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Synthesis and cytotoxicity of aminomethylselenopheno[3,2-*b*]thiophene sulfonamides

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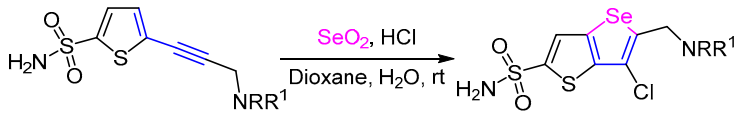


The reaction of 5-[(aminomethyl)ethynyl]thiophene-2-sulfonamides with *in situ* generated selenium(IV) chloride was used to synthesize 5-aminomethyl-substituted 6-chloroselenopheno[3,2-*b*]thiophene-2-sulfonamides. The cytotoxicity of these compounds was studied against HT-1080 (human fibrosarcoma), MH-22A (mouse hepatoma), CCL-8 (mouse sarcoma), MES-SA (human uterine sarcoma), MCF-7 (estrogen receptor-positive human breast adenocarcinoma) cell lines, as well as the normal NIH 3T3 cell line (mouse fibroblasts).

Keywords: selenium, selenophene, sulfonamide, thiophene, cytotoxicity, electrophilic addition, intramolecular cyclization.

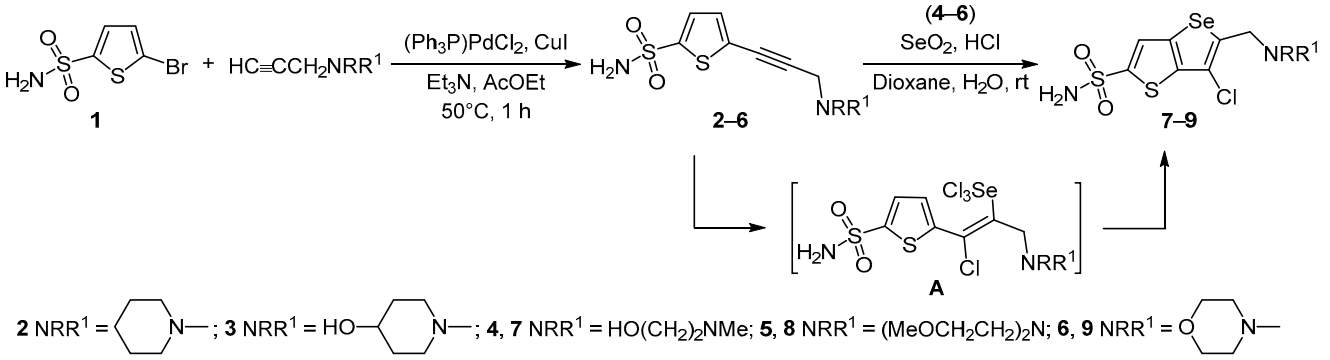
Selenium, its organic and inorganic derivatives are generally known to exhibit a broad range of biological activity. It has been established that selenium as nutritional supplement strengthens the immune system and reduces the incidence of oncological diseases.[1,2] Certain organic selenium derivatives show antitumor,[3–8] antiviral,[9] and antimicrobial[10,11] activity. It has been shown that selenium-containing compounds have a remarkable ability to affect the activity of redox enzymes, such as glutathione peroxidase and reductase, by modulation of thioredoxin system activity.[12–14] It should be noted that selenium halides are widely used as reagents in organic synthesis that add at double and triple bonds.[15–18]

In general the interest toward chemistry of selenium-containing heterocyclic systems is largely motivated by the diverse biological activity of such compounds[19–21] and by the possible use as synthons in the construction of π-systems.[22–24] For example, selenopheno[3,2-*b*]thiophenes have attracted attention because their structural analogs, 5-substituted thieno[3,2-*b*]thiophene-2-sulfonamides, are active as drugs for the treatment of glaucoma; the best results were observed with bis(2-methoxyethyl)aminomethyl-substituted derivatives.[25,26] However, the methods of synthesis and biological properties of the structurally related selenophenothiophenes have not yet been described.

This work was devoted to the synthesis and cytotoxicity studies of new heterocyclic compounds, derivatives of 6-chloroselenopheno[3,2-*b*]thiophene sulfonamides. We developed a new two-step synthetic route for the preparation of these compounds. The first step was a reaction of 5-bromo-2-thiophene sulfonamide (**1**) with terminal alkynes, catalyzed by bis(triphenylphosphino)-palladium dichloride and copper(I) iodide, which gave substituted 5-[(aminomethyl)ethynyl]thiophene-2-sulfonamides **2**–**6** in 69–99% yields (Scheme 1).[27] The sulfonamide group did not require additional protection when the reaction was performed in a mixture of anhydrous ethyl acetate and triethylamine. The avoidance of more toxic solvents, such as dimethylformamide, dimethyl acetamide or secondary amines, provided environmental and energy-saving advantages to this method. The selected reaction conditions allowed to decrease the reaction time for the formation of ethynylthiophenes **2**–**6** to 1 h.

In the second step, dioxane solutions of ethynylthiophenes **2**–**6** were added dropwise to a solution of selenium tetrachloride, which was prepared *in situ* from selenium dioxide and concentrated hydrochloric acid. The addition of $SeCl_4$ to the multiple bonds of compounds **2**–**6** probably produced intermediates **A**, which underwent intramolecular cyclization to selenophenes **7**–**9**. It should be noted that the *in situ* prepared selenium(IV) chloride was less reactive than $SeBr_4$.[27,28] However, that should be considered as an advantage in this case, providing for fewer side reactions. As a result, the isolation of chloro-substituted selenophenothiophenes **7**–**9** did not present any major difficulties, but the yields (31–69%) were somewhat

Scheme 1



lower, compared to the bromo-substituted analog of compound **8** (84%).[27] The yield of compound **7** was lower than in the case of compounds **8** and **9**, as there was a possibility of selenium tetrachloride reaction with the hydroxyethyl group.

Prior to biological testing, the synthesized compounds **2**–**6** were treated with HCl solution in ether, giving the respective salts **2**·HCl–**6**·HCl, well soluble under physiological conditions.

The cytotoxic properties of compounds **2**–**6**, **8**, **9** were studied *in vitro* with the following tumor cell lines: HT-1080 (human fibrosarcoma), MH-22A (mouse hepatoma), CCL-8 (mouse sarcoma), MES-SA (human uterine sarcoma), MCF-7 (estrogen receptor-positive human breast adenocarcinoma), as well as the normal NIH 3T3 cell line (mouse fibroblasts). The concentrations of study compounds that were lethal to 50% of the cells *in vitro* (IC_{50}) (Table 1) were determined according to the standard method by the intensity of cell wall staining in the presence of Crystal violet and mitochondrial enzyme staining in the presence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.[29]

According to the obtained data, 5-[(aminomethyl)ethynyl]thiophene-2-sulfonamides **2**–**6** did not show pronounced cytotoxic activity against tumor cells. It should be taken into account that the studied derivatives had lower acute toxicity *in vitro* (LD_{50} 1684–2195 mg/kg). At the same time, the introduction of selenophene ring noticeably increased the cytotoxic effect. Thus, the mouse hepatoma cell line MH-22A and human uterine sarcoma cell line MES-SA were sensitive to bis(2-methoxyethyl)aminomethyl and morpholine derivatives of 6-chloroselenopheno[3,2-*b*]thiophene-2-sulfonamide (compounds **8** and **9**, IC_{50} 68–79 μM). In addition, the derivative **8** showed inhibitory activity against human fibrosarcoma cell line HT-1080 (IC_{50} 65 μM) and mouse sarcoma cell line CCL-8 (IC_{50} 92 μM). The investigated selenophenothiophene sulfonamides were not toxic to normal cells (LD_{50} 1159–1559 mg/kg).

Thus, we have developed a convenient method for the synthesis of new selenophene-containing fused heterocycles, 3-chloroselenopheno[3,2-*c*]thiophene sulfonamides. The method for the preparation of selenophenothiophenes is simple, does not require unusual reaction conditions and can be applied to the synthesis of a wider range of compounds. The cytotoxic activity of the synthesized 3-chloroselenopheno[3,2-*c*]thiophenes was studied *in vitro.* The obtained results indicate that this type of compounds is nontoxic to normal cells *in vitro* and therefore may serve as a promising lead for further studies.

Experimental

^1H and ^{13}C NMR spectra were acquired on a Varian Mercury 400 instrument (400 and 100 MHz, respectively), with residual proton signals (δ 2.50 ppm for DMSO-d_6, δ 7.26 ppm for $CDCl_3$) or signals of ^{13}C nuclei of the deuterated solvent (δ 39.4 ppm for DMSO-d_6, δ 77.2 ppm for $CDCl_3$) as internal standard. Mass spectra were recorded with a Waters Micromass 3100 Mass Detector coupled to a Waters Acquity UPLC system, using an

Table 1. The *in vitro* cytotoxic activity of sulfonamides **2-9**

Compound	Cell line						
	HT-1080	MH-22A	MES-SA	MCF-7	CCL-8	NIH 3T3	NIH 3T3
	IC_{50}, μM	IC_{50}, μM	IC_{50}, μM	IC_{50}, μM	IC_{50}, μM	IC_{50}, μM	LD_{50}, mg/kg
2·HCl	311	>400	>400	>400	310	302	1989
3·HCl	279	>400	>400	>400	>400	>400	1684
4·HCl	>400	>400	>400	>400	>400	>400	2195
5·HCl	279	>400	>400	>400	>400	>400	1927
6·HCl	>400	>400	>400	>400	>400	>400	2177
8	65	68	73	208	92	182	1159
9	240	77.5	79	>400	236	>400	1559

Acquity UPLC BEH C18 column (1.7 μm, 2.1×50 mm, 0.4 ml/min), MeCN (from 5 to 100%, 8 min) – HCOOH (0.5% in H_2O). Elemental analysis was performed on a Carlo Erba EA 1108 elemental analyzer. The reaction progress and purity of the obtained compounds were controlled by TLC on Merck Kieselgel plates, visualization under UV light. Propargylamines were obtained by reactions of propargyl bromide with secondary amines. The tumor cell lines were obtained from the collection of ATCC (American Type Culture Collection).

Synthesis of 5-[(aminomethyl)ethynyl]thiophene-2-sulfonamides 2–6 and their hydrochlorides (General method). $Pd(PPh_3)_2Cl_2$ (26.2 mg, 0.1 mmol) and CuI (9.5 mg, 0.05 mmol) were added to triethylamine (1 ml) under argon atmosphere and stirred for 5 min. Then a solution of 5-bromo-2-thiophene sulfonamide (**1**) (242 mg, 1 mmol) in EtOAc (5 ml) and the appropriate tertiary propargylamine (2 mmol) were added. The reaction mixture was stirred for 1 h at 50°C under inert atmosphere. The reaction mixture was then cooled, diluted with EtOAc, and treated with a few drops of aqueous ammonia, stirred for 5–10 min and filtered through a thin layer of silica gel. The solution was evaporated to dryness and the product was isolated by silica gel column chromatography (eluent CH_2Cl_2–EtOAc–MeOH). In order to obtain hydrochlorides, the isolated product was dissolved in 1:1 EtOAc–Et_2O mixture and treated with anhydrous ethereal HCl solution to pH 1. The mixture was stirred until the reaction was complete (TLC control), evaporated to dryness, and diluted with anhydrous ether. The precipitate was collected by filtration and dried.

5-[3-(Piperidin-1-yl)prop-1-yn-1-yl]thiophene-2-sulfonamide (2). Yield 98%, beige powder, mp 118–120°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 1.31–1.34 (2H, m, CH_2); 1.52–1.59 (4H, m, $2CH_2$); 2.49–2.58 (4H, m, CH_2NCH_2); 3.60 (2H, s, CH_2N); 7.30 (1H, d, *J* = 3.7, H-4), 7.46 (1H, d, *J* = 3.7, H-3); 7.80 (2H, s, NH_2). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 23.4; 25.4; 47.5; 52.6; 76.4, 93.0, 126.3; 129.9; 135.9; 145.5. Mass spectrum, *m/z* (I_{rel}, %): 285 $[M+H]^+$ (100). **5-[3-(Piperidin-1-yl)prop-1-yn-1-yl]thiophene-2-sulfonamide hydrochloride (2·HCl)**. Yield 99%, white powder, mp 175–178°C. Found, %: C 44.81; H 5.50; N 8.26. $C_{12}H_{17}ClN_2O_3S_2$. Calculated, %: C 44.92; H 5.34; N 8.73.

5-[3-(4-Hydroxypiperidin-1-yl)prop-1-yn-1-yl]thiophene-2-sulfonamide (3). Yield 75%, beige powder, mp 138–140°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 1.18–1.40 (2H, m, CH_2); 1.51–1.71 (2H, m, CH_2); 2.28–2.48 (2H, m) and 2.75–2.88 (2H, m, CH_2NCH_2); 3.50 (1H, br. s, C<u>H</u>OH); 3.68 (2H, s, CH_2N); 4.64 (1H, br. s, OH); 7.31 (1H, d, *J* = 3.9, H-4); 7.46 (1H, d, *J* = 3.9, H-3); 7.80 (2H, s, NH_2). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 33.6; 45.6; 47.9; 49.5; 65.4; 109.9; 125.9; 129.9; 132.4; 145.9. Mass spectrum, *m/z* (I_{rel}, %): 301 $[M+H]^+$(100). **5-[3-(4-Hydroxypiperidin-1-yl)prop-1-yn-1-yl]thiophene-2-sulfonamide hydrochloride hydrate (3·HCl·H_2O)**. Yield 99%, white powder, mp 80–82°C. Found, %: C 40.70; H 5.09; N 7.42. $C_{12}H_{17}ClN_2O_3S_2$. Calculated, %: C 40.61; H 5.40; N 7.89.

5-{3-[2-Hydroxyethyl(methyl)amino]prop-1-yn-1-yl}thiophene-2-sulfonamide (4). Yield 69%, beige powder, mp 128–130°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 2.27 (3H, s, NCH_3), 2.47–2.48 (2H, m, C<u>H</u>$_2$OH); 3.47–3.51 (2H, m, NC<u>H</u>$_2$CH$_2$); 3.61 (2H, s, CH_2N); 4.44–4.47 (1H, m, OH); 7.28 (1H, d, *J* = 3.8, H-4); 7.45 (1H, d, *J* = 3.8, H-3); 7.79 (2H, s, NH_2). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 41.9; 46.3, 57.7; 58.9; 76.6; 92.8; 126.3, 129.9; 132.0; 145.5. Mass spectrum, *m/z* (I_{rel}, %): 275 $[M+H]^+$ (100). **5-{3-[2-Hydroxyethyl(methyl)amino]-prop-1-yn-1-yl}thiophene-2-sulfonamide hydrochloride (4·HCl)**. Yield 99%, white powder, mp 188–190°C.

5-{3-[Bis(2-methoxyethyl)amino]prop-1-yn-1-yl}thiophene-2-sulfonamide (5). Yield 99%, yellow oil. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 2.67 (4H, t, *J* = 5.7, 2NCH$_2$C<u>H</u>$_2$O); 3.24 (6H, s, $2OCH_3$); 3.42 (4H, t, *J* = 5.7, 2NC<u>H</u>$_2$CH$_2$O); 3.71 (2H, s, CH_2N); 7.27 (1H, d, *J* = 3.8, H-4); 7.45 (1H, d, *J* = 3.8, H-3); 7.79 (2H, s, NH_2). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 43.7; 52.8; 57.9; 70.5; 76.4; 93.1; 126.4; 129.9; 132.0; 145.5. Mass spectrum, *m/z* (I_{rel}, %): 333 $[M+H]^+$ (100). **5-{3-[Bis-(2-methoxyethyl)-amino]prop-1-yn-1-yl}thiophene-2-sulfonamide hydrochloride (5·HCl)**. Yield 99%, white powder, mp 140–145°C. Found, %: C 42.23; H 5.83; N 7.28. $C_{13}H_{21}ClN_2O_4S_2$. Calculated, %: C 42.33; H 5.74; N 7.59.

5-[3-(Morpholin-4-yl)prop-1-yn-1-yl]thiophene-2-sulfonamide (6). Yield 60%, beige powder, mp 120–122°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 2.62 (4H, t, *J* = 4.7, CH_2OCH_2); 3.54 (2H, s, CH_2N); 3.76 (4H, t, *J* = 4.7, CH_2NCH_2); 4.98 (2H, br. s, NH_2); 7.09 (1H, d, *J* = 3.8, H-4); 7.49 (1H, d, *J* = 3.8, H-3). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 47.0; 51.6; 65.9; 76.8; 92.3; 126.1; 129.9; 132.2; 145.7. Mass spectrum, *m/z* (I_{rel}, %): 287 $[M+H]^+$. **5-[3-(Morpholin-4-yl)prop-1-yn-1-yl]thiophene-2-sulfonamide hydrochloride (6·HCl)**. Yield 99%, white powder, mp 175–178°C. Found, %: C 40.62; H 4.71; N 8.26. $C_{11}H_{15}ClN_2O_3S_2$. Calculated, %: C 40.92; H 4.68; N 8.68.

Synthesis of selenophenothiophenes 7–9 (General method). A solution of the appropriate 5-[(aminomethyl)-ethynyl]thiophene-2-sulfonamide **4**–**6** (0.7 mmol) in dioxane (10 ml) was added dropwise to a solution of $SeCl_4$ that was prepared *in situ* from SeO_2 (46 mg, 0.42 mmol) and 10 drops of concd. HCl. The reaction progress was controlled by TLC. After the cyclization reaction was complete, the reaction mixture was treated with EtOAc and solid Na_2CO_3. The mixture was filtered through a thin layer of silica gel. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness.

6-Chloro-5-{[2-hydroxyethyl(methyl)amino]methyl}-selenopheno[3,2-*b*]thiophene-2-sulfonamide (7). Yield 31%, yellow powder, mp 84–86°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 2.46 (3H, s, CH_3); 2.58–2.68 (2H, m, C<u>H</u>$_2$OH); 3.52–3.61 (2H, m, NC<u>H</u>$_2$CH$_2$); 3.85–3.40 (2H, m, CH_2N); 4.51–4.60 (1H, m, OH); 7.84 (2H, s, NH_2); 7.97 (1H, s, H Ar). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 52.7; 56.3; 58.9; 67.3; 102.4; 127.5; 133.8; 140.3; 144.5; 146.9. Mass spectrum, *m/z* (I_{rel}, %): 385 $[M+H]^+$ (20), 387 $[M+H]^+$ (40), 389 $[M+H]^+$ (100), 391 $[M+H]^+$ (30).

6-Chloro-5-{[bis(2-methoxyethyl)amino]methyl}selenopheno[3,2-*b*]thiophene-2-sulfonamide (8). Yield 69%, yellow powder, mp 86–88°C. ^{1}H NMR spectrum ($CDCl_3$),

δ, ppm (*J*, Hz): 2.86 (4H, t, *J* = 5.7, $2NCH_2C\underline{H_2}O$); 3.33 (6H, s, $2OCH_3$); 3.52 (4H, t, *J* = 5.7, $2NC\underline{H_2}CH_2O$); 4.02 (2H, s, CH_2N); 5.21 (2H, br. s, NH_2); 7.81 (1H, s, H Ar). ^{13}C NMR spectrum ($CDCl_3$) δ, ppm: 54.3; 54.5; 58.7; 74.1; 113.5; 127.6; 133.3; 142.3; 143.4; 150.8. Mass spectrum, *m/z* (I_{rel}, %): 443 $[M+H]^+$ (20), 445 $[M+H]^+$ (50), 447 $[M+H]^+$ (100), 449 $[M+H]^+$ (30).

6-Chloro-5-(morpholin-4-ylmethyl)selenopheno[3,2-*b*]-thiophene-2-sulfonamide (9). Yield 59%, yellow powder, mp 170–172°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 2.76–3.30 (2H, m, CH_2N); 3.59–3.84 (4H, m, CH_2OCH_2); 4.06–4.71 (4H, m, CH_2NCH_2); 7.91 (2H, s, NH_2); 8.02 (1H, s, H Ar). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 54.2; 58.1; 70.8; 111.5; 127.5; 133.8; 140.6; 144.1; 151.3. Mass spectrum, *m/z* (I_{rel}, %): 397 $[M+H]^+$ (20), 399 $[M+H]^+$ (50), 401 $[M+H]^+$ (100), 403 $[M+H]^+$ (30).

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