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Synthesis and antiproliferative activity *in vitro* of diacetylenic thioquinolines*

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A series of new acetylenic thioquinolines containing propargyl, 2-butynyl, or 4-bromo-2-butynyl groups has been prepared and tested for antiproliferative activity *in vitro* against the cells of human [SW707 (colon cancer), CCRF/CEM (leukemia)] and murine [P388 (leukemia), B16 (melanoma)] cancer lines. All the compounds obtained exhibited antiproliferative activity. The most active compounds **4h** and **4l–m** have ID_{50} values ranging from 0.2 to 3.6 μg/ml, comparable to that of the reference compound cisplatin.

1. Introduction

Acetylenic derivatives are an important class of compounds that has attracted increasing attention as a source of new anticancer agents. The synthetic methods for their preparation are of interest especially with regard to the synthesis of biologically active enediyne antitumor antibiotics or similar model molecules (Grissom et al. 1996; Jones and Found 2002; Nicolaou and Dai 1991). The limited availability of the natural enediyne systems, as well as problems with their total synthesis, have prompted several research groups to design, prepare, and test new simplified, fully synthetic analogues, characterized by a similar mode of action. Several cyclic and acyclic models have recently been developed, some of them including pyridine and quinoline units (Kumar et al. 2001; Rawat et al. 2001; Wu et al. 1996).

We have previously reported a simple and efficient method for the synthesis of thioquinolines which possess O, S, Se-propargyl groups (Boryczka et al. 2002a, 2002b). The new propargyl thioquinolines obtained exhibit antiproliferative activity *in vitro* against a broad panel of human and murine cancer cell lines. The most cytotoxic compounds of these series, 4-(3-hydroxypropoxy)-3′-propargylthio-3,4′-diquinoline sulfide and 3-methylthio-4-propargylselenoquinoline, approached the activity of cisplatin (Boryczka et al. 2002a, 2002b). It seems most likely that a propargyl group may be essential for antiproliferative activity of these compounds. A structure-activity relationship study showed a significant correlation between antiproliferative activity and the electronic properties expressed as ^{13}C NMR chemical shift and lipophilicity (Boryczka et al. 2002a, 2003).

Here we report on the synthesis and modification of diacetylenic thioquinolines with the aim of obtaining more information about the influence of substituents on antiproliferative activity in this class of compounds.

2. Investigations, results and discussion

2.1. Chemistry

The synthesis of title compounds **4** and **5** was achieved starting with 4-chloro-3-methylthioquinoline **3a** and 4-chloro-3-alkynylthioquinolines **3b–c**.

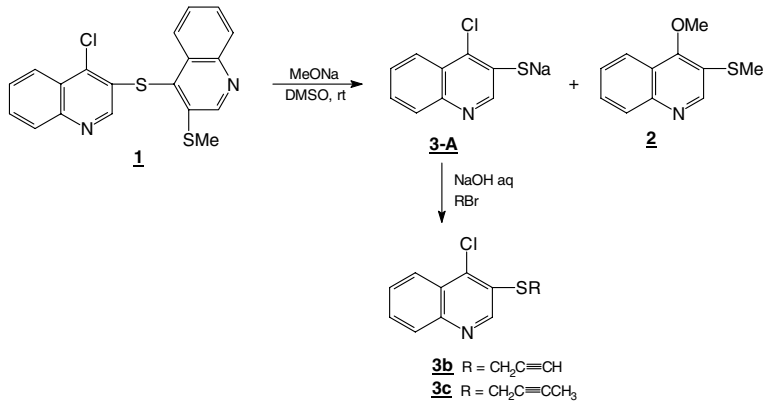
Compound **3a** was prepared according to our previously reported method (Maślankiewicz and Boryczka 1993). 4-Chloroquinolines **3b** and **3c** were synthesized as shown in Scheme 1. The starting compound **1** was prepared according to our published procedure (Maślankiewicz and Boryczka 1993). Treatment of **1** with sodium methoxide in DMSO at 20 °C gave sodium 4-chloro-3-quinolinethiolate **3-A** and 4-methoxy-3-methylthioquinoline **2**, which was removed by extraction. The sodium salt **3-A** after S-alkylation using propargyl bromide or 2-butynyl bromide gave **3b** and **3c** with 68% and 70% yields, respectively.

Compounds **3a–c** were converted into **4a–m** and **5a–b** by nucleophilic displacement of chloride by thiourea or selenourea in ethanol, hydrolysis of the uronium salt **4-A** and subsequent S- or Se-alkylation of the sodium salt **4-B** with propargyl bromide or 2-butynyl bromide or 1,4-dibromo-2-butyne (Scheme 2). The crude products were isolated from aqueous sodium hydroxide by filtration or extraction and separated by column chromatography.

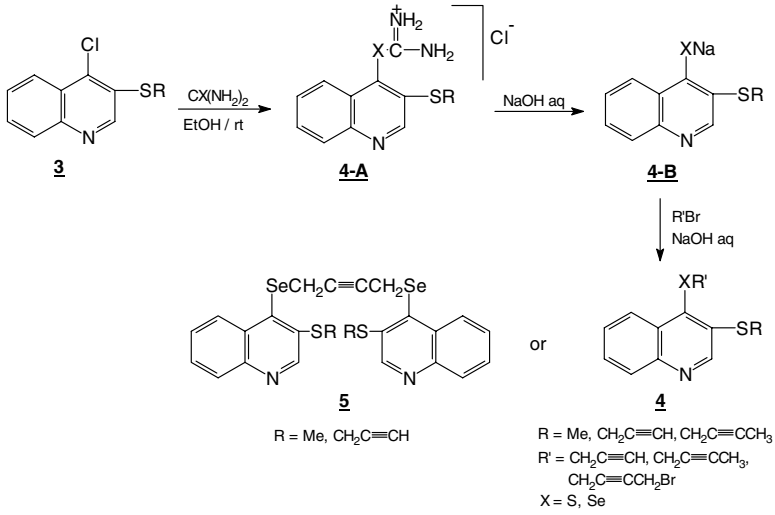
2.2. Antiproliferative activity

The compounds obtained were tested with an SRB or MTT (in the case of leukemia cells) assay for their antiproliferative activity *in vitro* against two human cancer cell lines: SW707 (colon cancer), CCRF/CEM (leukemia) and two murine cancer cell lines: P388 (leukemia), B16 (melanoma). The results of cytotoxic activity *in vitro* were expressed as the ID_{50} (μg/ml), i.e. the concentration of a

Scheme 1



Scheme 2



compound, which inhibits the proliferation of tumor cells by 50% as compared to the control untreated cells. Cisplatin was used as a reference cytotoxic agent (positive test control). A value of less than 4 μg/ml is considered to be an antiproliferative activity criterion for synthetic compounds. The cytotoxic activity of the synthesized compounds was also compared with that of **4o**, which was reported previously (Boryczka et al. 2002b). The results of the cytotoxicity studies are summarized in the Table.

In general all the compounds obtained exhibited potent antiproliferative activity against the cells of human and murine cancer lines used. 4-Chloro-3-(2-butynylthio)quinoline **3c** possessed rather low cytotoxic activity.

Among the S-methyl derivatives, **4a** was inactive against SW707 and B16 cells in the concentration range studied but retained high activity against CCRF/CEM. The replacement of the sulfur atom by selenium, in compound **4b**, resulted in increased activity, especially against cells of the SW707, CCRF/CEM and P388 cancer lines.

In the series of compounds **4c–f** containing a thiopropargyl substituent at position 3, **4d** showed the most potent activity against cells of all the cancer lines studied. Its sulfur analogue **4c** exhibited weak or no antiproliferative activity (against B16 cells). The substitution of the 2-butynyl group with a propargyl group at position 4 led to a decrease of activity in the case of sulfur compound **4e**. A similar change of cellular response was observed for selenium derivatives **4d** and **4f** but only against cells of the SW707 and B16 lines.

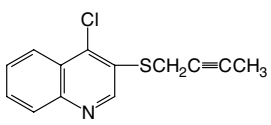
In the series of compounds **4g–j** having a thiobutynyl group at position 3, **3h** showed the most potent activity with ID_{50} values ranging from 0.8 to 3.7 μg/ml, comparable to that of cisplatin. The replacement of the selenium atom by sulfur, in compounds **4g** and **4i**, resulted in decreased activity, especially in the case of activity against cells of the B16 melanoma line. The replacement of the propargyl group at position 4 with a 2-butynyl group (**4i–j**) resulted in a decrease of activity.

Compounds **4k–m** with a 4-bromo-2-butynyl group at the 4 position seem to be the most active. Among them, **4m** was more cytotoxic than cisplatin and more active in comparison to **4k** and **4l** and the previously reported **4o** (Boryczka et al. 2002b). These results indicate that the 4-bromo-2-butynyl group significantly affects the cytotoxic activity of the compounds studied.

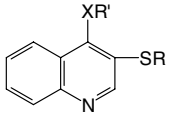
Considering the overall activities of **4a–l** it can be postulated that substitution of selenium (X=Se) with sulfur (X=S) reduces cytotoxic activity except for activity of compounds **4k–l** against CCRF/CEM and P388 cells.

Another noteworthy feature of the compounds obtained results from the observation that melanoma cells (B16) appear to be relatively resistant to the cytotoxic effects of the com-

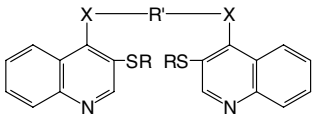
Table: Antiproliferative activity *in vitro* of acetylenic thioquinolines 3c, 4, 5 and reference cisplatin against cells of human and murine cancer cell lines



3c



4



5

Compd.	R	R′	X	Cell line/ID_{50} (μg/ml)			
				Human		Murine	
				SW707	CCRF/CEM	P388	B16
3c	–	–	–	43.6 ± 14.5	15.1 ± 4.6	15.6 ± 13.7	37.1 ± 5.5
4a	Me	$CH_2C{\equiv}CCH_3$	S	Neg	2.9 ± 0.7	12.9 ± 9.3	Neg
4b	Me	$CH_2C{\equiv}CCH_3$	Se	3.4 ± 0.6	1.1 ± 0.6	2.3 ± 0.1	32.9 ± 25.1
4c	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CCH_3$	S	43.0 ± 9.0	5.8 ± 2.6	23.9 ± 6.2	Neg
4d	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CCH_3$	Se	3.8 ± 0.3	2.2 ± 1.1	3.7 ± 1.0	3.9 ± 1.0
4e	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CH$	S	Neg	8.3 ± 1.5	31.1 ± 16.5	Neg
4f	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CH$	Se	5.2 ± 0.9	1.5 ± 0.8	2.7 ± 0.4	6.6 ± 0.6
4g	$CH_2C{\equiv}CCH_3$	$CH_2C{\equiv}CH$	S	33.7 ± 5.6	4.0 ± 2.7	17.9 ± 2.1	Neg
4h	$CH_2C{\equiv}CCH_3$	$CH_2C{\equiv}CH$	Se	3.6 ± 0.3	0.8 ± 0.8	3.7 ± 1.0	1.4 ± 1.8
4i	$CH_2C{\equiv}CCH_3$	$CH_2C{\equiv}CCH_3$	S	48.0 ± 18.6	24.1 ± 13.2	18.0 ± 9.3	Neg
4j	$CH_2C{\equiv}CCH_3$	$CH_2C{\equiv}CCH_3$	Se	35.5 ± 6.1	4.6 ± 2.1	12.3 ± 10.4	Neg
4k	$CH_2C{\equiv}CCH_3$	$CH_2C{\equiv}CCH_2Br$	S	28.9 ± 8.3	0.5 ± 0.1	2.8 ± 0.4	44.8 ± 3.6
4l	$CH_2C{\equiv}CCH_3$	$CH_2C{\equiv}CCH_2Br$	Se	3.6 ± 0.2	1.1 ± 0.2	2.8 ± 0.3	3.6 ± 0.2
4m	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CCH_2Br$	S	2.8 ± 0.9	0.2 ± 0.1	0.2 ± 0.1	3.5 ± 0.2
4o*	Me	$CH_2C{\equiv}CH$	Se	2.5 ± 1.5	–	1.4 ± 2.2	3.2 ± 1.0
5a	Me	$CH_2C{\equiv}CCH_2$	Se	43.8 ± 6.4	27.6 ± 4.1	31.9 ± 10.4	Neg
5b	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CCH_2$	Se	45.5 ± 16.0	57.3 ± 8.5	25.4 ± 9.1	56.3 ± 15.0
Cisplatin				3.2 ± 0.5	1.6 ± 0.1	0.2 ± 0.1	2.7 ± 0.8

* (Boryczka et al. 2002b)

pounds studied as compared with the 3 other cancer cell lines used. Interestingly, compound **4h**, exhibited a higher cytotoxic activity against B16 cells than did cisplatin.

The 1,4-disubstituted butynes **5a–b** exhibited much the same activity as each others, but lower than the monosubstituted derivatives **4**.

In summary, the novel diacetylenic compounds **4** and **5** are easy to prepare in good yields and generally possess significant cytotoxic activity *in vitro*. Among the compounds prepared, **4h** and **4l–m** were found to be the most active, with ID_{50} values comparable to that of cisplatin, and were selected for further anticancer activity studies.

3. Experimental

3.1. General techniques

Melting points were determined in open capillary tubes on a Boetius apparatus and are uncorrected. ^{1}H NMR (300 MHz) spectra were recorded on a Bruker MSL 300 spectrometer in $CDCl_3$ solvents with tetramethylsilane as internal standard; chemical shifts are reported in ppm (δ) and J values in Hz. EI MS spectra were run on a Finnigan MAT 95 at 70 eV. CI MS spectra were recorded on a Finnigan MAT 95 using isobutane as a reagent and the temperature of the ion source 200 °C. Elemental C, H, Br, Cl, N, S analyses were obtained on a Carlo Erba Model 1108 analyzer. TLC was performed on silica gel 60254F plates (Merck) using a mixture of chloroform and ethanol (15 : 1, v/v) as an eluent. UV light and iodine were used for visualization. CC was performed on silica gel 60, <63 μm (Merck) using a mixture of chloroform and ethanol (30 : 1, v/v) as an eluent. Solvents were dried and purified according to procedures in the literature.

3.2. Chemistry

4-Chloro-3′-methylthio-3,4′-diquinolinyl sulfide (**1**) was prepared from thioquinanthrene according to the procedure reported previously (Maślankiewicz and Boryczka 1993), m.p. 155–156 °C.

4-Chloro-3-(methylthio)quinoline (**3a**) was prepared from thioquinanthrene according to the procedure reported previously (Maślankiewicz and Boryczka 1993), m.p. 104–105 °C.

1,4-Dibromo-2-butyne was prepared in 69% yield from 2-butyn-1,4-diol according to the procedure reported previously (Machinek and Lüttke 1975), b.p. 80–82 °C/5 mm_{Hg}, $n_D^{20} = 1.5863$, lit. (Almenningen et al. 1961) b.p. 66–75 °C/1.5–2.5 mm_{Hg}, $n_D^{21} = 1.5898$.

3.2.1. General procedure for the synthesis of 4-chloro-3-(alkynylothio)quinolines 3b–c

A mixture of 0.74 g (2 mmol) of 4-chloro-3′-methylthio-3,4′-diquinolinyl sulfide (**1**) and 0.32 g (6 mmol) of sodium methoxide in 8 ml DMSO was stirred at rt for 30 min. The reaction mixture was poured into 20 ml of 5% aqueous sodium hydroxide and extracted with 4 × 5 ml of chloroform. The combined extracts were washed with water, dried with anhydrous magnesium sulfate and evaporated to give crude **2**. To the water layer 2 mmol of propargyl or 2-butynyl bromide was added and stirred for 30 min. The mixture was extracted with 4 × 5 ml of chloroform. The combined organic layer was washed with water and dried with anhydrous magnesium sulfate. After removal of the solvent the residue was purified by column chromatography to give pure products **3b–c**.

3.2.1.1. 4-Chloro-3-(propargylthio)quinoline (**3b**)

Yield 68%, m.p. 75–76 °C. ^{1}H NMR (300 MHz) δ: 2.24 (t, J = 2.4 Hz, 1 H, CH), 3.77 (d, J = 2.4 Hz, 2 H, CH_2S), 7.64–7.79 (m, 2 H, H-6 and H-7), 8.10–8.26 (m, 2 H, H-5 and H-8), 9.06 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 235 ($M^+ + 2$, 43), 233 (M^+, 100), 198 (83).
$C_{12}H_8ClNS$ (233.7)

3.2.1.2. 4-Chloro-3-(2-butynylthio)quinoline (**3c**)

Yield 70%, m.p. 72–73 °C. ^{1}H NMR (300 MHz) δ: 1.76 (t, J = 2.5 Hz, 3 H, CH_3), 3.76 (q, J = 2.5 Hz, 2 H, CH_2S), 7.62–7.77 (m, 2 H, H-6 and H-7), 8.09–8.24 (m, 2 H, H-5 and H-8), 8.99 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 249 ($M^+ + 2$, 38), 247 (M^+, 100), 212 (96).
$C_{13}H_{10}NSCl$ (247.7)

3.2.2. General procedure for the synthesis of acetylenic thioquinolines 4 and 5

A mixture of (0.2 mmol, 0.42 g) 4-chloro-3-methylthioquinoline **3a** or (0.2 mmol, 0.45 g) 4-chloro-3-propargylthioquinoline **3b** or (0.2 mmol, 0.49 g) 4-chloro-3-(2-butynylthio)quinoline **3c** and selenourea (0.26 g,

2.1 mmol) or thiourea (0.16 g, 2.1 mmol) in 99.8% ethanol (8 ml) was stirred at RT for 1 h. The reaction mixture was poured into 20 ml of 5% aqueous sodium hydroxide. Propargyl bromide (0.26 g, 2.2 mmol) or 2-butynyl bromide (0.29 g, 2.2 mmol) or 1,4-dibromo-2-butyne (0.25 g, 1.1 mmol) was added dropwise to the aqueous layer, and the mixture was stirred for 15 min. The resultant solid was filtered off, washed with water and air-dried to give crude products **4a–j** or **5a–b**. Compounds **4k–m** were obtained when the aqueous layer containing the sodium salt **4-B** was added dropwise to an excess of 1,4-dibromo-2-butyne (0.53 g, 2.5 mmol) in 1 ml ethanol. The crude products obtained were separated by column chromatography and then crystallized from a mixture of benzene and hexane to give pure products **4** or **5**.

3.2.2.1. 4-(2-Butynylthio)-3-methylthioquinoline (**4a**)

Yield 68%, m.p. 76–77 °C. ^{1}H NMR (300 MHz) δ: 1.59 (t, J = 2.4 Hz, 3 H, CH_3), 2.67 (s, 3 H, CH_3S), 3.63 (q, J = 2.4 Hz, 2 H, CH_2S), 7.59–7.70 (m, 2 H, H-6 and H-7), 8.07–8.58 (m, 2 H, H-5 and H-8), 8.78 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 259 (M^+, 18), 244 (100), 206 (75).
$C_{14}H_{13}NS_2$ (259.3)

3.2.2.2. 4-(2-Butynylseleno)-3-methylthioquinoline (**4b**)

Yield 62%, m.p. 66–67 °C. ^{1}H NMR (300 MHz) δ: 1.60 (t, J = 2.4 Hz, 3 H, CH_3), 2.66 (s, 3 H, CH_3S), 3.55 (q, J = 2.4 Hz, 2 H, CH_2Se), 7.58–7.70 (m, 2 H, H-6 and H-7), 8.08–8.54 (m, 2 H, H-5 and H-8), 8.74 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 307 (M^+, 19), 292 (100), 254 (45).
$C_{14}H_{13}NSSe$ (306.4)

3.2.2.3. 4-(2-Butynylthio)-3-(propargylthio)quinoline (**4c**)

Yield 66%, m.p. 92–93 °C. ^{1}H NMR (300 MHz) δ: 1.61 (t, J = 2.5 Hz, 3 H, CH_3), 2.27 (t, J = 2.5 Hz, 1 H, CH), 3.64 (q, J = 2.5 Hz, 2 H, CH_2S), 3.83 (d, J = 2.5 Hz, 2 H, CH_2S), 7.61–7.73 (m, 2 H, H-6 and H-7), 8.08–8.59 (m, 2 H, H-5 and H-8), 9.00 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 282 (M^+, 26), 268 (51), 230 (100).
$C_{16}H_{13}NS_2$ (283.4)

3.2.2.4. 4-(2-Butynylseleno)-3-(propargylthio)quinoline (**4d**)

Yield 61%, m.p. 89–90 °C. ^{1}H NMR (300 MHz) δ: 1.61 (t, J = 2.6 Hz, 3 H, CH_3), 2.27 (t, J = 2.5 Hz, 1 H, CH), 3.55 (q, J = 2.6 Hz, 2 H, CH_2Se), 3.81 (d, J = 2.5 Hz, 2 H, CH_2S), 7.61–7.70 (m, 2 H, H-6 and H-7), 8.06–8.54 (m, 2 H, H-5 and H-8), 8.98 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 330 (M^+, 8), 292 (51), 198 (100).
$C_{16}H_{13}NSSe$ (330.3)

3.2.2.5. 3,4-Di(propargylthio)quinoline (**4e**)

Yield 60%, m.p. 94–95 °C. ^{1}H NMR (300 MHz) δ: 2.09 (t, J = 2.6 Hz, 1 H, CH), 2.27 (t, J = 2.4 Hz, 1 H, CH), 3.72 (d, J = 2.6 Hz, 2 H, CH_2S), 3.84 (d, J = 2.4 Hz, 2 H, CH_2S), 7.62–7.75 (m, 2 H, H-6 and H-7), 8.11–8.60 (m, 2 H, H-5 and H-8), 9.01 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 268 (M^+, 17), 230 (100), 186 (82).
$C_{15}H_{11}NS_2$ (269.3)

3.2.2.6. 3-(Propargylthio)-4-(propargylseleno)quinoline (**4f**)

Yield 66%, m.p. 98–99 °C. ^{1}H NMR (300 MHz) δ: 2.15 (t, J = 2.4 Hz, 1 H, CH), 2.29 (t, J = 2.7 Hz, 1 H, CH), 3.63 (d, J = 2.7 Hz, 2 H, CH_2), 3.85 (d, J = 2.4 Hz, 2 H, CH_2), 7.60–7.73 (m, 2 H, H-6 and H-7), 8.10–8.55 (m, 2 H, H-5 and H-8), 8.97 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 316 (M^+, 12), 278 (73), 198 (100).
$C_{15}H_{11}NSSe$ (316.2)

3.2.2.7. 3-(2-Butynylthio)-4-(propargylthio)quinoline (**4g**)

Yield 64%, m.p. 91–92 °C. ^{1}H NMR (300 MHz) δ: 1.79 (t, J = 2.4 Hz, 3 H, CH_3), 2.08 (t, J = 2.5 Hz, 1 H, CH), 3.75 (d, J = 2.5 Hz, 2 H, CH_2S), 3.83 (q, J = 2.4 Hz, 2 H, CH_2S), 7.61–7.79 (m, 2 H, H-6 and H-7), 8.23–8.61 (m, 2 H, H-5 and H-8), 9.01 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 282 (M^+, 21), 268 (32), 230 (100).
$C_{16}H_{13}NS_2$ (283.4)

3.2.2.8. 3-(2-Butynylthio)-4-(propargylseleno)quinoline (**4h**)

Yield 58%, m.p. 96–97 °C. ^{1}H NMR (300 MHz) δ: 1.79 (t, J = 2.4 Hz, 3 H, CH_3), 2.11 (t, J = 2.6 Hz, 1 H, CH), 3.61 (d, J = 2.6 Hz, 2 H, CH_2Se), 3.80 (q, J = 2.4 Hz, 2 H, CH_2S), 7.62–7.75 (m, 2 H, H-6 and H-7), 8.13–8.55 (m, 2 H, H-5 and H-8), 8.97 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 331 (M^+, 6), 278 (100), 212 (92).
$C_{16}H_{13}NSSe$ (330.3)

3.2.2.9. 3,4-Di-(2-butynylthio)quinoline (**4i**)

Yield 83%, m.p. 100–101 °C. ^{1}H NMR (300 MHz) δ: 1.59 (t, J = 2.4 Hz, 3 H, CH_3), 1.79 (t, J = 2.4 Hz, 3 H, CH_3), 3.69 (q, J = 2.4 Hz, 2 H, CH_2S), 3.81 (q, J = 2.4 Hz, 2 H, CH_2S), 7.65–7.78 (m, 2 H, H-6 and H-7), 8.23–8.62 (m, 2 H, H-5 and H-8), 9.01 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 296 (M^+, 6), 282 (39), 244 (100).
$C_{17}H_{15}NS_2$ (297.0)

3.2.2.10. 3-(2-Butynylthio)- 4-(2-butynylseleno)quinoline (**4j**)

Yield 59%, m.p. 103–104 °C. ^{1}H NMR (300 MHz) δ: 1.61 (t, J = 2.6 Hz, 3 H, CH_3), 1.79 (t, J = 2.4 Hz, 3 H, CH_3), 3.54 (q, J = 2.6 Hz, 2 H, CH_2), 3.79 (q, J = 2.4 Hz, 2 H, CH_2), 7.60–7.73 (m, 2 H, H-6 and H-7), 8.10–8.55 (m, 2 H, H-5 and H-8), 8.97 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 345 (M^+, 12), 292 (42), 212 (100).
$C_{17}H_{15}NSSe$ (344.3)

3.2.2.11. 4-(4-Bromo-2-butynylthio)-3-(2-butynylthio)quinoline (**4k**)

Yield 53%, m.p. 90–91 °C. ^{1}H NMR (300 MHz) δ: 1.79 (t, J = 2.5 Hz, 3 H, CH_3), 3.64 (t, J = 2.4 Hz, 2 H, CH_2), 3.76 (t, J = 2.4 Hz, 2 H, CH_2), 3.83 (q, J = 2.5 Hz, 2 H, CH_2S), 7.65–7.76 (m, 2 H, H-6 and H-7), 8.16–8.60 (m, 2 H, H-5 and H-8), 9.01 (s, 1 H, H-2). EI MS (70 eV) m/z (rel. intensity) 378 (M^+ + 2, 6), 376 (M^+, 8), 296 (100), 243 (81).
$C_{17}H_{14}BrNS_2$ (376.3)

3.2.2.12. 4-(4-Bromo-2-butynylseleno)-3-(2-butynylthio)quinoline (**4l**)

Yield 65 %, m.p. 102–103 °C. ^{1}H NMR (300 MHz) δ: 1.79 (t, J = 2.5 Hz, 3 H, CH_3), 3.64 (t, J = 2.5 Hz, 2 H, CH_2), 3.66 (t, J = 2.5 Hz, 2 H, CH_2), 3.80 (q, J = 2.5 Hz, 2 H, CH_2S), 7.61–7.73 (m, 2 H, H-6 and H-7), 8.08–8.53 (m, 2 H, H-5 and H-8), 8.99 (s, 1 H, H-2). CI MS m/z (rel. intensity) 423 (M^+, 49), 271 (24), 294 (100).
$C_{17}H_{14}BrNSSe$ (423.2)

3.2.2.13. 4-(4-Bromo-2-butynylthio)-3-(propargylthio)quinoline (**4m**)

Yield 58%, m.p. 91–92 °C. ^{1}H NMR (300 MHz) δ: 2.27 (t, J = 2.6 Hz, 1 H, CH), 3.65 (t, J = 2.4 Hz, 2 H, CH_2), 3.75 (t, J = 2.4 Hz, 2 H, CH_2), 3.85 (d, J = 2.6 Hz, 2 H, CH_2S), 7.66–7.77 (m, 2 H, H-6 and H-7), 8.17–8.60 (m, 2 H, H-5 and H-8), 9.01 (s, 1 H, H-2). CI MS m/z (rel. intensity) 364 (M + 2, 94), 362 (M^+, 100), 244 (48).
$C_{16}H_{12}BrNS_2$ (362.2)

3.2.2.14. 1,4-Bis-(3-methylthio-4-quinolinylseleno)-2-butyne (**5a**)

Yield 64%, m.p. 157–158 °C. ^{1}H NMR (300 MHz) δ: 2.64 (s, 6 H, 2 × CH_3S), 3.36 (s, 4 H, 2 × CH_2Se), 7.53–7.66 (m, 4 H, 2 × H-6 and 2 × H-7), 8.02–8.38 (m, 4 H, 2 × H-5 and 2 × H-8), 8.72 (s, 2 H, 2 × H-2). EI MS (70 eV) m/z (rel. intensity) 558 (M^+, 4), 507 (26), 381 (59), 254 (100).
$C_{24}H_{20}N_2S_2Se_2$ (558.4)

3.2.2.15. 1,4-Bis-(3-propargylthio-4-quinolinylseleno)-2-butyne (**5b**)

Yield 67%, m.p. 147–148 °C. ^{1}H NMR (300 MHz) δ: 2.25 (t, J = 2.7 Hz, 2 H, 2 × CH), 3.39 (s, 4 H, 2 × CH_2Se), 3.79 (d, J = 2.7 Hz, 4 H, 2 × CH_2S), 7.55–7.71 (m, 4 H, 2 × H-6 and 2 × H-7), 8.06–8.39 (m, 4 H, 2 × H-5 and 2 × H-8), 8.95 (s, 2 H, 2 × H-2). EI MS (70 eV) m/z (rel. intensity) 606 (M^+, 6), 556 (12), 366 (16), 318 (100).
$C_{28}H_{20}N_2S_2Se_2$ (606.5)

3.3. Antiproliferative in vitro assay

3.3.1. Cells

The following established *in vitro* cancer cell lines were used: SW707 (human rectal adenocarcinoma), CCRF/CEM (human leukemia), P388 (mouse leukemia) and B16 (mouse melanoma). All lines were obtained from the American Type Culture Collection (Rockville, Maryland, USA) and maintained at the Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

Twenty-four hours before addition of the agents to be tested, the cells were plated in 96-well plates (Sarstedt, USA) at a density of 10^4 cells per well in 100 µl of culture medium. The cells were cultured in opti-MEM medium supplemented with 2 mM glutamine (Gibco, Warsaw, Poland), streptomycin (50 µg/ml), penicillin (50 U/ml) (both antibiotics from Polfa, Tarchomin, Poland) and 5% fetal calf serum (Gibco, Grand Island, USA). The cell cultures were maintained at 37 °C in a humid atmosphere saturated with 5% CO_2.

3.3.2. SRB assay

The details of this technique were described by Skehan et al. (1990). The cytotoxicity assay was performed after 72-hour exposure of the cultured cells to varying concentrations (from 0.1 to 100 µg/ml) of the test agents.

The cells attached to the plastic were fixed by gently layering cold 50% TCA (trichloroacetic acid, Aldrich-Chemie, Germany) on the top of the culture medium in each well. The plates were incubated at 4 °C for 1 h and then washed five times with tap water. The background optical density was measured in wells filled with culture medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B (SRB, Sigma, Germany) dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 min. Unbound dye was removed by rinsing (4×) with 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (POCh, Gliwice, Poland) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader with a Multiskan RC photometer (Labsystems, Helsinki, Finland). Each compound in a given concentration was tested in triplicate in each experiment, which was repeated 3–5 times.

3.3.3. MTT assay

This technique was used for cytotoxicity screening against mouse leukemia cells growing in a suspension culture. An assay was performed after 72-h exposure to varying concentrations (from 0.1 to 100 μg/ml) of the test agents. For the last 3–4 h of incubation 20 μl of MTT solution were added to each well (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; stock solution: 5 mg/ml). The mitochondria of viable cells reduce the pale yellow MTT to navy blue formazan: the more viable cells are present in a well, the more MTT will be reduced to formazan. When the incubation time was completed, 80 μl of the lysing mixture were added to each well (lysing mixture: 225 ml dimethylformamide, 67.5 g sodium dodecyl sulphate and 275 ml of distilled water). After 24 h, when formazan crystals had dissolved, the optical densities of the samples were read on an Multiskan RC photometer at 570 nm wavelength.

Each compound at a given concentration was tested in triplicate in each experiment, which was repeated 3–5 times.

The results of cytotoxic activity *in vitro* were expressed as ID_{50} – the dose of compound (in μg/ml) that inhibits the proliferation rate of tumor cells by 50% as compared to control untreated cells.

* Part XCII in the series of azinyl sulfides.

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