Novel Derivatives of Benzo[*b*]thieno[2,3-*c*]quinolones: Synthesis, Photochemical Synthesis, and Antitumor Evaluation

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Novel derivatives of benzo[*b*]thieno[2,3-*c*]quinolones **3a**−**j** were synthesized in a multistep synthesis starting from substituted benzo[*b*]thiophene-2-carbonyl chlorides, to their corresponding benzo[*b*]thiophene-2-carboxamides, which were photochemically dehydrohalogenated to their corresponding substituted benzo[*b*]thieno[2,3-*c*]quinolones. Compound **4** was prepared from **3i** by alkylation with 3-dimethylaminopropyl chloride in the presence of NaH. Compounds **7a**,**b** were prepared from **3g** in the multistep synthesis from compounds **5** and **6**. Compounds **3b**, **3c**−**f**, **3h, 7a**, and **7b** were found to exert cytostatic activity against malignant cell lines: pancreatic carcinoma (MiaPaCa2), breast carcinoma (MCF7), cervical carcinoma (HeLa), laryngeal carcinoma (Hep2), colon carcinoma (CaCo-2), melanoma (HBL), human fibroblast cell lines (WI-38). The compounds that bear a 3-dimethylaminopropyl substituent on the quinolone nitrogen (**3b**, **3c**−**f**, **3h**) showed higher antitumor activity than compounds bearing the same substituent on the amidic nitrogen (**7a** and **7b**). The compound **3h**, which has a 3-dimethylaminopropyl substituent on the quinolone nitrogen and a methoxycarbonyl substituent at position 9, had marked antitumor activity. Because of strong cytotoxic effect of compound **4** on melanoma cells (HBL, ME 67.3, and ME 67.1), a potential mechanism of action was examined. Analysis of DNA and Annexin-V-FLUOS staining indicated that compound **4** causes cell death by apoptosis.

Introduction

A lot of 2- and 4-quinolones substituted with different substituents showed in vitro inhibition of human tumor cell lines and also tubulin polymerization. This class of compounds are 2-phenyl-4-quinolones substituted with different substituents on the benzene part of the molecule. In these compounds a good correlation was found between cytotoxicity and inhibition of tubulin polymerization.[1−6] The same group of compounds displayed significant growth inhibitory action against a panel of tumor cell lines including human ileocecal carcinoma (HCT-8), murine leukemia (P-388), human melanoma (RPMI), and human central nervous system tumor (TE 671) cells. Some of these compounds displayed DNA topoisomerase I inhibitory activity in vitro and DNA topoisomerase II inhibitory activity in vitro.[7−9] Some new analogues of 4-quinolinones and 1,7-naphthyridin-4-ones have been synthesized and tested in vitro for their antitumor activity against CNS (SNB-75), breast (T-47D), and lung (NCI-H 522) cancer cell lines.[10] Substituted 3-hydroxy-2-quinolone has also been synthesized and evaluated by [^{3}H]-glycine and [^{3}H]-AMPA binding to rat cortical membranes.[11]

Few data have been publish describing the antitumor activity of heterocyclic condensed quinolones. The novel quinoline-quinone streptonigrin (SN) is an antitumor antibiotic that has activity against a broad range of tumors.[12−14] Although SN has been studied clinically as an antitumor agent, its use has been limited by reports of delayed myelotoxicity.[15,16] Nevertheless, positive results were reported for SN either as a single agent[17,18] or in combination chemotherapy.[19,20] SN is an excellent substrate for oxidoreductase (NQ01).[21]

Pyranoquinolin-2-ones were synthesized and evaluated for their in vitro cytotoxicity against a panel of human tumor cell lines,[22] and 2-arylquinazolinones had significant growth inhibitory action against tumor cell lines, some of them being potent inhibitors of tubulin polymerization. Some of them displayed selective activity against P-gp-expressing epidermoid carcinoma of the nasopharrynx.[23] Recently trifluoromethyl-substituted pyranoquinolinone was prepared and tested for its ability to modulate the transcriptional activity of the human androgen receptor (HAR).[24]

Searching for compounds related to these classes of biologically and pharmacologically active compounds, we have prepared new derivatives of thieno[3′,2′:4,5]thieno-[2,3-*c*]quinolones and examined their antitumor activity.[25]

In this work we prepared benzo[*b*]thieno[2,3-*c*]qinolones containing a 3-dimethylaminopropyl substituent on the quinolone nitrogen (**3b**−**f**, **3h**, and **4**) or a

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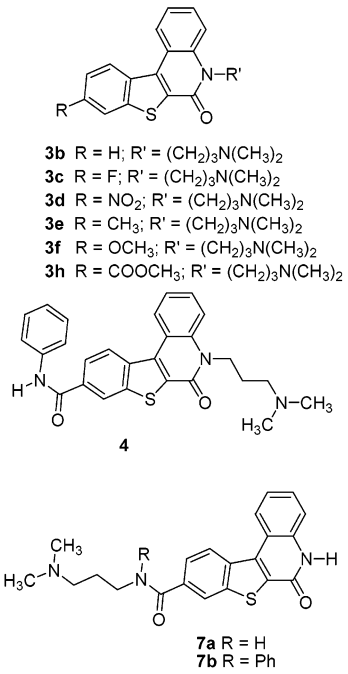


Figure 1. Benzo[*b*]thieno[2,3-*c*]qinolones (**3b**−**f**, **3h**, **4**, **7a**, and **7b**).

3-dimethyaminopropyl substituent in the amide part of the molecule (**7a**, **7b**) and evaluated their antitumor activity.

Chemistry

All compounds **3a**−**j** shown in Figure 1 were prepared in multistep synthesis according to Scheme 1. Starting from different 6-substituted 3-chlorobenzo[*b*]thiophene-2-carbonyl chlorides **1a**−**g**, which already were known from the literature.[26] The yield varied from 50 to 80% by refluxing of chlorides **1a**, **1b**, and **1c** with aniline in toluene. Corresponding 3-chlorobenzo[*b*]thiophene-2-carboxamides were obtained (**2a**[27] in 98% yield, **2g**[28] in 88.9% yield, and **2i**[29] in 35.4% yield).

On the other hand, *N*-[3-(dimethyamino)propyl]-substituted 3-chlorobenzo[*b*]thiophene-2-carboxamides **2b**−**f**, **2h**, and **2j** were obtained in yields of 40−70% from chlorides **1a**−**g** by the Shotten−Baumann method[30] with *N*-[3-(dimethyamino)propyl]aniline at 0 °C. The anilides **2a**−**j** were converted by the photochemical dehydrohalogenation reaction into their corresponding quinolones **3a**−**j** in 50−90% yields.[29] Better yield was achieved in cases where the *N*-atom on the anilide moiety of a molecule was not substituted with an *N*-[3-(dimethyamino)propyl] substituent.

Hydrolysis of ester group of compound **3g** and conversion of corresponding carboxylic acid **5** into carbonyl chloride **6** gave the intermediate which was used for preparation of both quinolones: *N*-[3-(dimethyamino)-propyl]-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinoline-9-carboxamide hydrochloride (**7a**) (reflux with 3-(dimethylamino)propylamine) and *N*-[3-(dimethyamino)-propyl]-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinoline-9-carboxanilide hydrochloride (**7b**).[28]

N-Alkylation of compound **3i** with 3-dimethylamino-propyl chloride hydrochloride in the presence of NaH, after stirring at room temperature, gave the expected quinolone derivative **4**.

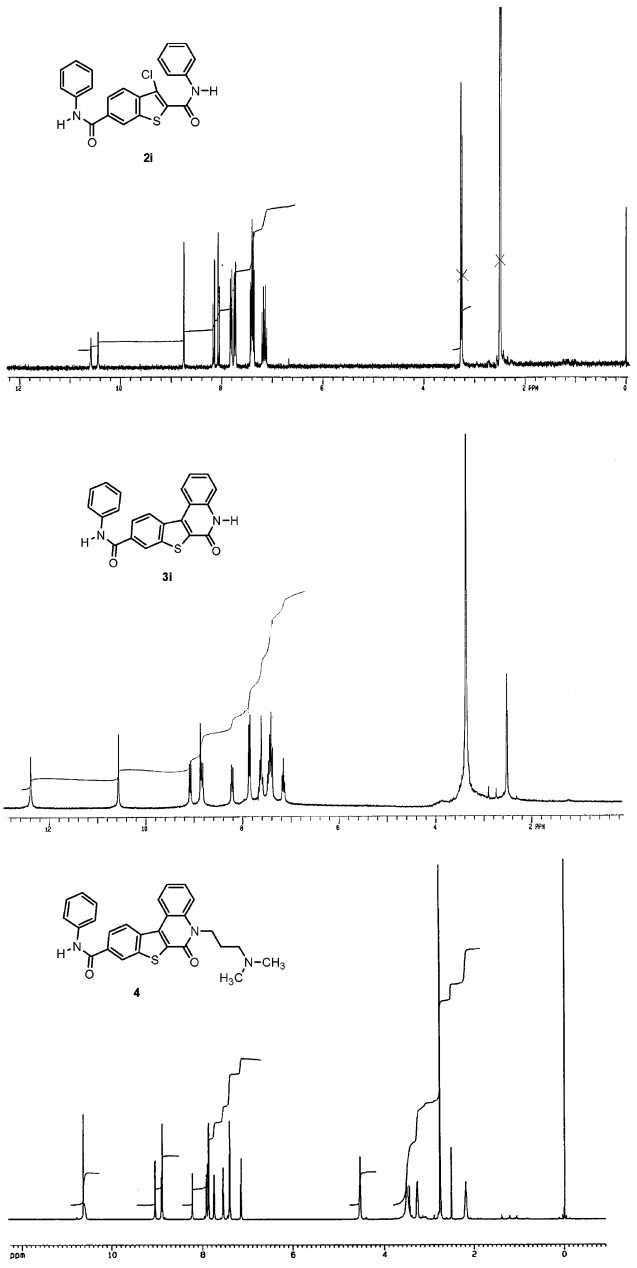


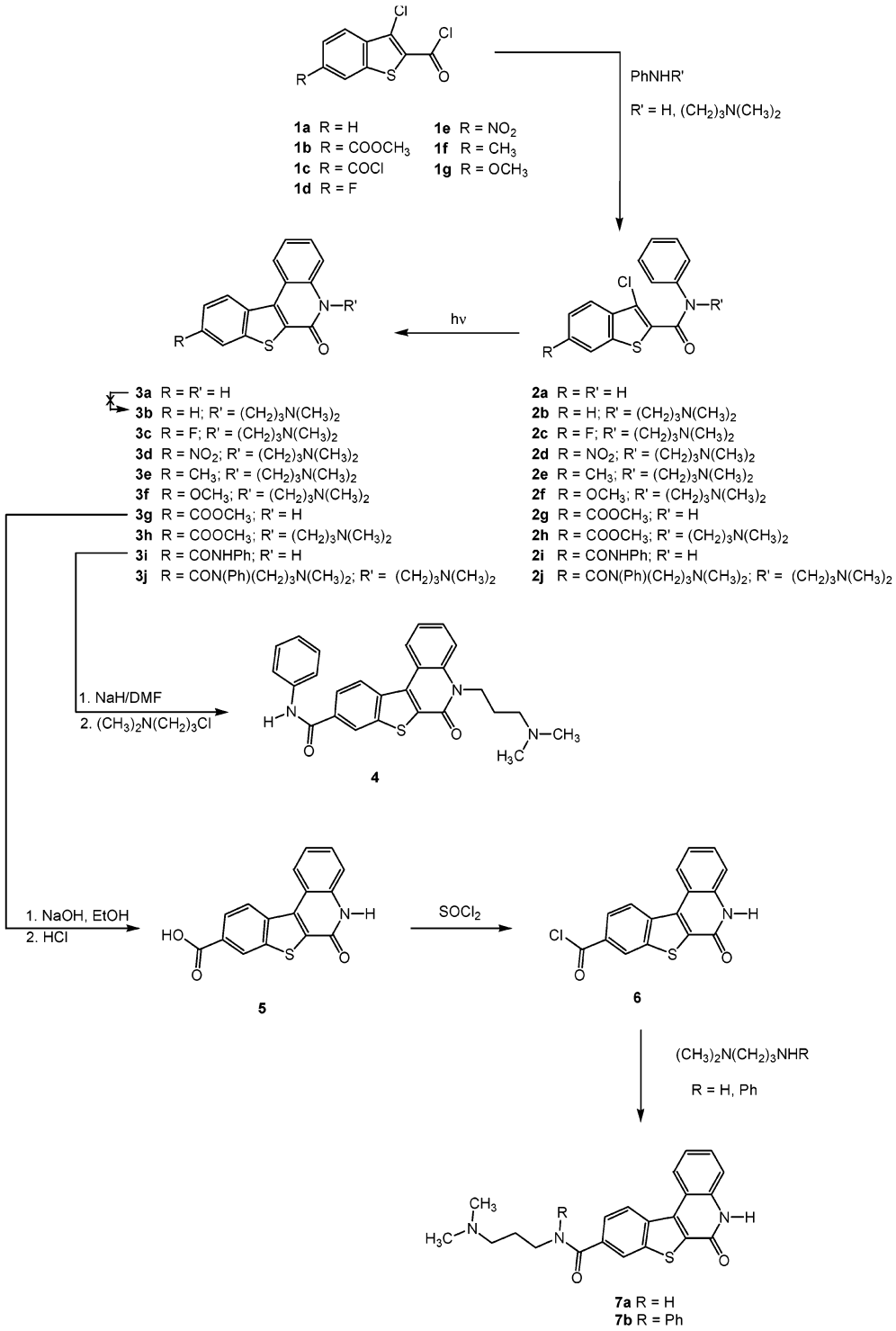
Figure 2. ^{1}H NMR spectra of compounds **2i**, **3i**, and **4**.

Figure 2 shows ^{1}H NMR spectra of compounds **2i**, **3i**, and **4** from which it is evident that a monoalkylation reaction had taken place and that the reaction occurred at the quinolone part of the molecule. The structures of the novel compounds were determined on the basis of analysis of chemical shifts and H−H coupling constants in ^{1}H NMR spectra and ^{13}C NMR spectra as well as by elemental analysis.

Biological Results and Discussion

Antitumor Activity. The effect of all compounds on cell proliferation was tested on the following malignant human tumor cell lines: cervical carcinoma (HeLa), breast carcinoma (MCF-7), colon carcinoma (CaCo-2), pancreatic carcinoma (MIA PaCa-2), melanoma (HBL), and laryngeal carcinoma (Hep-2), as well as on a human fibroblast cell line (WI-38). Compound **4** was tested also on additional melanoma cell lines, ME 67.1 and ME 67.3. The results are presented in Table 1 and Figure 3.

Scheme 1



All tested compounds exhibited strong antitumor activity with IC_{50} ranging from 0.01 to 34.7 μM. Compounds **3f**, **3h**, and **4** had the strongest inhibition. Compound **4** was the strongest inhibitor of growth for melanoma cell lines, HBL (IC_{50} = 0.01 μM), ME 67.3 (IC_{50} = 0.19 μM), and ME 67.1 (IC_{50} = 0.79 μM) and for CaCo-2 cells (IC_{50} = 0.49 μM). Compound **3h** also mostly inhibited HBL cells (IC_{50} = 0.35 μM), as well as MCF-7, CaCo-2, and Hep-2 cell lines (IC_{50} = 0.47−0.65 μM). Compound **3f** was not as toxic for HBL cells (IC_{50} = 1.58 μM) as for CaCo-2 (IC_{50} = 0.47 μM) and MIA PaCa-2 cells (IC_{50} = 0.51 μM). Compound **7b** had the most pronounced selectivity in cytostatic activity: HBL cells were inhibited the most (IC_{50} = 0.67 μM), HeLa (IC_{50} = 34.7 μM) and MCF-7 cells (IC_{50} = 19.5 μM) the least. No other compound showed so big a difference in inhibition. In comparison with antitumor activity of similar quinolones,[3−6,9,11] the substances tested in these experiments were very strong inhibitors of tumor cell proliferation at very low concentrations.

From results in Table 1, we noticed a very strong inhibitory effect of compound **4** on melanoma cell line HBL, and we speculated that this compound could act as a specific inhibitor for melanoma cells. For this

Table 1. Inhibitory Effect of Compounds on the Growth of Tumor Cell Lines and Normal Fibroblasts (WI-38)

	IC_{50} (μM)[a]						
compd	HeLa	MCF-7	CaCo-2	MIAPaCa-2	HBL	Hep-2	WI-38
3b	2.5	4.27	0.62	1.4	0.58	3.63	4.5
3c	0.65	4.6	0.98	2.5	3.98	3.16	2.9
3d	1.0	5.8	0.79	1.25	1.2	3.54	2.4
3e	1.0	4.5	1.7	1.1	1.1	3.63	2.45
3f	0.85	2.2	0.47	0.51	1.58	2.96	2.3
3h	1.3	0.59	0.47	1.0	0.35	0.65	1.6
4	2.8	2.63	0.49	1.0	0.01	1.77	2.3
7a	6.3	10.0	3.8	6.6	1.0	6.6	4.4
7b	34.7	19.5	4.79	4.79	0.67	3.16	10.0

[a] 50% inhibitory concentration or compound concentration required to inhibit cell proliferation by 50%.

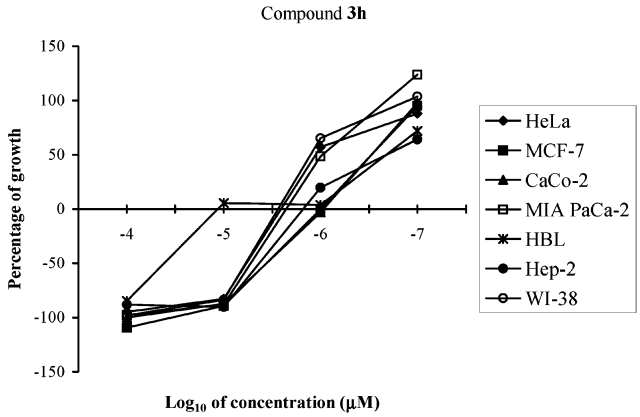


Figure 3. The inhibitory effect of different concentration of the most active substance (**3h**) on the growth of human malignant tumor cell lines (HeLa, MCF-7, CaCo-2, MIA PaCa-2, HBL, Hep-2) and normal human fibroblasts (WI-38).

Table 2. Influence of Compound **4** on Adhesion of HBL Cells

	time of incubation (h)			
concentration (M)	1	2	3	24
1×10^{-5}	87%	87%	81%	28%
5×10^{-6}	90%	98%	95%	62%

reason, inhibition of growth of additional melanoma cell lines (ME 67.1 and ME 67.3) was investigated. Both of these cell lines were sensitive to compound **4** (ME 67.3, $IC_{50} = 0.19$ μM; ME 67.1, $IC_{50} = 0.79$ μM). A potential mechanism of action was also examined for compound **4**. The influence of compound **4** on adhesion of HBL cells was examined. By MTT test we determined the cells attached on plastic substratum, after treatment with **4** at different time point. The results (Table 2) are expressed as a ratio (%) between treated and control cells. One of three parallel experiments is shown.

The influence of compound **4** on cell cycle was also examined. Melanoma cell line (HBL) was treated with compound **4**, and after staining with propidium iodide (PI), a flow cytometry analysis was performed (Figure 4). In comparison with control cells (G0/G1 = 60.19%; G2/M = 4.26%; S = 37.06%), the number of cells in G2/M phase increased about 2.25 times (G2/M = 9.6) and in S phase decreased about 1.33 times ($S = 27.78$), without significant changes in G0/G1 phase (62.62%) (the results are average from two independent experiments).

To better understand the mechanism of action, DNA from HBL cells was isolated after treatment with compound **4**. DNA was separated on an agarose gel, and fragmentation of DNA was observed (Figure 5). In comparison with control cells, fragmentation of DNA increased with concentration of compound **4**. The presence of DNA fragments in treated cells pointed to apoptosis as a mechanism of cell death induced by compound **4**. Induction of apoptosis by compound **4** was confirmed with Annexine-V-FLUOS staining. The number of apoptotic cells (green fluorescence) increased with concentration of **4** (Table 3).

Table 3. Percent of Apoptotic Cells after Treatment of HBL Cells with Compound **4** for 5 h

concentration (M)	apoptotic cells (%)
control	1
1×10^{-6}	9
5×10^{-6}	29
1×10^{-5}	35

Apoptosis is characterized by cell shrinkage, condensation of the nucleus, and DNA fragmentation.[31−35] A variety of cancer chemotherapeutic agents induce cell death by apoptosis. From the above results it is very difficult to say whether compound **4** acts as a specific inhibitor for melanoma cells, but we can say that compound **4** induced apoptosis in melanoma cells and influenced their adhesion. Whether induction of apoptosis caused detachment of the cells or the converse is not clear. Whether compound **4** exerts its effect directly on the cell cycle or indirectly through interaction with the cell membrane has not been determined. Even though compound **4** induced apoptosis in melanoma cells, other mechanisms responsible for cell death have not been excluded (e.g., inhibition of tubulin polymerization[36−39]).

Conclusions

All tested compounds exhibited strong inhibitory activities against all cell lines tested. The most sensitive were HBL cells, although cell sensitivity varied from compound to compound. The most potent antitumor effect against all cells lines was found for compounds **3f**, **3h**, and **4**. Compound **4** induced apoptosis in HBL cells and altered cell adhesion. Future study will be focused on more profound examination of the mechanisms of action for these compounds, as well as their in vivo effect.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot stage microscope and are uncorrected. IR spectra were recorded on a Nicolet Magna 760 spectrophotometer between sodium chloride plates. UV spectra were recorded on either a Perkin-Elmer 124 or a Hewlett-Packard 8452A spectrophotometer in methanol or ethanol. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian Gemini 300 or a Bruker Avance DPX 300 spectrometer using TMS as an internal standard in DMSO-d_6, $CDCl_3$, or D_2O. Mass spectra were recorded on either a Varian-MAT 311A (electron impact and fast atom bombardment) or a Micromass Platform LCZ (Electrospray). Elemental analysis for carbon, hydrogen, and nitrogen was performed on a Perkin-Elmer 2400 elemental analyzer. Where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4% of the theoretical value. Irradiation was performed at room temperature with a water-cooled immersion well fitted with an "Hanovia" 450 W medium pressure and with an "Original Hanau" 125 or 400 W high pressure mercury arc lamp using quartz or Pyrex filter. All compounds were routinely checked by TLC with Merck silica

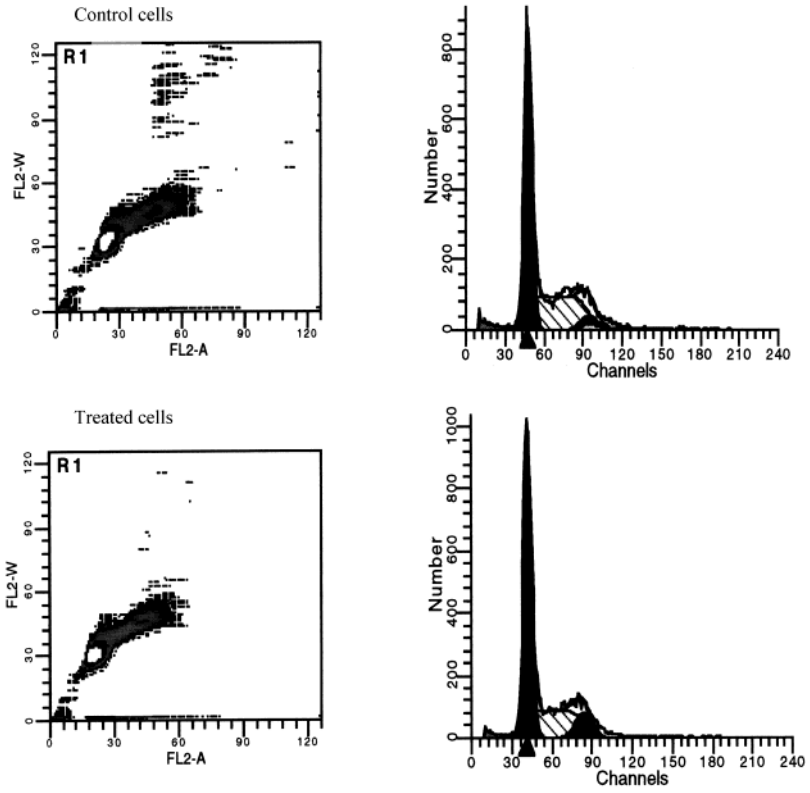


Figure 4. Flow cytometry analysis of HBL cells treated with compound **4** and stained with PI. The cells were grown under confluences and treated with compound **4** for 5 h.

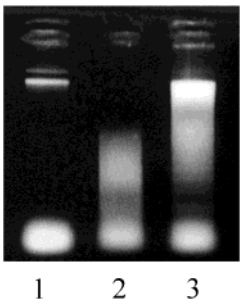


Figure 5. DNA analysis after treatment of HBL cells with compound **4**. Lane 1, control cells; lane 2, cells treated with compound **4** at concentration of 5×10^{-6} M; and lane 3, cells treated with compound **4** at concentration of 10^{-5} M.

gel 60F-254 glass plates or Merck aluminum oxide plates in KBr disks or as a liquid film.

General Method for the Synthesis of 6-Substituted 3-Chlorobenzo[*b*]thiophene-2-carboxamides (2a−j). Method A. A solution of 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**−**c**) (0.02 mol) and aniline (0.03 or 0.07 mol) in toluene (80 mL) was refluxed for 1 h. After cooling, precipitated crystals were filtered off and recrystallized. **Method B**. A solution of 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**−**g**) (0.02 mol) in chloroform (400 mL) was added dropwise to a cold mixture of *N*-[3-(dimethylamino)propyl]aniline (0.02 or 0.04 mol) and 5% NaOH (9 or 18 mL) at 2−5 °C. The resulting mixture was stirred at the same temperature for 30 min and then 1 h at room temperature. The organic layer was separated and washed first with 3% HCl and then with water and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product was purified by column chromatography (neutral aluminum oxide) or recrystallization.

***N*-Phenyl-3-chlorobenzo[*b*]thiophene-2-carboxamide (2a)**: from **1a** by method A, white crystals after recrystallization from chloroform:cyclohexane (1:2), yield 98.6%; mp 170 °C (lit.[27] mp 169 °C).

***N*-[3-(Dimethylamino)propyl]-*N*-phenyl-3-chlorobenzo[*b*]thiophene-2-carboxamide (2b)**: from **1a** by method B, brown oily product after purification by column chromatography (10% ethanol/toluene), yield 62.7%; IR (NaCl)(ν_{max}/cm^{-1}) 2766−2942 (CH_3, CH_2), 1651 (C═O); UV (methanol)(λ_{max}/nm) 230, 284; ^{1}H NMR ($CDCl_3$)(δ/ppm) 7.77−7.67 (m, 2H, J = 9.03, 7.70, 2.23 Hz), 7.43−7.39 (m, 2H, J = 8.79, 7.64, 2.21 Hz), 7.32−7.18 (m, 5H), 4.03 (t, 2H, J = 7.45 Hz), 2.49 (t, 2H, J = 7.37 Hz), 2.30 (s, 6H), 1.93 (tt, 2H, J = 7.40 Hz).; ^{13}C NMR ($CDCl_3$)(δ/ppm) 162.6 (s), 141.4 (s), 137.6 (s), 137.6 (s), 135.4 (s), 130.9 (s), 129.0 (d), 129.0 (d), 127.6 (d), 127.4 (d), 127.4 (d), 126.3 (d), 125.0 (d), 122.5 (d), 122.4 (d), 56.7 (t), 48.5 (t), 45.1 (q), 45.1 (q), 22.5 (t); MS m/z 373 (M + 1). Anal. ($C_{20}H_{21}ClN_2OS$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-*N*-phenyl-6-fluoro-3-chlorobenzo[*b*]thiophene-2-carboxamide (2c)**: from **1d** by method B, white crystals after recrystallization from chloroform:ethyl acetate (1:3), yield 65.6%; mp 203−204 °C; IR (KBr)(ν_{max}/cm^{-1}) 3417−2469 (CH_3, CH_2), 1640 (C═O); UV (Methanol)(λ_{max}/nm) 206, 232, 286; ^{1}H NMR (DMSO-d_6)(δ/ppm) 7.94 (dd, 1H, J = 9.08, 2.28 Hz), 7.72 (dd, 1H, J = 8.90, 5.07 Hz), 7.39−7.22 (m, 6H), 3.95 (t, 2H, J = 7.10 Hz), 3.13 (t, 2H, J = 8.06 Hz), 2.73 (s, 6H), 1.99 (tt, 2H, J = 7.45, 7.75 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 161.8 (s), 161.4 (s), 159.9 (s), 140.5 (s), 138.2 (s), 131.1 (s), 129.1 (d), 129.1 (d), 127.9 (d), 127.6 (d), 127.6 (d), 123.7 (d), 118.4 (s), 114.9 (d), 109.6 (d), 53.9 (t), 46.7 (t), 41.8 (q), 41.8 (q), 22.3 (t); MS m/z 391 (M + 1). Anal. ($C_{20}H_{20}ClFN_2OS$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-*N*-phenyl-6-fluoro-3-chlorobenzo[*b*]thiophene-2-carboxamide (2d)**: from **1e** by method B, yellow crystals after recrystallization from chloroform:ethyl acetate (1:3), yield 51.5%; mp 227−228 °C; IR (KBr)(ν_{max}/cm^{-1}) 3505−2467 (CH_3, CH_2), 1634 (C═O); UV (methanol)(λ_{max}/nm) 206, 226, 308; ^{1}H NMR (DMSO-d_6)(δ/ppm) 9.11 (s, 1H), 8.25 (dd, 1H, J = 8.91, 1.94 Hz), 7.90 (d, 1H, J = 8.90 Hz), 7.44−7.22 (m, 5H), 3.97 (t, 2H, J = 6.98 Hz), 3.15 (t, 2H, J = 7.87 Hz), 2.74 (s, 6H), 1.99 (tt, 2H, J = 7.54, 7.79 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 161.0 (s), 145.6 (s), 140.0 (s), 138.5 (s), 138.1 (s), 137.0 (s), 129.2 (d), 129.2 (d), 128.2 (d), 127.6

(d), 127.6 (d), 122.7 (d), 120.5 (d), 120.2 (d), 118.2 (s), 53.9 (t), 46.6 (t), 41.9 (q), 41.9 (q), 22.3 (t); MS *m*/*z* 418 (M + 1). Anal. ($C_{20}H_{20}ClN_3O_3S$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-*N*-phenyl-6-methyl-3-chlorobenzo[*b*]thiophene-2-carboxamide (2e)**: from **1f** by method B, white crystals after recrystallization from chloroform:ethyl acetate (1:3), yield 91.6%; mp 201−203 °C; IR (KBr)(ν_{max}/cm^{-1}) 3555−2476 (CH_3, CH_2), 1641 (C═O); UV (Methanol)(λ_{max}/nm) 208, 232, 292; ^{1}H NMR (DMSO-d_6)(δ/ppm): 7.75 (s, 1H), 7.58 (d, 1H, *J* = 8.23 Hz), 7.38−7.19 (m, 6H), 3.95 (t, 2H, *J* = 6.94 Hz), 3.13 (t, 2H, *J* = 7.99 Hz), 2.73 (s, 6H), 2.39 (s, 3H), 1.98 (tt, 2H, *J* = 7.50, 7.62 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 162.0 (s), 140.7 (s), 137.3 (s), 137.1 (s), 132.4 (s), 130.1 (s), 129.2 (d), 129.2 (d), 128.0 (d), 127.7 (d), 127.7 (d), 127.5 (d), 122.8 (d), 121.7 (d), 118.8 (s), 54.1 (t), 46.8 (t), 42.0 (q), 42.0 (q), 22.5 (t), 21.2 (q); MS *m*/*z* 387 (M+1). Anal. ($C_{21}H_{23}ClN_2OS$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-*N*-phenyl-6-methoxy-3-chlorobenzo[*b*]thiophene-2-carboxamide (2f)**: from **1g** by method B, white crystals after recrystallization from chloroform:ethyl acetate (1:3), yield 29.9%; mp 188−190 °C; IR (KBr)-(ν_{max}/cm^{-1}) 3436−2474 (CH_3, CH_2), 1642 (C═O); UV (Ethanol)-(λ_{max}/nm) 197, 233, 301; ^{1}H NMR (DMSO-d_6)(δ/ppm) 7.58 (d, 1H, *J* = 8.92 Hz), 7.55 (d, 1H, *J* = 2.12 Hz), 5.37−5.23 (m, 5H), 7.07 (dd, 1H, *J* = 8.88, 2.28 Hz), 3.94 (t, 2H, *J* = 7.11 Hz), 3.80 (s, 3H), 3.13 (t, 2H, *J* = 8.13 Hz), 2.74 (s, 6H), 1.96 (tt, 2H, *J* = 7.92, 8.34 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm): 162.0 (s), 158.9 (s), 140.9 (s), 139.0 (s), 133.4 (s), 129.3 (d), 129.3 (d), 128.3 (s), 128.0 (d), 127.8 (d), 127.8 (d), 122.9 (d), 119.1 (s), 116.1 (d), 105.5 (d), 55.8 (q), 54.2 (t), 46.9 (t), 42.2 (q), 42.2 (q), 22.5 (t); MS *m*/*z* 403 (M + 1). Anal. ($C_{21}H_{23}ClN_2O_2S$) C, H, N.

Methyl 2-(*N*-phenylcarbamoyl)-3-chlorobenzo[*b*]thiophene-6-carboxylate (2 g): from **1b** by method A, white crystals after recrystallization from acetone, yield 88.8%; mp 197−198 °C (lit.[28] mp 197−198 °C).

Methyl 2-{*N*-[3-(dimethylamino)propyl]-*N*-phenylcarbamoyl}-3-chlorobenzo[*b*]thiophene-6-carboxylate (2h): from **1b** by method B, yellow crystals after recrystallization from chloroform:ethyl acetate (1:2), yield 65.6%; mp 191−195 °C (lit.[28] mp 191−195 °C).

***N*,*N*′-Diphenyl-3-chlorobenzo[*b*]thiophene-2,6-dicarboxamide (2i)**: from **1c** by method A, light yellow crystals after recrystallization from DMF:water (1:2), yield 85.6%; mp 278 °C (lit.[29] mp 278 °C).

***N*,*N*′-Diphenyl-*N*,*N*′-bis[3-(dimethylamino)propyl]-3-chlorobenzo[*b*]thiophene-2,6-dicarboxamide (2j)**: from **1c** by method B, brown oily product after purification by column chromatography (5% ethanol/benzene), yield 35.4%; IR (NaCl)(ν_{max}/cm^{-1}) 2740−2920 (CH_3, CH_2), 1635 (C═O); UV (Methanol)(λ_{max}/nm) 220, 290; ^{1}H NMR ($CDCl_3$)(δ/ppm) 7.68 (s, 1H), 7.43 (d, 1H, *J* = 8.46 Hz), 7.20−7.08 (m, 9H), 7.00−6.98 (m, 2H), 3.99−3.94 (m, 4H), 2.37−2.30 (m, 4H), 2.17 (s, 12H), 1.83−1.79 (m, 4H); ^{13}C NMR ($CDCl_3$)(δ/ppm): 169.3 (s), 162.1 (s), 142.9 (s), 141.1 (s), 136.9 (s), 135.8 (s), 134.3 (s), 132.9 (s), 129.1 (d), 129.1 (d), 129.0 (d), 129.0 (d), 127.6 (d), 127.5 (d), 127.5 (d), 127.3 (d), 127.3 (d), 126.7 (d), 125.2 (d), 123.2 (d), 121.5 (d), 119.9 (s), 56.7 (t), 56.6 (t), 48.6 (t), 48.3 (t), 45.1 (q), 45.1 (q), 45.1 (q), 45.1 (q), 25.5 (t), 25.4 (t); MS *m*/*z* 577 (M + 1). Anal. ($C_{32}H_{37}ClN_4O_2S$) C, H, N.

General Method for the Synthesis of [1]Benzothieno-[2,3-*c*]quinolin-6-ones (3a−j). A solution of 6-substituted 3-chlorobenzo[*b*]thiophene-2-carboxamides (**2a**−**j**) (1.6 mmol) and triethylamine (1.6 mmol) in 9% methanol/toluene (benzene) or dioxane (550 mL) was irradiated with 450 W medium pressure or 125 or 400 W high-pressure mercury arc lamp using quartz or Pyrex filter at room temperature for 10 min up to 2 h. The air was bubbled through the solution. After evaporation of the solvent, the crude product was purified by column chromatography (neutral aluminum oxide) or recrystallization. Free base was then converted into the hydrochloride salt by stirring it with saturated ethanolic HCl.

6-Oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinolin-6-one (3a): from **2a** after 20 min of irradiation with 450 W medium-pressure mercury arc lamp using Pyrex filter in 9% methanol/toluene, light brown crystals after recrystallization from methanol, yield 65.6%; mp > 300 °C (lit.[40] mp > 310 °C).

5-[3-(Dimethylamino)propyl]-6-oxo-5,6-dihydro[1]-benzothieno[2,3-*c*]quinolin-6-one hydrochloride (3b): from **2b** after 30 min of irradiation with 400 W high-pressure mercury arc lamp using Pyrex filter in 9% methanol/benzene. After evaporation of the solvent, the crude product was purified by column chromatography (20% ethanol/toluene) and then converted into hydrochloride salt; light brown crystals, yield 50.3%; mp 260−263 °C; IR (KBr)(ν_{max}/cm^{-1}) 2475−2948 (CH_3, CH_2), 1615 (C═O); UV (ethanol)(λ_{max}/nm) 234, 256, 302, 318, 342, 360; ^{1}H NMR (DMSO-d_6)(δ/ppm) 10.92 (s, 1H), 8.94 (dd, 1H, *J* = 9.31 Hz), 8.88 (d, 1H, *J* = 7.67 Hz), 8.27 (dd, 1H, *J* = 9.28 Hz), 7.90 (d, 1H, *J* = 8.42 Hz), 7.76−7.67 (m, 3H), 7.53 (dd, 1H, *J* = 7.62 Hz), 4.53 (t, 2H, *J* = 7.13 Hz), 3.25 (dt, 2H, *J* = 5.05 Hz), 2.76 (s, 3H), 2.75 (s, 3H), 2.18 (tt, 2H, *J* = 7.27 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 157.7 (s), 141.7 (s), 137.4 (s), 135.4 (s), 135.2 (s), 131.8 (s), 129.5 (d), 127.8 (d), 126.2 (d), 126.2 (d), 124.6 (d), 124.3 (d), 123.2 (d), 118.9 (s), 116.3 (d), 54.2 (t), 42.2 (q), 42.2 (q), 39.1 (t), 22.8 (t); MS *m*/*z* 337 (free base M + 1). Anal. ($C_{20}H_{21}ClN_2OS$) C, H, N.

An attempt was made to synthesize compound **3b** by *N*-alkylation[41] of **3a**, but the reaction was unsuccessful and only starting material was isolated.

5-[3-(Dimethylamino)propyl]-9-fluoro-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinolin-6-one hydrochloride (3c): from **2c** after 20 min of irradiation with 400 W high-pressure mercury arc lamp using Pyrex filter in 9% methanol/toluene. After evaporation of the solvent, the crude product was purified by recrystallization from toluene:methanol (1:1) and then converted into hydrochloride salt; white crystals, yield 62.5%; mp 252 °C; IR (KBr)(ν_{max}/cm^{-1}) 3501−2483 (CH_3, CH_2), 1620 (C═O); UV (methanol)(λ_{max}/nm) 204, 232, 254, 302, 340, 356; ^{1}H NMR (DMSO-d_6)(δ/ppm) 10.39, (s, 1H), 8.97 (dd, 1H, *J* = 9.22, 5.05 Hz), 8.83 (d, 1H, *J* = 7.53 Hz), 8.21 (dd, 1H, *J* = 9.02, 2.54 Hz), 7.89 (d, 1H, *J* = 8.33 Hz), 7.74 (dd, 1H, *J* = 7.49, 7.45 Hz), 7.58−7.49 (m, 2H), 4.52 (t, 2H, *J* = 7.11 Hz), 3.24 (t, 2H, *J* = 7.90 Hz), 2.75 (s, 6H), 2.16 (tt, 2H, *J* = 7.22, 7.68 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 162.8 (s), 159.5 (s), 157.2 (s), 143.1 (s), 137.2 (s), 134.5 (s), 132.0 (s), 129.5 (d), 127.7 (d), 124.2 (d), 123.0 (d), 118.3 (s), 116.0 (d), 114.5 (d), 110.1 (d), 54.0 (t), 42.0 (q), 42.0 (q), 39.7 (t), 22.6 (t); MS *m*/*z* 355 (free base M+1). Anal. ($C_{20}H_{20}ClFN_2OS$) C, H, N.

5-[3-(Dimethylamino)propyl]-9-nitro-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinolin-6-one hydrochloride (3d): from **2d** after 20 min of irradiation with 400 W high-pressure mercury arc lamp using Pyrex filter in 9% methanol/toluene. After evaporation of the solvent, the crude product was purified by recrystallization from toluene:methanol (1:1) and then converted into hydrochloride salt; yellow crystals, yield 43.6%; mp 270 °C; IR (KBr)(ν_{max}/cm^{-1}) 3417−2360 (CH_3, CH_2), 1634 (C═O); UV (methanol)(λ_{max}/nm) 224, 278, 299, 370; ^{1}H NMR (DMSO-d_6)(δ/ppm) 10.79 (s, 1H), 9.07 (d, 1H, *J* = 2.15 Hz), 8.84 (d, 1H, *J* = 9.25 Hz), 8.55 (d, 1H, *J* = 7.96 Hz), 8.20 (dd, 1H, *J* = 8.94, 2.11 Hz), 7.82 (d, 1H, *J* = 8.39 Hz), 7.67 (dd, 1H, *J* = 7.25, 7.83 Hz), 7.40 (dd, 1H, *J* = 7.40, 7.19 Hz), 4.23 (t, 2H, *J* = 6.78 Hz), 3.27 (t, 2H, *J* = 7.68 Hz), 2.78 (s, 6H), 2.17 (tt, 2H, *J* = 6.90, 7.50 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 157.0 (s), 145.5 (s), 141.6 (s), 139.4 (s), 137.1 (s), 136.1 (s), 133.7 (s), 129.8 (d), 126.6 (d), 124.1 (d), 123.2 (d), 120.1 (d), 120.1 (d), 118.0 (s), 116.2 (d), 54.1 (t), 42.1 (q), 39.6 (t), 22.7 (t); MS *m*/*z* 382 (free base M+1). Anal. ($C_{20}H_{20}$-ClN_3O_3S) C, H, N.

5-[3-(Dimethylamino)propyl]-9-methyl-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinolin-6-one hydrochloride (3e): from **2e** after 20 min of irradiation with 400 W high-pressure mercury arc lamp using Pyrex filter in 9% methanol/toluene. After evaporation of the solvent, the crude product was purified by recrystallization from toluene:methanol (1:1) and then converted into hydrochloride salt; white crystals, yield 60.4%; mp 261−263 °C; IR (KBr)(ν_{max}/cm^{-1}) 3444−2479 (CH_3, CH_2), 1639 (C═O); UV (methanol)(λ_{max}/nm) 204, 234, 256, 308, 342, 358; ^{1}H NMR (DMSO-d_6)(δ/ppm) 10.31 (s, 1H), 8.85 (d, 1H, *J* = 7.27 Hz), 8.81 (d, 1H, *J* = 8.62 Hz),

8.05 (s, 1H), 7.88 (d, 1H, J = 8.40 Hz), 7.72 (dd, 1H, J = 7.29, 7.42 Hz), 7.54−7.50 (m, 2H), 4.52 (t, 2H, J = 7.02 Hz), 3.24 (t, 2H, J = 7.93 Hz), 2.75 (s, 6H), 2.53 (s, 3H), 2.16 (tt, 2H, J = 7.73, 7.44 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 157.7 (s), 142.0 (s), 137.9 (s), 137.3 (s), 135.2 (s), 133.0 (s), 131.0 (s), 129.4 (d), 127.8 (d), 125.7 (d), 124.6 (d), 123.8 (d), 123.1 (d), 118.8 (s), 116.1 (d), 54.2 (t), 42.2 (q), 42.2 (q), 39.6 (t), 22.8 (t), 21.2 (q); MS m/z 351 (free base M + 1). Anal. ($C_{21}H_{23}ClN_2OS$) C, H, N.

5-[3-(Dimethylamino)propyl]-9-methoxy-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinolin-6-one hydrochloride (3f): from **2f** after 20 min of irradiation with 400 W high-pressure mercury arc lamp using Pyrex filter in 9% methanol/toluene. After evaporation of the solvent, the crude product was purified by recrystallization from toluene:methanol (1:1) and then converted into hydrochloride salt; white crystals, yield 76.7%; mp 254−255 °C; IR (KBr)(ν_{max}/cm^{-1}) 3439−2681 (CH_3, CH_2), 1634 (C═O); UV (methanol)(λ_{max}/nm) 205, 234, 256, 164, 324, 336, 353; ^{1}H NMR (DMSO-d_6)(δ/ppm) 10.47 (s, 1H), 8.79 (d, 2H, J = 9.38 Hz), 7.88−7.83 (m, 2H), 7.71 (dd, 1H, J = 7.51 Hz), 7.50 (dd, 1H, J = 7.58 Hz), 7.24 (dd, 1H, J = 8.14, 2.04 Hz), 4.50 (t, 2H, J = 7.10 Hz), 3.92 (s, 3H), 3.24 (t, 2H, J = 7.95 Hz), 2.75 (s, 6H), 2.16 (tt, 2H, J = 7.92, 7.12 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 158.9 (s), 157.3 (s), 143.8 (s), 137.1 (s), 135.0 (s), 133.0 (s), 129.2 (d), 128.6 (s), 126.6 (d), 124.3 (d), 122.8 (d), 118.4 (s), 115.9 (d), 115.7 (d), 106.3 (d), 55.6 (q), 53.9 (t), 41.9 (q), 41.9 (q), 39.6 (t), 22.6 (t); MS m/z 367 (free base M + 1). Anal. ($C_{21}H_{23}ClN_2O_2S$) C, H, N.

Methyl 6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinolin-9-carboxylate (3g): from **2g** after 1 h of irradiation with 450 W medium-pressure mercury arc lamp using quartz filter in 9% methanol/benzene; white crystals after recrystallization from DMF, yield 87.0%; mp > 300 °C (lit.[28] mp > 310 °C).

Methyl5-[3-(dimethylamino)propyl]-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinoline-9-carboxylate hydrochloride (3h): from **2h** after 10 min of irradiation with 450 W medium-pressure mercury arc lamp using quartz filter in 9% methanol/benzene. After evaporation of the solvent, the crude product was purified by recrystallization from ethanol and then converted into hydrochloride salt; white crystals, yield 78.9%; mp 245−247 °C; IR (KBr)(ν_{max}/cm^{-1}) 2683−2922 (CH_3, CH_2), 1729, 1629 (C═O); UV (ethanol)(λ_{max}/nm) 232, 266, 282, 294, 306, 328, 354, 370; ^{1}H NMR (DMSO-d_6)(δ/ppm) 10.25 (s, 1H), 9.01 (d, 1H, J = 8.80 Hz), 8.86 (d, 1H, J = 1.55 Hz), 8.84 (d, 1H, J = 8.25 Hz), 8.14 (dd, 1H, J = 8.73, 1.63 Hz), 7.89 (d, 1H, J = 8.50 Hz), 7.74 (dd, 1H, J = 7.80, 7.88, 1.03 Hz), 7.53 (dd, 1H, J = 7.80, 7.45 Hz), 4.52 (t, 2H, J = 7.17 Hz), 3.95 (s, 3H), 3.25 (tt, 2H, J = 5.25 Hz), 2.76 (s, 3H), 2.75 (s, 3H), 2.16 (tt, 2H, J = 7.59 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm): 165.7 (s), 157.4 (s), 141.5 (s), 138.5 (s), 137.2 (s), 134.5 (s), 134.4 (s), 129.6 (d), 128.1 (s), 126.1 (d), 125.9 (d), 125.6 (d), 124.4 (d), 123.2 (d), 118.5 (s), 116.2 (d), 54.1 (t), 52.5 (q), 42.1 (q), 42.1 (q), 39.3 (t), 22.6 (t); MS m/z 395 (free base M + 1). Anal. ($C_{22}H_{23}ClN_2O_3S$) C, H, N.

6-Oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinolin-9-carboxanilide (3i): from **2i** after 2 h of irradiation with 450 W medium-pressure mercury arc lamp using quartz filter in dioxane; yellow crystals after recrystallization from DMF:water (1:2), yield 85.7%; mp > 300 °C (lit.[29] mp > 300 °C).

***N,N′*-Bis[3-(dimethylamino)propyl]-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinoline-9-carboxanilide dihydrochloride (3j)**: from **2j** after 2 h of irradiation with 400 W high-pressure mercury arc lamp using Pyrex filter in 9% methanol/benzene. After evaporation of the solvent, the crude product was purified by column chromatography (10% ethanol/benzene) and then converted into dihydrochloride salt; white crystals, yield 28.7%; mp 238−242 °C; IR (KBr)(ν_{max}/cm^{-1}) 2682−2957 (CH_3, CH_2), 1642, 1634 (C═O); UV (methanol)(λ_{max}/nm) 232, 260, 308, 348, 364; ^{1}H NMR (D_2O)(δ/ppm) 7.33−7.18 (m, 7H), 7.01 (d, 1H, J = 8.51 Hz), 6.66−6.61 (m, 2H), 6.34−6.31 (m, 2H), 4.06 (t, 2H, J = 7.57 Hz), 3.60 (t, 2H, J = 7.57 Hz), 3.22 (t, 2H, J = 7.48 Hz), 3.07 (t, 2H, J = 7.48 Hz), 2.87 (s, 6H), 2.79 (s, 6H), 2.05 (tt, 2H, J = 7.38 Hz), 1.80 (tt, 2H, J = 7.38 Hz); ^{13}C NMR (D_2O)(δ/ppm) 172.5 (s), 158.3 (s), 142.8 (s), 142.0 (s), 136.3 (s), 135.5 (s), 135.0 (s), 134.7 (s), 131.5 (s), 130.9 (d), 130.9 (d), 130.4 (d), 129.4 (d), 129.4 (d), 129.2 (d), 125.9 (d), 125.2 (d), 124.6 (d), 124.1 (d), 123.7 (d), 118.4 (s), 116.2 (d), 56.1 (t), 55.6 (t), 48.1 (t), 43.6 (q), 43.6 (q), 43.6 (q), 43.6 (q), 40.6 (t), 23.4 (t), 23.1 (t); MS m/z 541 (free base M + 1). Anal. ($C_{32}H_{38}Cl_2N_4O_2S$) C, H, N.

5-[3-(Dimethylamino)propyl]-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinoline-9-carboxanilide Hydrochloride (4). To a cold solution of **3i** (2.00 g, 5.4 mmol) in anhydrous DMF (100 mL) was added sodium hydride (1.00 g, 26.0 mmol) in three portions.[42] After the solution was stirred under nitrogen for 15 min, a solution of 3-(dimethylamino)-propyl chloride hydrochloride (2.23 g, 14.0 mmol) in DMF (50 mL) was added, and the reaction mixture was stirred for 2 h at 0 °C and 60 h at room temperature. The solid (sodium chloride) was separated by filtration, and the solvent was removed under reduced pressure. The crude product was purified by recrystallization from chloroform:cyclohexane (1:2), and free base was then converted into the hydrochloride salt by stirring it with saturated ethanolic HCl. Resulting precipitate was filtered off, washed with absolute ethanol, and dried in vacuo at 50 °C for 5 h to give yellow crystals (1.26 g); yield 47.4%; mp 173−175 °C; IR (KBr)(ν_{max}/cm^{-1}) 2670−3028 (CH_3, CH_2), 1629 (C═O); UV (ethanol)(λ_{max}/nm) 234, 264, 282, 310, 350, 368; ^{1}H NMR (DMSO-d_6)(δ/ppm) 10.62 (s, 1H), 10.59 (s, 1H), 9.04 (d, 1H, J = 8.83 Hz), 8.90 (d, 1H, J = 7.45 Hz), 8.88 (d, 1H, J = 1.62 Hz), 8.22 (dd, 1H, J = 8.72, 1.62 Hz), 7.91 (d, 1H, J = 8.65 Hz), 7.87 (d, 2H, J = 7.64 Hz), 7.75 (dd, 1H, J = 7.33, 8.38 Hz), 7.55 (dd, 1H, J = 7.49, 7.74 Hz), 7.40 (dd, 2H, J = 7.68, 8.14 Hz), 7.15 (dd, 1H, J = 7.37, 8.34 Hz), 4.53 (t, 2H, J = 7.19 Hz), 3.27 (dt, 2H, J = 5.32 Hz), 2.76 (s, 3H), 2.75 (s, 3H), 2.19 (tt, 2H, J = 7.66 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 164.8 (s), 157.5 (s), 141.4 (s), 139.2 (s), 137.3 (s), 137.3 (s), 134.7 (s), 133.7 (s), 133.6 (s), 129.7 (d), 128.7 (d), 128.7 (d), 125.9 (d), 125.4 (d), 124.6 (d), 124.0 (d), 123.7 (d), 123.2 (d), 120.6 (d), 120.6 (d), 118.7 (s), 116.2 (d), 56.1 (t), 42.1 (q), 42.1 (q), 39.4 (t), 22.7 (t); MS m/z 456 (free base M + 1). Anal. ($C_{27}H_{26}ClN_3O_2S$) C, H, N.

6-Oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinoline-9-carboxylic Acid (5). A solution of NaOH (0.60 g, 15.0 mmol) in water (300 mL) was added to the solution of **3g** (1.50 g, 5.0 mmol) in ethanol (250 mL). The resulting solution was refluxed for 1 h. Ethanol was removed under reduced pressure, and the residue was dissolved in water and acidified with concentrated HCl. The precipitate was filtered off, washed with water, and recrystallized from DMSO to give **6** (1.35 g) as white crystals; yield 95.1%; mp > 300 °C (lit.[28] mp > 300 °C).

6-Oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinoline-9-carbonyl Chloride (6). A mixture of **5** (0.40 g, 1.4 mmol) and thionyl chloride (10 mL, 137.7 mmol) was refluxed for 5 h. Excess of thionyl chloride was removed under reduced pressure to give **7** (0.45 g) as yellow crystals in quantitative yield; mp > 300 °C (lit.[28] mp > 300 °C).

***N*-[3-(Dimethylamino)propyl]-6-oxo-5,6-dihydro[1]benzothieno[2,3-*c*]quinoline-9-carboxamide Hydrochloride (7a)**. A solution of **6** (0.82 g, 2.6 mmol) and [3-(dimethylamino)propyl]amine (12 mL, 95.4 mmol) in toluene (35 mL) was refluxed for 30 min. After cooling, the precipitate was filtered off and washed with 20% HCl and then with water. Recrystallization from DMSO, followed by conversion into the hydrochloride salt by stirring it with saturated ethanolic HCl, gave light brown crystals (0.346 g); yield 62.0%; mp 230−235 °C; IR (KBr)(ν_{max}/cm^{-1}) 2711−2963 (CH_3, CH_2) 1661 (C═O); UV (methanol)(λ_{max}/nm) 234, 264, 306, 326, 350, 366; ^{1}H NMR (DMSO-d_6)(δ/ppm) 12.37 (s, 1H), 10.51 (s, 1H), 9.04 (t, 1H, J = 5.71 Hz), 9.01 (d, 1H, J = 8.80 Hz), 8.83 (d, 1H, J = 8.24 Hz), 8.77 (d, 1H, J = 1.50 Hz), 8.15 (dd, 1H, J = 8.69, 1.50 Hz), 7.65−7.57 (m, 2H), 7.45−7.42 (m, 1H), 3.43 (dt, 2H, J = 6.46, 6.03 Hz), 3.14 (t, 2H, J = 7.87 Hz), 2.77 (s, 6H), 2.00 (tt, 2H, J = 6.77, 7.81 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 165.6 (s), 157.6 (s), 141.0 (s), 137.6 (s), 137.2 (s), 135.5 (s), 134.0 (s), 132.8 (s), 129.0 (d), 125.5 (d), 124.7 (d), 123.6 (d), 123.1 (d), 122.7 (d), 117.1 (s), 116.7 (d), 54.4 (t), 41.9 (q), 41.9 (q), 36.5 (t), 24.1 (t); MS m/z 380 (free base M + 1). Anal. ($C_{21}H_{22}ClN_3O_2S$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-6-oxo-5,6-dihydro[1]-benzothieno[2,3-*c*]quinoline-9-carboxanilide Hydrochloride (7b)**. The chloride **6** was converted into free base of **7b** by the method B described earlier for the synthesis of 6-substituted 3-chlorobenzo[*b*]thiophene-2-carboxamides **2a**−**j**. Free base was then converted into the hydrochloride salt by stirring it with saturated ethanolic HCl. Resulting precipitate was filtered off, washed with absolute ethanol, and dried in vacuo at 50 °C for 5 h to give light yellow crystals; yield 47.7%; mp 235−240 °C; IR (KBr)(ν_{max}/cm^{-1}) 2865−3123 (CH_3, CH_2), 1660, 1651 (C=O); UV (ethanol)(λ_{max}/nm) 230, 260, 304, 350, 364; ^{1}H NMR (DMSO-d_6)(δ/ppm) 12.35 (s, 1H), 10.98 (s, 1H), 9.02 (d, 1H, J = 8.97 Hz), 8.85 (d, 1H, J = 1.43 Hz), 8.77 (d, 1H, J = 8.14 Hz), 8.17 (dd, 1H, J = 8.75, 1.65 Hz), 7.65−7.15 (m, 8H), 4.00 (t, 2H, J = 6.83 Hz), 3.20 (dt, 2H, J = 5.99 Hz), 2.75 (s, 3H), 2.73 (s, 3H), 2.01 (tt, 2H, J = 5.17 Hz); ^{13}C NMR (DMSO-d_6)(δ/ppm) 168.9 (s), 157.7 (s), 142.5 (s), 140.6 (s), 137.7 (s), 135.8 (s), 135.6 (s), 135.3 (s), 133.6 (s), 129.4 (d), 129.4 (d), 129.1 (d), 128.2 (d), 128.2 (d), 127.1 (d), 125.6 (d), 125.0 (d), 124.3 (d), 123.6 (d), 122.8 (d), 117.2 (s), 116.8 (d), 55.0 (t), 47.4 (t), 43.1 (q), 43.1 (q), 23.5 (t); MS *m*/*z* 456 (free base M + 1). Anal. ($C_{27}H_{26}ClN_3O_2S$) C, H, N.

Cell Lines and Culturing. Human tumor cell lines (HeLa, cervical carcinoma; MCF-7, breast carcinoma; CaCo-2, colon carcinoma; MIA PaCa-2, pancreatic carcinoma; HBL, ME 67.3 and ME 67.1, melanoma; Hep-2, laryngeal carcinoma) and normal human fibroblasts (WI-38) were tested for sensitivity in vitro. All cell lines were grown in DMEM medium (supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin) at 37 °C in a humidified atmosphere with 5% CO_2. For the purpose of the experiment, the cells were plated in quadruplicates in 96-microwell flat bottom plates at 2×10^4 cells/mL (all tumor cell lines) and 3×10^4 cells/mL (WI-38). The next day (24 h later) compounds were added to the cells at different concentrations (5×10^{-4} M, 10^{-4} M, and 10^{-5} M). Compounds were dissolved in DMSO at a concentration of 10^{-1} M and diluted with DMEM medium into working concentrations. The concentration of DMSO was too small to affect the growth. Control cells (without any compound) were growing under the same conditions.

Cell viability was measured immediately before (day 0) and 72 h after addition of compounds, using MTT assay, which detects dehydrogenase activity in viable cells.[43,44] For this purpose the medium was discarded and 40 μL of MTT (1%) was added to each well. After 4 h of incubation at 37 °C, the precipitates were dissolved in 160 μL of DMSO. The absorbance was measured on an ELISA reader at 570 nm, and the percentage of growth was calculated. Each number was the mean of three individual experiments done in quadruplicate. The cytotoxic effects of each compound were obtained as IC_{50} values, which represents the molar drug concentrations required to cause 50% inhibition.

Isolation of DNA−Apoptotic Fragments. Melanoma cells (HBL) were treated with compound **4**, at concentration of 5×10^{-6} M and 10^{-5} M for 24 h. The apoptotic fragments of DNA were isolated according to a method described by Herrmann et al.[45]

Flow Cytometry. Melanoma cells (HBL) were grown under confluence and treated with compound **4** (10^{-5} M) for 5 h. Fixation and DNA staining for cell cycle analysis was performed (ICBR Flow Cytometry Core Laboratory, http://www.biotech.ufl.edu). PI stained cells were analyzed by FACS CALIBUR (Becton Dickinson), using ModFitLT V2.0 (PMac) software.

Detection and Quantification of Apoptosis by Annexin-V-Staining. Melanoma cells (HBL) were plated at 2×10^5 cells/mL in a multiwell plate (2 mL). After 48 h, the cells were treated with 10^{-6} M, 5×10^{-6} M, and 10^{-5} M of compound **4** 5 h. Staining with Annexin-V-FLUOS (Staining kit, Roche Diagnostics GmbH, Mannheim, Germany) was performed according to the manufacture procedure, with slight modification. The cells were collected, washed with PBS, and centrifuged at 200*g* for 5 min. The cell pellets were suspended in 20 μL of staining solution and incubated for 15 min at ambient temperature. The evaluation was performed by fluorescence microscopy. The results are expressed as a ratio between apoptotic (green) and total number of the cells.

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References

(1) Li, L.; Wang, H.-K.; Kuo, S.-C.; Wu, T.-S.; Lednicer, D.; Lin, C. M.; Hamel, E.; Lee, K.-H. Antitumor Agents. 150. 2′,3′,4′,5′,5,6,7-Substituted 2-Phenyl-4-quinolones and Related Compounds: Their Synthesis, Cytotoxicity, and Inhibition of Tubulin Polymerization. *J. Med. Chem.* **1994**, *37*, 1126−1135.

(2) Li, L.; Wang, H.-K.; Kuo, S.-C.; Wu, T.-S.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K.-H. Antitumor Agents. 155. Synthesis and Biological Evaluation of 3′,6,7-Substituted 2-Phenyl-4-quinolones as Antimicrotubule Agents. *J. Med. Chem.* **1994**, *37*, 3400−3407.

(3) Chen, K.; Kuo, S.-C.; Hsieh, M.-C.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K.-H. Antitumor Agents. 174. 2′,3′,4′,5′,5,6,7-Substituted 2-Phenyl-1,8-naphthyridin-4-ones: Their Synthesis, Cytotoxicity, and Inhibition of Tubulin Polymerization. *J. Med. Chem.* **1997**, *40*, 2266−2275.

(4) Zhang, S.-X.; Bastow, K. F.; Tachibana, Y.; Kuo, S.-C.; Hamel, E.; Mauger, A.; Narayanan, V. L.; Lee, K.-H. Antitumor Agents. 196. Substituted 2-Thienyl-1,8-naphthyridin-4-ones: Their Synthesis, Cytotoxicity, and Inhibition of Tubulin Polymerization. *J. Med. Chem.* **1999**, *42*, 4081−4087.

(5) Zhang, S.-X.; Feng, J.; Kuo, S.-C.; Brossi, A.; Hamel, E.; Tropsha, A.; Lee, K.-H. Antitumor Agents. 199. Three-Dimensional Quantitative Structure−Activity Relationship Study of the Colchicine Binding Site Ligands Using Comparative Molecular Field Analysis. *J. Med. Chem.* **2000**, *43*, 167−176.

(6) Hour, M.-J.; Huang, L.-J.; Kuo, S.-C.; Xia, Y.; Bastow, K. F.; Nakanishi, Y.; Hamel, E.; Lee, K.-H. 6-Alkylamino- and 2,3-Dihydro-3′-methoxy-2-phenyl-4-quinazolinones and Related Compounds: Their Synthesis, Cytotoxicity, and Inhibition of Tubulin Polymerization. *J. Med. Chem.* **2000**, *43*, 4479−4487.

(7) Wang, H.-K.; Bastow, K. F.; Cosentino, M. L.; Lee, K.-H. Antitumor Agents. 166. Synthesis and Biological Evaluation of 5,6,7,8-Substituted-2-phenylthiochromen-4-ones. *J. Med. Chem.* **1996**, *39*, 1975−1980.

(8) Xia, Y.; Yang, Z.-Y.; Xia, P.; Bastow, K. F.; Tachibana, Y.; Kuo, S.-C.; Hamel, E.; Hackl, T.; Lee, K.-H. Antitumor Agents. 181. Synthesis and Biological Evaluation of 6,7,2′,3′,4′-Substituted-1,2,3,4-tetrahydro-2-phenyl-4-quinolones as a New Class of Antimitotic Antitumor Agents. *J. Med. Chem.* **1998**, *41*, 1155−1162.

(9) Tabarrini, O.; Cecchetti, V.; Fravolini, A.; Nocentini, G.; Barzi, A.; Sabatini, S.; Miao, H.; Sissi, C. Design and Synthesis of Modified Quinolones as Antitumoral Acridones. *J. Med. Chem.* **1999**, *42*, 2136−2144.

(10) Sit, S.-Y.; Ehrgott, F. J.; Gao, J.; Meanwell, N. A. 3-Hydroxyquinolin-2-ones: Inhibitors of [^{3}H]-glycine Binding to the Site Associated with the NMDA Receptor. *Bioorg. Med. Chem. Lett.* **1996**, *6* (5), 499−504.

(11) El-Subbagh, H. I.; Abadi, A. H.; Al-Khawad, I. E.; Al-Rashood, K. A. Synthesis and Antitumor Activity of some New Substituted Quinolin-4-one and 1,7-Naphthyridin-4-ones Analogues. *Arch. Pharm. Pharm. Med. Chem.* **1999**, 332, 19−24.

(12) Oleson, J. J.; Calderella, L. A.; Mjos, K. J.; Reith, A. R.; Thie, R. S.; Toplin, I. The Effects of Streptonigrin on Experimental Tumors. *Antibiot. Chemother.* **1961**, *11*, 158−164.

(13) Teller, M. N.; Wagshul, S. F.; Wolley, G. W. Transplantable Human Tumors in Experimental Chemotherapy. Effects of Streptonigrin on HS-1 and Hep-3 in the Rat. *Antibiot. Chemother.* **1961**, *11*, 165−173.

(14) Reilly, H. C.; Sigura, K. An Antitumor Spectrum of Streptonigrin. *Antibiot. Chemother.* **1961**, *11*, 174−177.

(15) Hackethal, C. A.; Golbey, R. B.; Tan, C. T. C.; Karnofsky, D. A.; Burchenal, J. H. Clinical Observation on the Effects of Streptonigrin in Patients wizh Neoplastiv Disease. *Antibiot. Chemother.* **1961**, *11*, 178−183.

(16) Rivers, S. L.; Whittington, R. M.; Medrek, T. J. Treatment of Malignant Lymphomas with Methyl Ester of Streptonigrin (NSC 45384). *Cancer* **1966**, *19* (10), 1377−1385.

(17) Kaung, D. T.; Whittington, R. M.; Spencer, H. H.; Patno, M. E. Comparison of Chlorambucil and Streptonigrin (NSC-45383) in the Treatment of Chronic Lymphocytic Leukemia. *Cancer* **1969**, *23* (3), 597−600.

(18) Kaung, D. T.; Whittington, R. M.; Spencer, H. H.; Patno, M. E. Comparison of Chlorambucil and Streptonigrin (NSC-45383) in the Treatment of Malignant Lymphomas. *Cancer* **1969**, *23* (6), 1280−1283.

(19) Banzet, P.; Jacquillat, C.; Civatte, J.; Puissant, A.; Maral, J.; Chastang, C.; Israel, L.; Belaich, S.; Jourdain, J.-C.; Weil, M.; Auclerc, G. Adjuvant Chemotherapy in the Management of Primary Malignant Melanoma. *Cancer* **1978**, *41* (4), 1240−1248.
(20) Forcier, R. J.; McIntyre, O. R.; Nissen, N. I.; Pajak, T. F.; Glidewell, O.; Holland, J. F. Combination Chemotherapy of non-Hodgkin Lymphoma. *Med. Pediatr. Oncol.* **1978**, *4* (4), 351−362.
(21) Fryatt, T.; Goroski, D. T.; Nilson, Z. D.; Moody, C. J.; Beall, H. D. Novel Quinolinequinone Antitumor Agents: Structure-Metabolism Studies with NAD(P)H: Quinone Oxidoreductase (NQO1). *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2195−2198.
(22) Yang, Z.-Y.; Xia, Y.; Xia, P.; Tachibana, Y.; Bastow, K. F.; Lee, K.-H. Antitumor Agents. 187. Synthesis and Cytotoxicity of Substituted 8,8-Dimethyl-2*H*,8*H*-pyrano[6,5-*h*]quinoline-2-one and Related Compounds. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 713−716.
(23) Xia, Y.; Yang, Z.-Y.; Hour, M.-J.; Kuo, S.-C.; Xia, P.; Bastow, K. F.; Nakanishi, Y.; Nampoothiri, P., Hackl, T.; Hamel, E.; Lee, K.-H. Antitumor Agents. Part 204: Synthesis and Biological Evaluation of Substituted 2-Aryl Quinazolinones. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1193−1196.
(24) Edwards, J. P.; Higuchi, R. I.; Winn, D. T.; Pooley, C. L. F.; Caferro, T. R.; Hamann, L. G.; Zhi, L.; Marschke, K. B.; Goldman, M. E.; Jones, T. K. Nonsteroidal Androgen Receptor Agonists Based on 4-(Trifluoromethyl)-2*H*-pyrano[3, 2-*g*]quinolin-2-one. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1003−1008.
(25) Dogan Koruznjak, J.; Slade, N.; Zamola, B.; Pavelić, K.; Karminski-Zamola, G. Synthesis, photochemical synthesis and antitumor evaluation of novel derivatives of thieno[3′,2′:4,5]thieno[2,3-*c*]quinolones. *Chem. Pharm. Bull.* **2002**, *50*, 656−660.
(26) Ried, W.; Oremek, G.; Ocakcioglu, B. Synthese von Substituierten Benzo[*b*]thiophenen. *Liebigs Ann. Chem.* **1980**, 1424−1427.
(27) Krubsack, A. J.; Higa, T. Mechanism of the Abnormal Reaction of Thionyl Chloride with Carboxylic Acids. *Tetrahedron Lett.* **1968**, *49*, 5149−5152.
(28) Dogan, J.; Karminski-Zamola, G.; Boykin, D. W. Synthesis and Photosynthesis of Substituted Benzo[*b*]thieno[3, 2-*c*]quinolones. *Heterocycl. Commun.* **1996**, *2*, 213-217.
(29) Dogan, J.; Karminski-Zamola, G.; Boykin, D. W. Photosynthesis of Some New Heteropolycyclic Quinolones. *Heterocycles* **1995**, *41*, 1659−1667.
(30) Baum, E. Brenzschleimsäurechlorid als Acrylirungsmittel. Synthese der Pyromycursäure. *Ber.* **1904**, 2949−2961.
(31) Kerr, J. F. R.; Harmon, B. V. Definition and incidence of apoptosis and historical perspective. In *Apoptosis: The molecular biology of cell death*; Tomei, L. D.; Cope, F. O., Eds.; Cold Spring Harbor Laboratory Press: Plainview, New York, 1991; pp 5−30.
(32) Lazebnik, Y. A.; Cole, S.; Cook, C. A.; Nelson, G. W.; Earnshaw, W. C. Nuclear events of apoptosis in vitro in cell-free mitotic extracts: a model system for analysis of the active phase of apoptosis. *J. Cell Biol.* **1993**, *123*, 7−22.
(33) Evans, D. L.; Dive, C. Effects of cisplatin on the induction of apoptosisin proliferating hepatoma cells and nonproliferating immature thymocytes. *Cancer Res.* **1993**, *53*, 2133-2139.
(34) Kuribayashi, N.; Sakagami, H.; Sakagami, T.; Niimi, E. I.; Shiokawa, D.; Ikekita, M., Takeda, M.; Tanuma, S. T. Induction of DNA fragmentation in human myelogenous leukemic cell lines by sodium 5,6-benzylidene-L-ascorbate and its related compounds. *Anticancer Res.* **1994**, *14*, 969−976.
(35) Grdiša, M.; Lopotar, N.; Pavelić, K. Effect of 17-member azalide on tumor cells growth. *Exp. Chemother.* **1998**, *44*, 331−336.
(36) Zhao, S.; Smith, K. S.; Deveau, A. M.; Dickhaus, C. M.; Johnson, M. A.; Macdonald, T. L.; Cook, J. M. Biological activity of the tryprostatins and their diastereomers on human carcinoma cell lines. *J. Med Chem.* **2002**, *45*, 1559−1562.
(37) Kanthou, C.; Tozer, G. M. The tumor vascular targeting agent combretastatin A-4-phosphate induces reorganization of the actin cytoskeleton and early membrane blebbing in human endothelial cells. *Blood* **2002**, *99*, 2060−2069.
(38) Li, Q.; Wooda, K. W.; Claiborne, A.; Gwaltney, S. L.; Barr, K. J.; Liu, G.; Gehrke, L.; Credo, R. B.; Hui, Y. H.; Lee, J.; Warner, R. B.; Kovar, P.; Nukkala, M. A.; Zielinski, N. A.; Tahir, S. K.; Fitzgerald, M.; Kim, K. H.; Marsh, K.; Frost, D.; Mg, S. C.; Rosenberg, S.; Sham, H. L. Synthesis and biological evaluation of 2-indolyloxazolines as a new class of tubulin polymerization inhibitors. Discovery of A-289099 as an orally active antitumot agent. *Bioorg., Med. Chem. Lett.* **2002**, *12*, 465−469.
(39) Bacher, G.; Beckers, T.; Emig, P.; Klenner, T.; Kutscher, B.; Nickel, B. New smal molecule tubulin inhibitors. *Pure Appl. Chem.* **2001**, *73*, 1459−1464.
(40) Ried, W.; Ochs, W. Umsetzung Asymmetrisch Substituierter Alkine mit Dischwefeldichlorid. *Chem. Ber.* **1974**, *107*, 1334−1343.
(41) Kanaoka, Y.; Itoh, K.; Hatanaka, Y.; Flippen, J. L.; Karle, I. L.; Witkop, B. Synthetic Photochemistry with Heterocyclic Anilides. Stereochemistry of the Intramolecular 1,5-Hydrogen Shifts in Nonoxidative Photocyclization of Benzo[*b*]thiophene-2-carboxanilides. *J. Org. Chem.* **1975**, *40* (20), 3001−3003.
(42) Gronowitz, S.; Timari, G. Some reactions of Thieno-fused Quinoline *N*-oxides. *J. Heterocycl. Chem.* **1990**, *27*, 1501−1504.
(43) Slater, T. F.; Sawyer, B.; Sträuli, U. Studies on Succinate-tetrazolium Reductase Systems. III Points of Coupling of Four Different Tetrazolium Salts. *Biochim. Biophys. Acta* **1963**, *77*, 383−393.
(44) Mickisch, G.; Fajta, S.; Keilhauer, G.; Schlick, E.; Tschada, R.; Alken, P. Chemosensitivity Testing on Primary Human Cell Carcinoma by a Tetrazolium Based Microculture Assay (MTT). *Urol. Res.* **1990**, *18*, 131−136.
(45) Herrmann, M.; Lorenz, H. M.; Voll, R.; Grunke, M.; Woith, W.; Kalden, J. R. A rapid and simple method for isolation of apoptotic DNA fragments, *Nucl. Acids Res.* **1994**, *22*, 5506−5507.

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