 4.1 Hz, H_5); 7.46 (dd, 1H, $J_{5'-4'}$ = 7.5 Hz, $J_{5'-6'}$ = 4.1 Hz, $H_{5'}$); 8.14 (d, 1H, H_4); 8.43 (d, 1H, $H_{4'}$); 8.53 (d, 1H, $H_{6'}$); 8.62 (d, 1H, H_6); 13.88 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{10}Cl_2N_4O_2Se$ (%):C, 40.41; H, 2.42; N, 13.46. Found:C, 40.23; H, 2.34; N, 13.22.

Methyl N,N′-bisbenzoylimidothiocarbamate, **3c**:IR (KBr):3430, 1700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ):2.69 (s, 3H, S–CH_3); 7.51 (m, 2H, $H_3 + H_5$); 7.58 (m, 3H, $H_{3'} + H_{5'} + H_4$); 7.67 (m, 1H, $H_{4'}$); 8.07 (d, 2H,$J_{2'-3'} = J_{6'-5'}$ = 8.4 Hz, $H_{2'} + H_{6'}$); 8.37 (d, 2H, $J_{2-3} = J_{6-5}$ = 8.4 Hz, $H_2 + H_6$). Anal. Calcd for $C_{16}H_{14}N_2O_2S$ (%): C, 62.54; H, 4.72; N, 9.12. Found: C, 62.54; H, 4.48; N, 8.99.

Methyl N,N′-bisbenzoylimidoselenocarbamate, **3d**: IR (KBr): 3446, 1691 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 2.53 (s, 3H, Se–CH_3); 7.51 (m, 2H, $H_3 + H_5$); 7.58 (m, 3H, $H_{3'} + H_{5'} + H_4$); 7.67 (m, 1H, $H_{4'}$); 8.07 (d, 2H, $J_{2'-3'} = J_{6'-5'}$ = 7.1 Hz, $H_{2'} + H_{6'}$); 8.38 (d, 2H, $J_{2-3} = J_{6-5}$ = 7.1 Hz, $H_2 + H_6$); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 8.9 (Se–CH_3); 128.5 ($C_4 + C_6$); 128.8 ($C_{4'} + C_{6'}$); 129.6 ($V_3 + C_{3'} + C_7 + C_{7'}$); 130.9 ($C_5$); 131.8 ($C_{5'}$); 134.2 ($C_2$); 136.9 ($C_{2'}$); 166.3 ($C_1$); 172.8 (C–Se); 176.6 ($C_{1'}$). Anal. Calcd for $C_{16}H_{14}N_2O_2Se$ (%): C, 55.65; H, 4.06; N, 8.12. Found: C, 55.57; H, 4.19; N, 8.03.

Ethyl N,N′-bisbenzoylimidoselenocarbamate, **3e**: IR (KBr): 3452,1694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 1.62 (t, 3H, J = 7.5 Hz, Se–CH_2-CH_3); 3.21 (q, 2H, J = 7.5 Hz, Se–CH_2-CH_3); 7.51 (m, 2H, $H_3 + H_5$); 7.58 (m, 3H, $H_{3'} + H_{5'} + H_4$); 7.67 (m, 1H, $H_{4'}$); 8.07 (d, 2H, $J_{2'-3'} = J_{6'-5'}$ = 7.4 Hz, $H_{2'} + H_{6'}$); 8.36 (d, 2H, $J_{2-3} = J_{6-5}$ = 7.4 Hz, $H_2 + H_6$). Anal. Calcd for $C_{17}H_{16}N_2O_2Se$ (%): C, 56.82; H, 4.46; N, 7.80. Found: C, 56.64; H, 4.46; N, 7.85.

Isopropyl N,N′-bisbenzoylimidoselenocarbamate, **3f**: IR (KBr): 3430, 1694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 1.65 [d, 6H, J = 7.0 Hz, Se–CH–(CH_3)$_2$]; 4.26 [sept, 1H, J = 7.0 Hz, Se–CH–(CH_3)$_2$]; 7.51 (m, 2H, $H_3 + H_5$); 7.58 (m, 3H, $H_{3'} + H_{5'} + H_4$); 7.67 (m, 1H, $H_{4'}$); 8.07 (d, 2H, $J_{2'-3'} = J_{6'-5'}$ = 7.2 Hz, $H_{2'} + H_{6'}$); 8.36 (d, 2H, $J_{2-3} = J_{6-5}$ = 7.2 Hz, $H_2 + H_6$). Anal. Calcd for $C_{18}H_{18}N_2O_2Se$ (%): C, 57.91; H, 4.83; N, 7.51. Found: C, 57.70; H, 4.86; N, 7.51.

Methyl N,N′-bis(3,5-dimethoxybenzoyl)imidoselenocarbamate, **3g**: IR (KBr): 3417, 1688 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$,δ): 2.51 (s, 3H, Se–CH_3); 3.89 (s, 12H, OCH_3); 6.70 (s, 1H, H_4); 6.72 (s, 1H, $H_{4'}$); 7.17 (s, 2H, $H_{2'} + H_{6'}$); 7.75 (s, 2H, $H_2 + H_6$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.9 (Se–CH_3); 56.0 (4[OCH_3]); 106.0 ($C_{5'}$); 108.1 (C_5); 129.2 ($C_{3'} + C_{7'}$); 131.3 ($C_3 + C_7$); 133.8 ($C_{2'}$); 138.9 (C_2); 161.0 ($C_4 + C_6 + C_{4'} + C_{6'}$); 166.2 ($C_1$); 172.5 (C–Se); 176.0 ($C_{1'}$). Anal. Calcd for $C_{20}H_{22}N_2O_6Se$ (%): C, 51.61; H, 4.73; N, 6.02. Found (%): C, 51.29; H, 4.48; N, 5.78.

Isopropyl N,N′-bis(3,5-dimethoxybenzoyl)imidoselenocarbamate, **3h**: IR (KBr): 3414, 1689 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 1.64 [s, 6H, Se–CH–(CH_3)$_2$]; 3.89 (s, 12H, O–CH_3); 4.21 [m, 1H, Se–CH–(CH_3)$_2$]; 6.69 (d, 1H, $J_{4-2} = J_{4-6}$ = 2 Hz, H_4); 6.72 (d, 1H, $J_{4'-2'} = J_{4'-6'}$ = 2 Hz,$H_{4'}$); 7.17 (d, 2H, $H_{2'} + H_{6'}$); 7.52 (d, 2H, $H_2 + H_6$). Anal. Calcd for $C_{22}H_{26}N_2O_6Se$ (%): C, 53.55; H, 5.27; N, 5.68. Found: C, 53.23; H, 4.98; N, 5.58.

Methyl N,N′-bis(4-chlorobenzoyl)imidothiocarbamate, **3i**: IR (KBr): 1703 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 2.67 (s, 3H, S–CH_3); 7.47 (d,2H, $H_3 + H_5 J_{3-4}$ = 8.6 Hz); 7.55 (d, 2H, $H_{3'} + H_{5'}$); 7.99 (d, 2H, $H_{2'} + H_{6'}$); 8.28 (d, 2H, $H_2 + H_6$). Anal Calcd for $C_{16}H_{12}Cl_2N_2O_2S$ (%): C, 52.32; H, 3.27; N, 7.63. Found: C, 52.41; H, 3.26; N, 7.78.

Methyl N,N′-bis(4-chlorobenzoyl)imidoselenocarbamate, **3j**: IR (KBr): 3413, 1691 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 2.51 (s, 3H, Se–CH_3); 7.47 (br s, 2H, $H_3 + H_5$); 7.54 (br s, 2H, $H_{3'} + H_{5'}$); 7.99 (br s, 2H, $H_{2'} + H_{6'}$); 8.27 (br s, 2H, $H_2 + H_6$); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 9.0 (Se–CH_3); 129.3 ($C_{3'} + C_{7'} + C_4 + C_6 + C_{4'} + C_{6'}$); 129.9 ($C_3 + C_7$); 132.2 ($C_2$); 135.2 ($C_{2'}$); 140.0 ($C_5$); 140.8 ($C_{5'}$); 165.2 ($C_1$); 173.6 (C–Se); 175.7 ($C_{1'}$). Anal. Calcd for $C_{16}H_{12}Cl_2N_2O_2Se$ (%): C, 46.38; H, 2.90; N, 6.76. Found: C, 46.06; H, 2.81; N, 6.72.

Isopropyl N,N′-bis(4-chlorobenzoyl)imidoselenocarbamate, **3k**: IR (KBr): 3414, 1692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 1.64 [d, 6H, Se–CH–(CH_3)$_2$, J = 7.0 Hz]; 4.21 [m, 1H, Se–CH–(CH_3)$_2$]; 7.48 (d, 2H, J_{3-2} = 6.6 Hz, $H_3 + H_5$);7.55 (d, 2H, $J_{3'-2'}$ = 6.6 Hz, $H_{3'} + H_{5'}$); 7.99 (d, 2H, $H_{2'} + H_{6'}$); 8.26 (d, 2H, $H_2 + H_6$). Anal. Calcd for $C_{18}H_{16}Cl_2N_2O_2Se$ (%): C, 48.87; H, 3.62; N, 6.33. Found: C, 48.60; H, 3.47; N, 6.21.

Methyl N,N′-bis(4-nitrobenzoyl)imidoselenocarbamate, **3l**: IR (KBr): 3414, 1691, 1529 cm^{-1}: ^1H NMR (400 MHz, DMSO-d_6, δ): 2.55 (s, 3H, Se–CH_3); 8.11 (m, 4H, $H_3 + H_5 + H_{3'} + H_{5'}$); 8.36 (br s, 4H, $H_2 + H_6 + H_{2'} + H_{6'}$). Anal. Calcd for $C_{16}H_{12}N_4O_6Se{\cdot}HCl$ (%): C, 40.72; H, 2.76; N, 11.88. Found: C, 40.73; H, 2.57; N, 11.78.

Methyl N,N′-bis(4-trifluoromethylbenzoyl)imidoselenocarbamate, **3m**: IR (KBr): 3451, 1692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 2.54 (s, 3H, Se–CH_3); 7.76 (d, 2H, $J_{3-2} = J_{5-6}$ = 7.3 Hz, $H_3 + H_5$); 7.86 (d, 2H, $J_{3'-2'} = J_{5'-6'}$ = 7.5 Hz, $H_{3'} + H_{5'}$); 8.17 (d, 2H, $H_{2'} + H_{6'}$); 8.45 (d, 2H, $H_2 + H_6$). Anal. Calcd for $C_{18}H_{12}F_6N_2O_2Se$ (%): C, 44.92; H, 2.51; N, 5.82. Found: C, 44.96; H, 2.56; N, 5.92.

Methyl N,N′-bis(4-cyanobenzoyl)imidoselenocarbamate, **3n**: IR (KBr): 3416, 2230, 1697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 2.56 (s, 3H, Se–CH_3); 7.81 (d, 2H, $J_{3-2} = J_{5-6}$ = 8.0 Hz, $H_3 + H_5$); 7.90 (d, 2H, $J_{3'-2'} = J_{5'-6'}$ = 8.0 Hz, $H_{3'} + H_{5'}$); 8.16 (d, 2H,

$H_{2'} + H_{6'}$); 8.45 (d, 2H, $H_2 + H_6$). Anal. Calcd for $C_{18}H_{12}N_4O_2Se$ (%): C, 54.70; H, 3.06; N, 14.18. Found: C, 54.74; H, 3.24; N, 14.13.

Methyl N,N′-bis(4-methylbenzoyl)imidothiocarbamate, **3o**: IR (KBr): 3450, 1688 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 2.46 (s, 6H, Ph–CH_3), 2.66 (s, 3H, S–CH_3), 7.29 (d, 2H, $J_{3-2} = 8.2$ Hz, $H_3 + H_5$), 7.36 (d, 2H, $J_{3'-2'} = 8.2$ Hz, $H_{3'} + H_{5'}$), 7.95 (d, 2H, $H_{2'} + H_{6'}$), 8.25 (d, 2H, $H_2 + H_6$). Anal. Calcd for $C_{18}H_{18}N_2O_2S$ (%): C, 66.26; H, 5.52; N, 8.59. Found: C, 66.01; H, 5.64; N, 8.37.

Methyl N,N′-bis(4-methylbenzoyl)imidoselenocarbamate, **3p**: IR (KBr): 3446, 1679 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 2.46 (s, 6H, Ph–CH_3); 2.51 (s, 3H, Se–CH_3); 7.30 (d, 2H, $J_{3-2} = 7.4$ Hz, $H_3 + H_5$); 7.36 (d, 2H, $J_{3'-2'} = 7.4$ Hz, $H_{3'} + H_{5'}$); 7.96 (d, 2H, $H_{2'} + H_{6'}$); 8.27 (d, 2H, $H_2 + H_6$). Anal. Calcd for $C_{18}H_{18}N_2O_2Se$ (%): C, 57.92; H, 4.86; N, 7.51. Found: C, 58.33; H, 4.64; N, 7.56.

Methyl N,N′-bis(4-tert-butylbenzoyl)imidoselenocarbamate, **3q**: IR (KBr): 3448, 2957–2867, 1692 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ): 1.38 [s, 18H, C-$(CH_3)_3$]; 2.51 (s, 3H, Se–CH_3); 7.53 (d, 2H, $J_{3-2} = 8.1$ Hz, $H_3 + H_5$); 7.59 (d, 2H, $J_{3'-2'} = 8.1$ Hz, $H_{3'} + H_{5'}$); 8.00 (d, 2H, $H_{2'} + H_{6'}$); 8.31 (d, 2H, $H_2 + H_6$). Anal. Calcd for $C_{24}H_{30}N_2O_2Se$ (%): C, 63.02; H, 6.61; N, 6.12. Found: C, 62.97; H, 6.74; N, 6.07.

37. *Materials and methods for cytotoxicity assay (MTT assay):* PC-3 cells were seeded in 96-well plates (*Millipore, Eschborn, Germany*) at a density of 5×10^3 cells per well in 200 μL of complete medium and the samples were incubated at 37 °C under 5% CO_2 overnight prior to the addition of the compounds. After 72 h of incubation with the compounds, 10 μL MTT solution (5 mg/mL in PBS) was added to each well and these were stored for an additional 4 h at 37 °C, 5% CO_2. The absorbance of formazan at $\lambda = 570$ nm was measured on a *Polarstar Galaxy plate reader* (*BMG LabTechnologies GmbH*). The percentage of viable cells was calculated to obtain IC_{50}-values in comparison to untreated control cells. PC-3 cells (human tumorigenic and metastatic prostate cancer cells) were obtained from American Type Culture Collection (ATCC), Manassas, USA, and cultured under standard conditions (Dulbecco's RPMI 1640 medium, with GlutamaxTM 1, Invitrogen) supplemented with 10% fetal bovine serum (Fetalclone III, SH30109.03, HYCLONE) and 1% Penicillin–Streptomycin (Invitrogen). RWPE-1 cells (non-tumorigenic human prostate cells) were also obtained from ATCC and cultured in keratinocyte-SFM Kit (Invitrogen) supplemented with 1% fetal bovine serum, Fetalclone III, SH30109.03, HYCLONE, and 1% Penicillin–Streptomycin (Invitrogen).
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