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Synthesis and antiproliferative evaluation of bis-styrylbenzothiazol-3-ium salts

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Abstract A series of bis-styrylbenzothiazol-3-ium salts containing different substituents **4–12** were synthesized from 2-methylbenzothiazole by condensation of quaternary salts **1–3** with *p*-substituted benzaldehydes. All compounds were characterized by IR, ^{1}H and ^{13}C NMR, MS, and elemental analysis. Synthesized compounds **4–12** were screened for antitumor activities. Based on presented *in vitro* screening results we may conclude that compounds **4–6** and **9** showed the best cell growth inhibitory activity.

Keywords Heterocycles; Antitumor activity; Aldol reaction.

Introduction

In the western world cancer is a disease of striking significance. Despite advances in diagnosis and treatment, overall survival of patients still remains low. Therefore, an area of major pharmaceutical interest over the last several decades has been development of small molecules with antitumor activity [1–5].

Many 2-substituted benzothiazoles when quaternized to benzothiazolium salts are especially active as antimicrobial [6, 7], antihelminitic [8] and antineoplastic [9] agents. Reported structural features of biologically active benzothiazolium salts are substituents bonded to the *p*-position of the phenyl ring and a conjugated bridge between benzothiazolium and phenyl ring [10]. These types of compounds have received considerable attention, not just due to their potential as anti-tumor drugs, but also as tools to study and visualize *DNA* [11]. Because of their capacity to penetrate through cell membranes they were proposed for the *DNA* visualization in fluorescence microscopy [12].

Due to a broad spectrum of above mentioned activities, we synthesized a series of new compounds from the benzothiazole series and tested their antitumor activity *in vitro*.

Results and discussion

The two step synthesis of the requisite target compounds **4–12** in the Scheme 1 started from 2-methylbenzothiazole and ω,ω'-dibromoalkane. In the first reaction step quaternary salts **1–3** were obtained. Compounds **1–3** were used in the second step in the condensation with substituted aldehydes. A series of novel polymethylene bis-(styrylbenzothiazol-3-ium) dibromides **4–12** were obtained [13].

The structures of new compounds were confirmed by MS, IR, ^{1}H, and ^{13}C NMR spectra.

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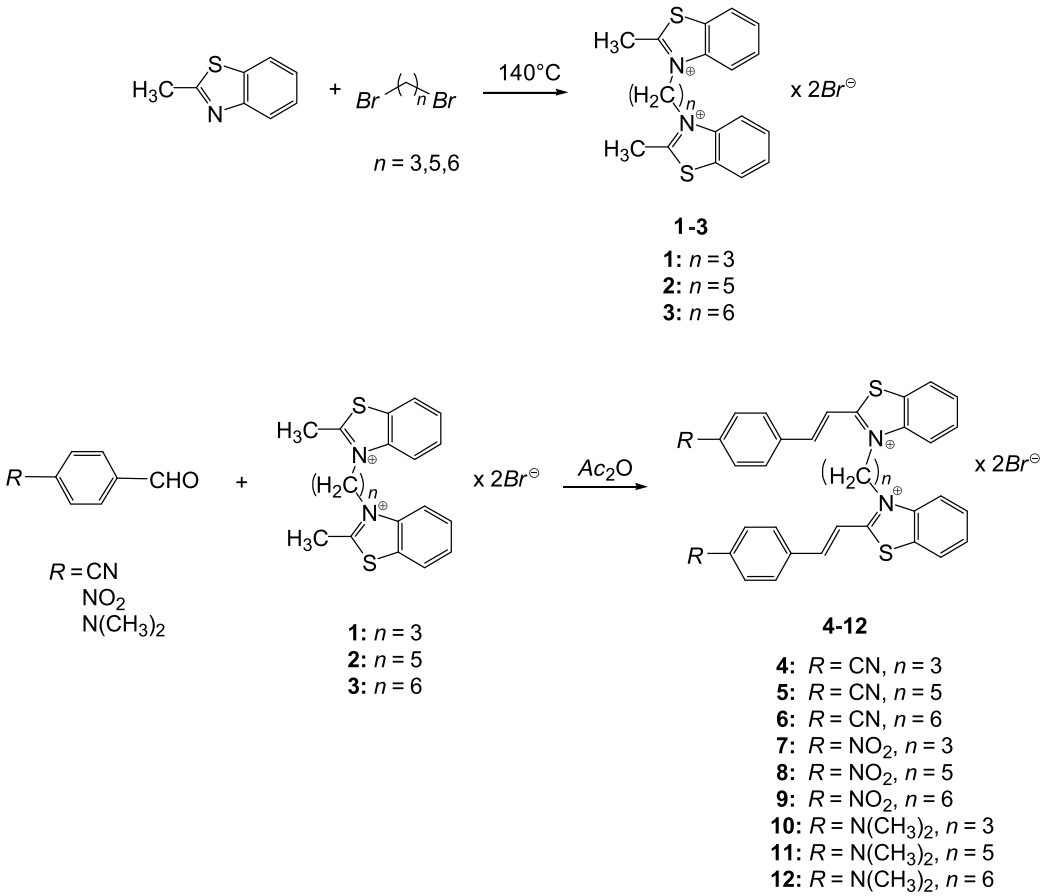

Scheme 1

Antiproliferative effect of compounds in vitro

Compounds **4**–**12** were evaluated for their cytostatic activities against five human tumor cell lines: cervical carcinoma (HeLa), breast carcinoma (MCF-7), colon carcinoma (SW 620), pancreatic carcinoma (MiaPaCa-2), lung carcinoma (H 460), as well as

Table 1 Growth inhibitory effects of compounds **4**–**12** on the growth of malignant tumor cell lines and normal human diploid fibroblast (WI 38)

Comp.	$IC_{50}/\mu M$*					
	Cell lines					
	WI 38	HeLa	MiaPaCa-2	SW 620	MCF-7	H 460
4	70 ± 4	74 ± 26	79 ± 17	52 ± 19	91 ± 1	>100
5	48 ± 5	>100	79 ± 19	32 ± 0.5	89 ± 2	>100
6	37 ± 11	48 ± 42	47 ± 0.1	35 ± 23	74 ± 22	72 ± 27
7	>10	>10	>10	>10	>10	>10
8	>100	>100	>100	39 ± 6	>100	>100
9	>100	>100	28 ± 12	25 ± 13	62 ± 13	59 ± 40
10	>100	>100	>100	>100	>100	>100
11	>10	≥10	≥10	>10	>10	>10
12	>10	>10	>10	5 ± 1	>10	>10

* IC_{50} The concentration that causes 50% growth inhibition

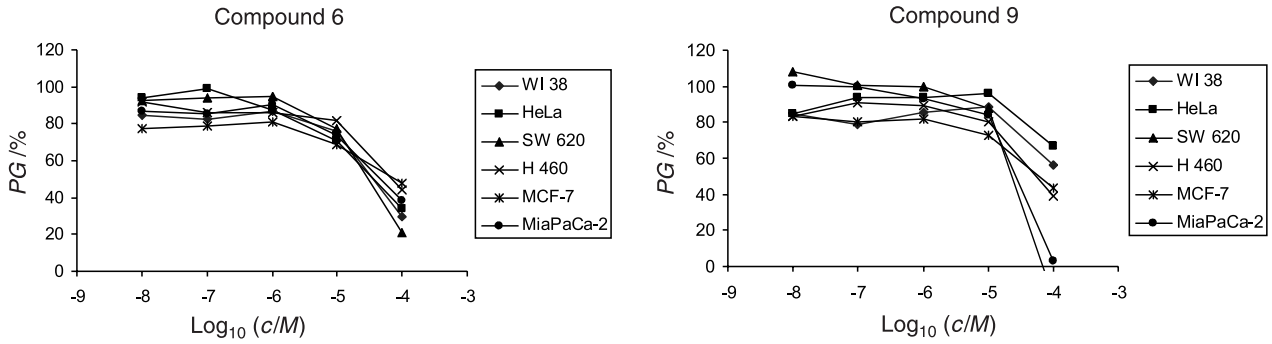

Fig. 1 Dose-response profiles for compounds **6** and **9** tested *in vitro*. *PG* Percentage of growth

normal human fibroblasts (WI 38). Almost all tested compounds showed antiproliferative effects on the presented panel cell lines (Table 1 and Fig. 1).

The compounds **7**, **10**, and **11** produced no growth inhibition on the presented panel cell lines in the tested concentration range. Except these three compounds, all other compounds inhibited the growth of SW 620 cells (IC_{50} values ranging from 5 ± 1 to $52 \pm 19\ \mu M$). Compounds **4–6** showed moderate inhibitory effect on all cell lines, but mostly at the highest concentration tested. Among tested compounds **8**, **9**, and **12** were the most selective ones, since they did not inhibit the growth of normal fibroblasts.

It should be also taken into consideration that the compounds **7**, **10–12** precipitated in the cell culture medium during the 72 h period, forming red-colored precipitates at the highest concentration tested ($10^{-4}\ M$). Since MTT test is a colorimetric assay where red/violet insoluble formazan crystals are formed by reduction of yellow tetrazolium salt in mitochondria of viable cells (see Experimental section), red precipitates gave false-positive results (*i.e.*, mimicked viable cells). However, we examined the cells under the microscope just prior the addition of MTT reagent and noticed a weak proliferation status of these cells pointing to a certain inhibitory activity of these compounds, not measurable by the MTT test. Thus, we decided to narrow the concentration range, having $10^{-5}\ M$ as the highest concentration tested.

In conclusion, we prepared a series of polymethylene bis(styrylbenzothiazol-3-ium) dibromides and tested their antiproliferative activity. Based on the presented *in vitro* screening results we may conclude that compounds **4–6** and **9** showed the best cell growth inhibitory activity (Fig. 1).

Experimental

Chemistry

Melting points were obtained on an Original Kofler Mikroheitztisch apparatus (Reichert, Wien). IR spectra were recorded on a Nicolet Magna 760 spectrophotometer in KBr discs. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 or a Bruker Avance DPX 300 spectrometer at 300 and 75 MHz. All NMR spectra were measured in *DMSO*-d_6 solutions using *TMS* as an internal standard. Elemental analysis for carbon, hydrogen and nitrogen were performed on a *Perkin-Elmer* 2400 elemental analyzer, their results were found to be in good agreement ($\pm 0.2\%$) with the calculated values. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates and HPLC-MS.

*General procedure for synthesis of polymethylene bis(2-methylbenzothiazolium) dibromides **1–3***

Reaction mixture of 13 mmol appropriate ω,ω'-dibromoalkane and 8.0 g 2-methylbenzothiazole (54 mmol) was refluxed for 6 h at 140°C. The product was treated with warm toluene and acetone and recrystallized from methanol.

3,3′-Trimethylenebis(2-methylbenzothiazolium) dibromide (**1**, $C_{19}H_{20}Br_2N_2S_2$)

Yield 5.03 g (75.0%), pale yellow powder, mp 257–258°C (Ref. [13] 256°C); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta =$ 178.07, 140.72, 129.31, 128.98, 127.98, 124.68, 116.95, 46.08, 26.01, 17.44 ppm.

3,3′-Pentamethylenebis(2-methylbenzothiazolium) dibromide (**2**, $C_{21}H_{24}Br_2N_2S_2$)

Yield 5.25 g (76.5%), pale yellow powder, mp 260–261°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.49$ (d, 2H, $J = 8.07$ Hz), 8.36 (d, 2H, $J = 8.16$ Hz), 7.91–7.78 (m, 4H), 4.77–4.79 (m, 4H, CH_2N^+), 3.26 (s, 6H, CH_3), 1.99–1.89 (m, 2H, CH_2), 1.68–1.57 (m, 2H, CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 177.67$, 141.33, 129.82, 129.59, 128.56, 125.20, 117.39, 49.45, 27.82, 23.48, 17.50 ppm; IR (KBr): $\bar{\nu} = 3389$, 1640, 1617 cm^{-1}.

3,3′-Hexamethylenebis(2-methylbenzothiazolium) dibromide (**3**, $C_{22}H_{26}Br_2N_2S_2$)
Yield 5.00 g (70.9%), white powder, mp 290–292°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.47$ (d, 2H, $J = 8.07$ Hz), 8.36 (d, 2H, $J = 8.34$ Hz), 7.79–7.78 (m, 4H), 4.76–4.69 (m, 4H, CH_2N^+), 3.22 (s, 6H, CH_3), 1.90–1.78 (m, 4H, CH_2), 1.55–1.44 (m, 4H, CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 177.58$, 141.29, 129.81, 129.59, 128.55, 125.13, 117.34, 49.55, 27.99, 25.83, 17.39 ppm; IR (KBr): $\bar{\nu} = 6416$, 1638, 1617 cm^{-1}.

General procedure for synthesis of polymethylene bis(styrylbenzothiazol-3-ium) dibromides
Bis(styrylbenzothiazol-3-ium) dibromides **4**–**12** were prepared from the quaternary salts **1**–**3**. A suspension of 0.4 g corresponding quaternary salt in 10 cm^3 acetic anhydride and 5 cm^3 corresponding *p*-substituted benzaldehyde were heated at 160°C. After 6 h the reaction mixture was cooled and 15 cm^3 ether were added. The resulting precipitate was filtered off and washed with ether. Crude product was twice refluxed with 20 cm^3 methanol and filtered off.

(E,E)-3,3′-Trimethylenebis[2-(4-cyanostyryl)benzothiazol-3-ium] dibromide (**4**, $C_{35}H_{26}Br_2N_4S_2$)
Yield 0.859 g (59.2%), yellow powder, mp 266–268°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.75$ (d, 2H, $J = 8.4$ Hz), 8.48–8.43 (m, 4H), 8.34 (d, 4H, $J = 8.4$ Hz), 8.26 (d, 2H, $J = 16.2$ Hz, C*H*=CH), 7.98 (d, 4H, $J = 7.8$ Hz), 7.9–7.76 (m, 4H), 5.6–5.4 (m, 4H, CH_2N^+), 2.5† (m, 4H, CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 171.9$, 146.59, 141.26, 138.01, 132.74, 130.40, 129.66, 128.8, 128.70, 124.69, 118.27, 117.17, 117.09, 113.51, 46.22, 28.00 ppm; MS: m/z (%) = 567 ((M$^+$ + 1)-2Br, 283 ((M + 1)-2Br-284, 100), 1); IR (KBr): $\bar{\nu} = 1600$, 2200 cm^{-1}.

(E,E)-3,3′-Pentamethylenebis[2-(4-cyanostyryl)benzothiazol-3-ium] dibromide (**5**, $C_{37}H_{30}Br_2N_4S_2$)
Yield 0.645 g; (45.3%), pale green powder, mp 260–262°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.53$ (d, 2H, $J =$ 7.53 Hz), 8.34 (d, 2H, $J = 8.07$ Hz), 8.14–8.1 (m, 8H), 8.03 (d, 4H, $J = 8.34$ Hz), 7.89–7.79 (m, 4H), 5.02–4.92 (m, 4H, CH_2N^+), 2.02–1.85 (m, 4H, CH_2), 1.57–1.43 (m, 2H, CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 171.82$, 146.82, 141.72, 138.38, 133.29, 130.68, 130.25, 129.31, 129.24, 125.20, 119.00, 117.75, 116.97, 114.13, 49.70, 29.06, 22.74 ppm; MS: m/z (%) = 595 ((M + 1)-2Br, 1), 297 ((M + 1)-2Br-298, 100); IR (KBr): $\bar{\nu} = 3414$, 1616, 2226 cm^{-1}.

(E,E)-3,3′-Hexamethylenebis[2-(4-cyanostyryl)benzothiazol-3-ium] dibromide (**6**, $C_{38}H_{32}Br_2N_4S_2$)
Yield 0.874 g (61.7%), white powder, mp 278–280°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.5$ (d, 2H, $J = 7.89$ Hz), 8.35 (d, 2H, $J = 8.4$ Hz), 8.29–8.26 (m, 6H), 8.21 (d, 2H, $J = 16.0$ Hz, C*H*=CH), 8.02 (d, 4H, $J = 8.4$ Hz), 7.89–7.79 (m, 4H), 5.04–4.99 (m, 4H, CH_2N^+), 1.90–1.76 (m, 4H, CH_2), 1.52–1.42 (m, 4H, CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 171.74$, 146.74, 141.77, 138.58, 133.36, 130.66, 130.21, 129.26, 129.24, 125.18, 118.9, 117.63, 117.31, 113.99, 49.72, 29.29, 25.84 ppm; MS: m/z (%) = 609 ((M + 1)-2Br, 1), 304 ((M + 1)-2Br-305, 100); IR (KBr): $\bar{\nu} = 3414$, 2225, 1615 cm^{-1}.

(E,E)-3,3′-Trimethylenebis[2-(4-nitrostyryl)benzothiazol-3-ium] dibromide (**7**, $C_{33}H_{26}Br_2N_4O_4S_2$)
Yield 0.852 g (55.6%), light green powder, mp 258–259°C white powder; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.83$ (d, 2H, $J = 8.35$ Hz), 8.59 (d, 2H, $J = 15.97$ Hz, C*H*=CH), 8.53 (d, 2H, $J = 8.35$ Hz), 8.49 (d, 4H, $J = 8.89$ Hz), 8.38 (d, 2H, $J = 15.97$ Hz, CH=C*H*), 8.31 (d, 4H, $J = 8.89$ Hz), 7.95 (t, 2H, $J = 7.91$ Hz), 7.84 (t, 2H, $J = 7.72$ Hz), 5.59–5.54 (m, 4H, CH_2N^+), 2.5† (m, 4H, CH_2) ppm; MS: m/z (%) = 607 ((M + 1)-2Br, 1), 303 ((M + 1)-2Br-304, 100); IR (KBr): $\bar{\nu} = 1580$, 1505 cm^{-1}.

(E,E)-3,3′-Pentamethylenebis[2-(4-nitrostyryl)benzothiazol-3-ium] dibromide (**8**, $C_{35}H_{30}Br_2N_4O_4S_2$)
Yield 0.786 g (52.3%), yellow-green powder, mp 258–260°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.46$ (d, 2H, $J = 8.1$ Hz), 8.28 (d, 6H, $J = 8.76$ Hz), 8.17–8.11 (m, 8H), 7.81–7.73 (m, 4H), 4.96–4.91 (m, 4H, CH_2N^+), 1.90–1.83 (m, 4H, CH_2), 1.48–1.41 (m, 2H, CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 171.26$, 148.82, 145.70, 141.26, 139.74, 130.78, 129.79, 128.87, 128.83, 124.72, 123.97, 117.29, 117.25, 49.29, 28.60, 22.37 ppm; MS: m/z (%) = 635 ((M + 1)-2Br, 2), 317 ((M + 1)-2Br-318, 100); IR (KBr): $\bar{\nu} = 3368$, 3413, 1616 cm^{-1}.

(E,E)-3,3′-Hexamethylenebis[2-(4-nitrostyryl)benzothiazol-3-ium] dibromide (**9**, $C_{36}H_{32}Br_2N_4O_4S_2$)
Yield 0.738 g (49.5%), light green powder, mp 270–272°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.50$ (d, 2H, $J = 7.53$ Hz), 8.37–8.28 (m, 12H), 8.22 (d, 2H, $J = 15.93$ Hz, C*H*=CH), 7.92–7.8 (m, 4H), 5.05–4.97 (m, 4H, CH_2N^+), 1.94–1.81 (m, 4H, 2CH_2), 1.55–1.46 (m, 4H, 2CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 171.68$, 149.23, 146.10, 141.79, 140.37, 131.16, 130.25, 129.35, 129.32, 125.20, 124.59, 117.95, 117.64, 49.74, 29.29, 25.86 ppm; MS: m/z(%) = 649 ((M + 1)-2Br, 2), 324 ((M + 1)-2Br-325, 100); IR (KBr): $\bar{\nu} = 3415$, 1615 1595 cm^{-1}.

(E,E)-3,3′-Trimethylenebis[2-4-(N,N-dimethylamino)-benzothiazol-3-ium] dibromide (**10**, $C_{37}H_{38}Br_2N_4S_2$)
Yield 1.05 g (68.9%), dark violet powder, mp 292–294°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.48$ (d, 2H, $J = 8.2$ Hz), 8.30 (d, 2H, $J = 8.25$ Hz), 8.11 (d, 2H, $J = 15.12$ Hz, C*H*=CH), 8.03 (d, 4H, $J = 8.83$ Hz), 7.85–7.78 (m, 4H), 7.67 (t, 2H, $J = 7.63$ Hz), 6.79 (d, 4H, $J = 8.94$ Hz), 5.23 (brm, 4H, CH_2N^+), 2.5† (m, 4H, CH_2) ppm; MS: m/z (%) = 603 ((M + 1)-2Br, 3), 301 ((M + 1)-2Br-302, 100); IR (KBr): $\bar{\nu} = 3400$, 1640, 1590 cm^{-1}.

† The signals of the CH_2 protons are overlapped by [$^2H_6$]*DMSO*

(E,E)-3,3′-Pentamethylenebis[2-4-(N,N-dimethylamino)-benzothiazol-3-ium] dibromide (**11**, $C_{39}H_{42}Br_2N_4S_2$)
Yield 0.864 g (57.7%), dark violet powder, mp 210–212°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.28$ (d, 2H, $J = 7.68$ Hz), 8.07 (d, 2H, $J = 8.16$ Hz), 7.92 (d, 2H, $J = 15.2$ Hz, C*H*=CH), 7.84 (d, 4H, $J = 8.76$ Hz), 7.73–7.61 (m, 4H), 7.54 (d, 2H, $J = 15.2$ Hz, CH=C*H*), 6.81 (d, 4H, $J = 8.76$ Hz), 4.81–4.77 (m, 4H, CH_2N^+), 3.13 (s, 12H, CH_3), 1.87 (brm, 4H, CH_2), 1.49 (brm, 2H, CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 171.63$, 154.06, 151.07, 141.58, 133.58, 129.35, 127.88, 127.43, 124.41, 121.87, 116.37, 112.33, 106.09, 48.23, 40.29, 28.65, 23.14 ppm; MS: m/z (%) = 631 ((M + 1)-2Br, 1), 315 ((M + 1)-2Br-316, 100); IR (KBr): $\bar{\nu} = 3416$, 1575, 1529 cm^{-1}.

(E,E)-3,3′-Hexamethylenebis[2-4-(N,N-dimethylamino)-benzothiazol-3-ium] dibromide (**12**, $C_{40}H_{44}Br_2N_4S_2$)
Yield 0.968 g (65.2%), dark violet powder, mp 281–283°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.28$ (d, 2H, $J =$ 7.65 Hz), 8.07 (d, 2H, $J = 8.28$ Hz), 8.03 (d, 2H, $J = 15.13$ Hz, C*H*=CH), 7.89 (d, 4H, $J = 8.34$ Hz), 7.75–7.61 (m, 4H), 7.57 (d, 2H, $J = 15.13$ Hz, CH=C*H*), 6.70 (d, 4H, $J = 8.7$ Hz), 4.84–4.73 (m, 4H, CH_2N^+), 3.17 (s, 12H, CH_3), 1.78 (brm, 4H, CH_2), 1.46 (brm, 4H, CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 171.56$, 154.00, 150.98, 141.59, 133.46, 129.33, 127.86, 127.52, 124.45, 121.84, 116.27, 112.37, 106.14, 49.05, 48.30, 28.69, 25.91 ppm; MS: m/z (%) = 645 ((M + 1)-2Br, 0.5), 322 ((M + 1)-2Br-323, 100); IR (KBr): $\bar{\nu} = 3415$, 1576, 1528 cm^{-1}.

Antitumor activity assay
The cervical carcinoma (HeLa), breast carcinoma (MCF-7), colon carcinoma (SW 620), pancreatic carcinoma (MiaPaCa-2), lung carcinoma (H 460) and diploid fibroblasts (WI 38) cells were cultured as monolayers and maintained in *Dulbecco*'s modified *Eagle*'s medium supplemented with 10% fetal bovine serum (FBS), 2 m*M* L-glutamine, 100 U cm^{-3} penicillin and 100 μg cm^{-3} streptomycin in a humidified atmosphere with 5% CO_2 at 37°C. The growth inhibition activity was assessed according to the slightly modified procedure performed at the National Cancer Institute, Developmental Therapeutics Program [14, 15]. The cells were inoculated onto standard 96-well microtiter plates on day 0. The cell concentrations were adjusted according to the cell population doubling time (*PDT*): 1×10^4 cm^{-3} for HeLa, H 460, MiaPaCa-2 and SW 620 cell lines ($PDT = 20$–24 h), 2×10^4 cm^{-3} for MCF-7 cell lines ($PDT = 33$ h) and 3×10^4 cm^{-3} for WI 38 ($PDT = 47$ h). Test agents were then added in five, 10-fold dilutions (10^{-8} to 10^{-4} mol dm^{-3}) and incubated for further 72 h. Working dilutions were freshly prepared on the day of testing. The solvent (*DMSO*) was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in working concentrations. After 72 h of incubation, the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells. The absorbance (*OD*, optical density) was measured on a microplate reader at 570 nm. The percentage of growth (*PG*) of the cell lines was calculated according to one or the other of the following two expressions:

If (mean OD_{test} − mean $OD_{t\,zero}$) ≥ 0 then:

$PG = 100 \times$ (mean OD_{test} − mean $OD_{t\,zero}$)/(mean OD_{ctrl} − mean $OD_{t\,zero}$).

If (mean OD_{test} − mean $OD_{t\,zero}$) < 0 then:

$PG = 100 \times$ (mean OD_{test} − mean $OD_{t\,zero}$)/$OD_{t\,zero}$.

where mean $OD_{t\,zero}$ = the average of optical density measurements before exposure of cells to the test compound, mean OD_{test} = the average of optical density measurements after the desired period of time, mean OD_{ctrl} = the average of optical density measurements after the desired period of time with no exposure of cells to the test compound.

Each test point was performed in quadruplicate in three individual experiments. The results are expressed as IC_{50}, which is the concentration necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from dose-response curves using linear regression analysis by fitting the test concentrations that give *PG* values above and below the reference value (*i.e.*, 50%). If however, for a given cell line all of the tested concentrations produce *PG*s exceeding the respective reference level of effect (*e.g.*, *PG* value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a ">" sign. Each result is a mean value from three separate experiments.

References

1. Shi DF, Bradshaw TD, Wrigley S, McCall CJ, Lelieveld P, Fichtner J, Stevens MFG (1996) J Med Chem 39:3375
2. Delmas F, Di Giorgio C, Robin M, Azas N, Gasquet M, Detang C, Costa M, Timon-David P, Galy J-P (2002) Antimicrob Agents Ch 46:2588
3. Ohtsuka T, Aoki Y (2003) Drugs Future 28:143
4. Amino N, Ideyama Y, Yamano M, Kuromitsu S, Tajinda K, Samizu K, Matsuhisa A, Kudoh M, Shibasaki M (2006) Cancer Lett 238:119
5. Edwards P (2006) Drug Discov Today 11:671
6. Guillet EG, Kimmich GA (1981) J Membr Biol 59:1
7. Afsah SA, Nayyer SA (1996) Asian J Chem 8:419
8. Garmaise DL, Paris GY, Komolossy J, *et al.* (1969) J Med Chem 12:30
9. Krieg M, Bilitz J (1996) Biochem Pharmacol 51:1461
10. Zahradnik P, Foltinova P, Halgaš J (1996) Environm Res 5:51
11. Carlson HJ, Bergqvist MH, Lincoln P, Westman G (2004) Bioorg Med Chem 12:2369
12. UK Patent Application (1981) 2074340 A
13. Gromov SP, Fedorova OA, Ushakov EN, Buevich AV, Baskin II, Pershina YV, Eliasson B, Edlund U, Alfimov MV (1999) J Chem Soc Perkin Trans 2:1323
14. Boyd MR, Paull KD (1995) Drug Dev Res 34:91
15. Marjanović M, Kralj M, Supek F, Frkanec L, Piantanida I, Šmuc T, Tušek-Božić LJ (2007) J Med Chem 50:1007