Synthesis, Photochemical Synthesis and Antitumor Evaluation of Novel Derivatives of Thieno[3′,2′:4,5]thieno[2,3-*c*]quinolones

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The novel derivatives of thieno[3′,2′:4,5]thieno[2,3-*c*]quinolones 6a, 6b, 7, 10a and 10b were synthesized in multistep synthesis starting from thiophene-3-carboxaldehyde and malonic acid reacting in aldol condensation or from 3-bromothiophenes or methyl 4-bromothiophene-2-carboxylate reacting in Heck reaction. They resulted in corresponding substituted thienylacrylic acids 3a—c, which were cyclized into thieno[2,3-*c*]thiophene-2-carbonyl chlorides 4a—c and converted into thieno[2,3-*c*]thiophene-2-carboxamides 5a—d. Prepared carboxamides were photochemically dehydrohalogenated into corresponding substituted thieno[3′,2′:4,5]thieno[2,3-*c*]-quinolones 6a—d. Compound 7 was prepared from 6d by alkylation with *N*-[3-(dimethylamino)propyl]chloride hydrochloride in the presence of NaH. Compounds 10a and 10b were prepared from 6c in the multistep synthesis over acid 8 and acid chloride 9. Compounds 6a, 6b, 7, 10a and 10b were found to exert cytostatic activities against malignant cell lines: pancreatic carcinoma (MiaPaCa2), breast carcinoma (MCF7), cervical carcinoma (HeLa), laryngeal carcinoma (Hep2), colon carcinoma (CaCo-2), melanoma (HBL), and human fibroblast cell lines (WI-38). The compound 6b, which bears the 3-dimethylaminopropyl substituent on quinolone nitrogen and methoxycarbonyl substituent on position 9, exhibiedt marked antitumor activity. On the contrary, compound 7, which also bears the 3-dimethylaminopropyl substituent on the quinolone nitrogen but anilido substituent on position 9, exhibited less antitumor activity than the others.

Key words quinolone; thiophene; antitumor activity; Heck reaction; photocyclization; alkylation

There is little literature data describing the antitumor activity of heterocyclic quinolones. Some new analogs of 4-quinolinones and 1,7 naphthyridin-4-ones were synthesized and their *in vitro* antitumor activity against central nervous system (CNS) (SNB-75), breast (T-47D) and lung (NCI-H 522) cancer cell lines was tested.[2)] One of the first quinolinequinones, streptonigrin (SN) is an antitumor antibiotic, which has activity against a broad range of tumors.[3—5)] SN was studied clinically as an antitumor agent, but its use was limited because of reports of delayed myelotoxicity.[6,7)] Nevertheless, positive results were reported for SN either as a single agent[8,9)] or in combination chemotherap,[10,11)] SN is an excellent substrate for oxidoreductase (NQ01).[12)]

Pyranoquinoline-2-ones were synthesized and evaluated for their *in vitro* cytotoxicity against a panel of human tumor cell lines,[13)] while 2-arylquinazolinones displayed significant growth inhibitory action against tumor cell lines and some of them were potent inhibitor of tubulin polymerization. Some of them displayed selective activity against P-gp-expressing epidermoid carcinoma of the nasopharynx.[14)] Recently trifluoromethyl substituted pyranoquinolinone was tested for its ability to modulate the transcriptional activity of the human androgen receptor (HAR).[15)]

Searching for compounds related to these classes of biologically and pharmacologically active compounds, we prepared new substituted thieno[3′,2′:4,5]thieno[2,3-*c*]-quinolones containing *N*-[3-(dimethyamino)propyl] substituent on the quinolone nitrogen (**6a**, **6b**, and **7**) and those containing *N*-[3-(dimethyamino)propyl] substituent in the amide part of the molecule (**10a** and **10b**).

Results and Discussion

Chemistry Compounds **6a** and **6b** were prepared in multistep synthesis according to Chart 1. Starting from thiophene-3-carboxaldehyde and malonic acid by already known aldol condensation reaction,[16)] or from 3-bromothiophene by Heck reaction,[17)] or from 4-bromothiophene-2-carboxaldehyde by oxidation of carboxaldehyde group[18)] and esterification of carboxylic acid,[19)] followed by Heck reaction, 5-substituted 3-thienyl-acrylic acids **3a**—**c** were prepared. Cyclisation of **3a**—**c** with thionyl chloride and a catalytic amount of pyridine, by the known method[20,21)] gave substituted 3-chlorothieno[2,3-*b*]thiophene-2-carbonyl chlorides **4a**—**c**. By refluxing of chlorides **4b** and **4c** with aniline in toluene corresponding 3-chlorothieno[2,3-*b*]thiophene-2-carboxamides **5c** and **5d** were obtained.

On the other hand, corresponding substituted 3-chlorothieno[2,3-*b*]thiophene-2-carboxamides **5a** and **5b** were obtained from chlorides **4a**—**c** by the Shotten–Baumann method[22)] with *N*-[3-(dimethyamino)propyl]aniline at 0 °C.

All prepared anilides **5a**—**d** were converted by photochemical dehydrohalogenation reaction into corresponding quinolones **6a**—**d**.[20)] Better yields were achieved in cases where *N*-atom on the anilide part of the molecule was not substituted with *N*-[3-(dimethyamino)propyl] substituent. Quinolone **7** was obtained by alkylation of **6d** with *N*-[3-(dimethyamino)propyl]chloride hydrochloride in the presence of NaH/*N*,*N*-dimethylformamide (DMF).[23)] Hydrolysis of ester group in position 9 of the quinolone **6c** gave acid **8** which, in the reaction with $SOCl_2$, gave acid chloride **9**, which was then converted into the quinolones **10a** in the reaction with *N*-[3-(dimethyamino)propyl]amine and **10b** in

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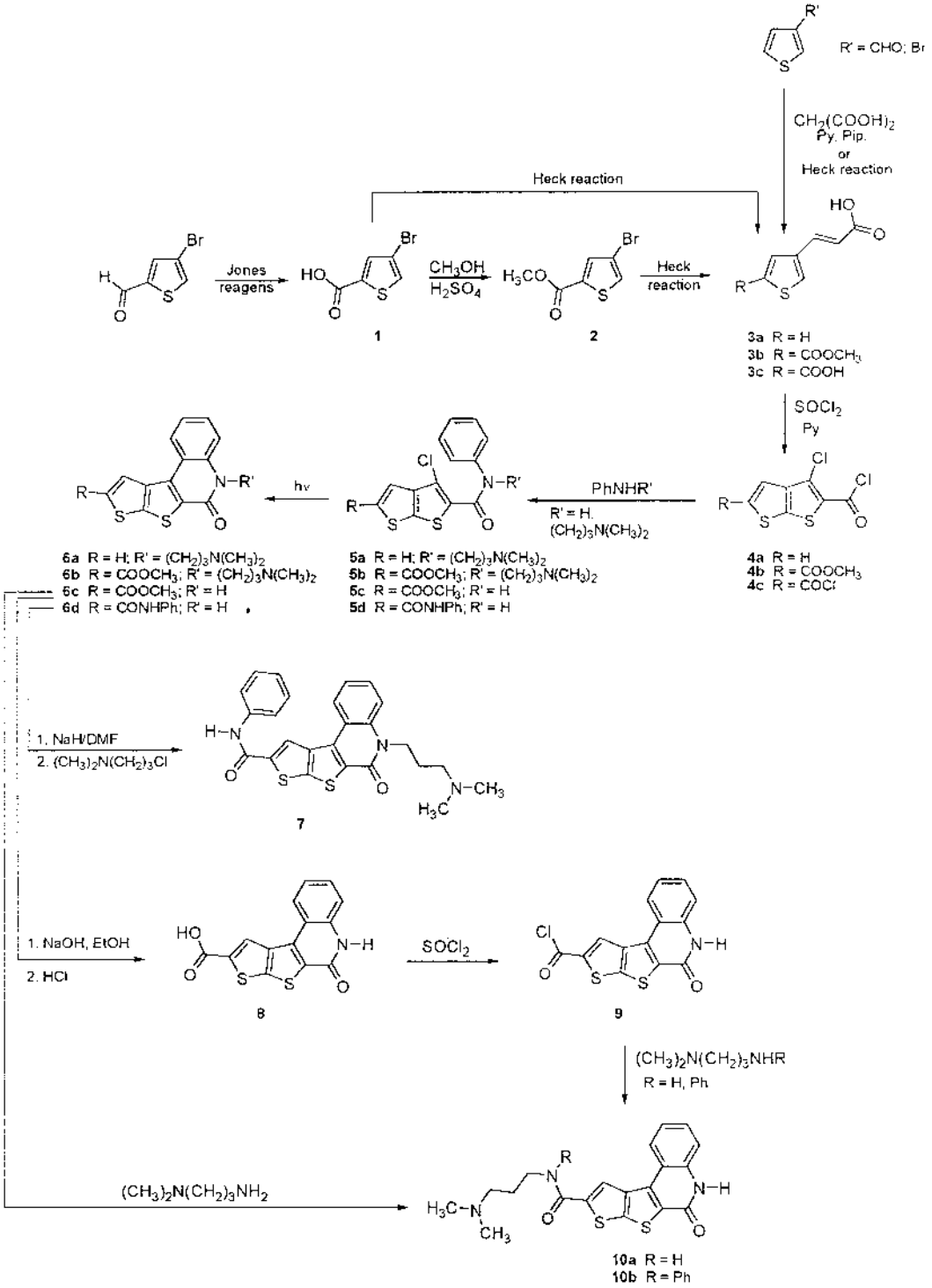

Chart 1

the reaction with *N*-[3-(dimethyamino)propyl]aniline.[24] Compound **10a** was also prepared directly from quinolone **6c** in the reaction with *N*-[3-(dimethyamino)propyl]amine.[25]

The structures of the novel compounds were determined on the basis of analysis of chemical shifts and H–H coupling constants in ^{1}H- and ^{13}C-NMR spectra as well as by elemental analysis.

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 human fibroblast cell line (WI-38).

Inhibitory effects are shown in Table 1 and Fig. 1. All tested compounds exhibited strong antitumor activity with IC_{50} ranging from 0.1 to 51 μM. Only compound **7** showed weaker inhibition than the others, IC_{50} ranging from 4.6 μM for HBL to 51 μM for Hep-2 cells. Compounds **6a** and **10a** were the strongest inhibitors of growth for HeLa cells

Table 1. Inhibitory Effects of Compounds **6a**, **6b**, **7**, **10a**, and **10b** on the Growth of Tumor Cell Lines and Normal Fibroblasts (WI-38)

Compd.	IC_{50} (μM)[a]						
	HeLa	MCF-7	CaCo-2	Mia PaCa-2	HBL	Hep-2	WI-38
6a	0.1	7.41	0.23	4.57	0.46	5.82	2.88
6b	2.2	6.23	1.0	1.0	6.17	2.5	4.67
7	31.6	47.9	38.0	34.7	4.5	51.0	7.59
10a	0.1	2.5	0.15	0.74	7.24	1.0	4.9
10b	2.5	6.3	3.8	2.34	0.59	4.17	5.76

a) 50% inhibitory concentration, or compound concentration required to inhibit cell proliferation by 50%.

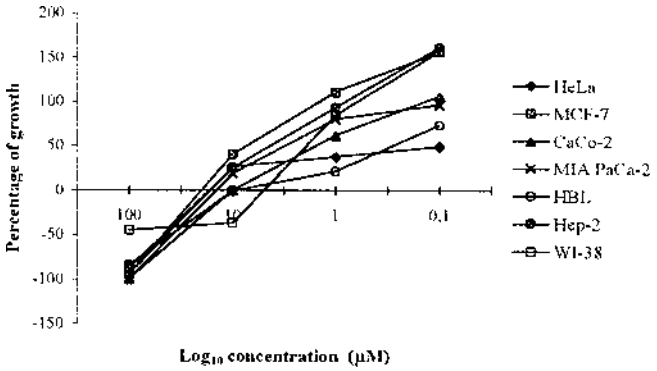

Fig. 1. The Inhibitory Effect of Different Concentrations of One of the Most Active Compounds (**6a**) on the Growth of Human Malignant Tumor Cell Lines (HeLa, MCF-7, CaCo-2, Mia PaCa-2, HBL, Hep-2) and Normal Human Fibroblasts (WI-38)

(IC_{50}=0.1 μM) and for CaCo-2 cells (IC_{50}=0.23 and 0.15 μM, respectively). **6a** and **10b** were also strong inhibitors of the growth of HBL cells (IC_{50}=0.46 and 0.59 μM). Compounds **6a** and **10a** showed the most pronounced selectivity in cytostatic activity—IC_{50} from 0.1 μM for HeLa cells to 7.41 and 7.24 μM for MCF-7 and HBL, respectively.

In comparison with antitumor activity of other quinolones[2,26—30] the substances tested in these experiments are very strong inhibitors of tumor cell proliferation at very small concentrations.

Conclusions

All tested compounds exhibited strong inhibitory activities against all cell lines tested. The cell sensitivity varied with the compound applied. All compounds examined were of similar cytotoxicity, except compound **7** which exhibited a less cytotoxic effect against all cell lines.

Experimental

Chemistry Melting points were determined on a Kofler hot stage microscope and are uncorrected. IR spectra were recorded on a Nicolet Magna 760 spectrophotometer in KBr discs or as a liquid film between sodium chloride plates. UV spectra were recorded on either a Perkin-Elmer 124 or a Hewlett-Packard 8452A spectrophotometer-meter 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 in DMSO-d_6 or $CDCl_3$. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, and coupling constants (J) are given in hertz (Hz). 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 analyser. Elemental compositions of the compounds agreed to within 0.4% of the calculated value. Irradiation was performed at room temperature with a water cooled immersion well fitted with an "Original Hanau" 400 W high pressure mercury arc lamp using a quartz or pyrex filter. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates or Merck aluminium oxide plates.

3-(3-Thienyl)acrylic Acid (3a) Method A: A solution of thiophene-3-carboxaldehyde (10 ml, 0.11 mol), malonic acid (11.40 g, 0.11 mol) and piperidine (1.5 ml) in pyridine (100 ml) was refluxed for 18 h. After cooling to room temperature the reaction mixture was poured into ice-water (*ca.* 300 g) and acidified with concentrated HCl with vigorous stirring. The precipitated crystals were filtered, washed with water and crystallized from 50% ethanol to give **3a** (10.17 g, 60.0%) as white crystals, mp 150—152 °C (lit.[16] mp 151 °C).

Method B[17]: A mixture of 3-bromothiophene (3 ml, 0.03 mol), cyclohexylammonium acrylate (20 g, 0.12 mol), palladium(II)acetate (0.10 g, 0.45 mmol), triphenylphosphine (0.36 g, 1.37 mmol), triethylamine (30 ml) and acetonitrile (70 ml) was sealed in a glass tube and heated at 120 °C for 20 h. After evaporation of the solvent, the residue was dissolved in water and boiled with charcoal. After filtration, the filtrate was acidified with diluted HCl (1 : 1). The resulting precipitate was filtered off and recrystallised from 50% ethanol to give **3a** (1.25 g, 25.3%) as white crystals.

3-(5-Methoxycarbonyl-3-thienyl)acrylic Acid (3b) Compound **3b** was synthesised according to method B starting from methyl 4-bromothiophene-2-carboxylate (**2**) in 70.2% yield, mp 197—200 °C. IR (KBr) cm^{-1}: 1725 (C=O), 1681 (C=O), 1630 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 12.86 (1H, s), 8.23 (1H, d, J=1.25 Hz), 8.10 (1H, d, J=1.24 Hz), 7.57 (1H, d, J=15.88 Hz), 6.47 (1H, d, J=15.87 Hz), 3.84 (3H, s). ^{13}C-NMR (DMSO-d_6) δ: 168.0 (s), 163.0 (s), 138.2 (s), 137.5 (d), 136.2 (s), 134.8 (d), 131.8 (d), 120.2 (d), 52.6 (q). MS *m/z*: 213 (M+1). *Anal.* Calcd for $C_9H_8O_4S$: C, 50.94; H, 3.80. Found: C, 49.28; H, 3.57.

3-(5-Carboxy-3-thienyl)acrylic Acid (3c) Compound **3c** was synthesized according to method B starting from 4-bromothiophene-2-carboxylic acid (**1**) in 84.7% yield, mp 264—266 °C (lit.[20] mp 264—266 °C).

3-Chlorothieno[2,3-*b*]thiophene-2-carbonyl Chloride (4a) The solution of acid **3a** (10 g, 0.07 mol), thionyl chloride (50 ml) and pyridine (2 ml) in chlorobenzene (100 ml) was heated at 140 °C for 18 h. Excess of thionyl chloride was removed under reduced pressure and the residue extracted with boiling cyclohexane. After cooling, the precipitated yellow crystals were filtered off to give **4a** (3.23 g, 40.5%), mp 127—130 °C (lit.[21] mp 127—130 °C).

Methyl 3-Chloro-2-chlorocarbonylthieno[2,3-*b*]thiophene-5-carboxylate (4b) Starting from acid **3b** using the same procedure as above, compound **4b** was obtained as yellow crystals in 56.6% yield, mp 135—138 °C. IR (KBr) cm^{-1}: 1771 (C=O), 1727 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.24 (1H, s), 4.00 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 163.8 (s), 161.8 (s), 141.2 (s), 136.9 (s), 134.7 (d), 134.1 (s), 129.8 (s), 124.6 (s), 52.7 (q). MS *m/z*: 295 (M+1). *Anal.* Calcd for $C_9H_4Cl_2O_3S_2$: C, 36.62; H, 1.37. Found: C, 36.60; H, 1.34.

3-Chlorothieno[2,3-*b*]thiophene-2,5-dicarbonyl Dichloride (4c) Starting from acid **3c** using the same procedure as above, compound **4c** was obtained as yellow crystals in 42.0% yield, mp 156—160 °C (lit.[20] mp 156—161 °C).

***N*-[3-(Dimethylamino)propyl]-3-chlorothieno[2,3-*b*]thiophene-2-carboxanilide (5a)** A solution of **4a** (4.40 g, 0.02 mol) in chloroform (250 ml) was added dropwise to a cold mixture of *N*-[3-(dimethylamino)propyl]aniline (3.56 g, 0.02 mol) and 5% NaOH (9 ml) at 2—3 °C. The resulting mixture was stirred at the same temperature for 30 min and then for 1 h at room temperature. The organic layer was separated and washed first with 10% HCl, then with water and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product was purified by column chromatography (neutral aluminium oxide) with toluene–ethanol mixture (9 : 1, v/v) to yield 6.44 g (89.5%) of compound **5a**. IR (NaCl) cm^{-1}: 2767—2944 (CH_2), 1634 (C=O). UV λ_{max} (methanol) nm: 230, 288, 346. ^{1}H-NMR (DMSO-d_6) δ: 7.67 (1H, d, J=5.29 Hz), 7.37—7.21 (5H, m), 7.15 (1H, d, J=5.32 Hz), 3.89 (2H, t, J=7.37 Hz), 2.38 (2H, t, J=7.12 Hz), 2.18 (6H, s), 1.74 (2H, tt, J=7.16, 7.39 Hz). ^{13}C-NMR (DMSO-d_6) δ: 161.3 (s), 142.5 (s), 141.6 (s), 137.4 (s), 133.6 (s), 131.7 (d), 129.2 (d), 129.2 (d), 127.6 (d), 127.6 (d), 127.5 (d), 118.5 (d), 116.2 (s), 56.1 (t), 48.0 (t), 44.8 (q), 44.8 (q), 24.8 (t). MS *m/z*: 379 (M+1). *Anal.* Calcd for $C_{18}H_{19}ClN_2OS_2$: C, 57.05; H, 5.05; N, 7.39. Found: C, 56.99; H, 5.10; N, 7.45.

Methyl 2-{*N*-[3-(Dimethylamino)propyl]-*N*-phenylcarbamoyl}-3-chlorothieno[2,3-*b*]thiophene-5-carboxylate (5b) Starting from compound **4b** using the same procedure as above, compound **5b** was obtained, after crystallisation from ethanol, as white crystals in 37.1% yield, mp 134—135 °C. IR (KBr) cm^{-1}: 2721—2946 (CH_2), 1716 (C=O), 1630 (C=O). UV λ_{max} (methanol) nm: 208, 270. ^{1}H-NMR (DMSO-d_6) δ: 7.75

(1H, s), 7.33—7.28 (4H, m), 7.23 (1H, dd, J=7.01 Hz), 3.87 (2H, t, J=7.31 Hz), 3.83 (3H, s), 2.25 (2H, t, J=6.97 Hz), 2.08 (6H, s), 1.67 (2H, tt, J=7.31, 7.07 Hz). ^{13}C-NMR (DMSO-d_6) δ: 162.4 (s), 161.6 (s), 143.6 (s), 142.3 (s), 142.2 (s), 137.6 (s), 136.0 (s), 130.0 (d), 130.0 (d), 128.4 (d), 128.4 (d), 128.4 (d), 125.0 (d), 117.4 (s), 57.1 (t), 53.4 (q), 49.0 (t), 45.9 (q), 45.9 (q), 26.0 (t). MS m/z: 437 (M+1). *Anal.* Calcd for $C_{20}H_{21}ClN_2O_3S_2$: C, 54.97; H, 4.84; N, 6.41. Found: C, 54.94; H, 4.80; N, 6.40.

Methyl 2-[*N*-Phenylcarbamoyl]-3-chlorothieno[2,3-*b*]thiophene-5-carboxylate (5c) A solution of **4b** (2.55 g, 8.6 mmol) and aniline (1 ml, 11.0 mmol) in toluene (50 ml) was refluxed for 3 h. After cooling, precipitated crystals were filtered off and recrystallised from acetone to give **5c** (1.81 g, 59.6%) as pale yellow crystals, mp 213—216 °C. IR (KBr) cm^{-1}: 1716 (C=O), 1651 (C=O). UV λ_{max} (methanol) nm: 206, 270. ^{1}H-NMR (DMSO-d_6) δ: 10.34 (1H, s), 7.98 (1H, s), 7.69 (2H, d, J=7.65 Hz), 7.38 (2H, dd, J=7.66 Hz), 7.15 (1H, dd, J=7.38 Hz), 3.89 (3H, s). ^{13}C-NMR (DMSO-d_6) δ: 161.6 (s), 158.6 (s), 143.0 (s), 142.6 (s), 138.0 (s), 136.9 (s), 135.7 (s), 128.9 (d), 128.9 (d), 124.7 (d), 124.5 (d), 120.3 (d), 120.3 (d), 117.5 (s), 52.8 (q). MS m/z: 352 (M+1). *Anal.* Calcd for $C_{15}H_{10}ClNO_3S_2$: C, 51.21; H, 2.86; N, 3.98. Found: C, 51.06; H, 2.86; N, 3.86.

3-Chlorothieno[2,3-*b*]thiophene-2,5-dicarboxanilide (5d) Starting from compound **4c** using the same procedure as above, compound **5d** was obtained, after crystallization from DMF–water mixture (1 : 2, v/v), as pale yellow crystals in 33.0% yield, mp 236—238 °C (lit.[20] mp 236—238 °C).

5-[3-(Dimethylamino)propyl]-6-oxo-5,6-dihydrothieno[3′,2′:4,5]-thieno[2,3-*c*]quinolin-6-one Hydrochloride (6a) A solution of anilide **5a** (0.65 g, 1.7 mmol) and triethylamine (0.23 ml, 1.7 mmol) in a methanol–benzene mixture (1 : 10, v/v) (550 ml) was irradiated with a 400 W high pressure mercury arc lamp using a pyrex filter at room temperature for 30 min. During the irradiation the air was bubbled through the solution. After evaporation of the solvent, the crude product was purified by column chromatography (neutral aluminium oxide) with toluene–ethanol mixture (9 : 1, v/v). The oily residue was stirred with saturated ethanolic HCl (10 ml) for 15 h to give **6a** (0.12 g, 31.3%) as white crystals, mp 260—263 °C. IR (KBr) cm^{-1}: 2474—3049 (CH_2), 1634 (C=O). UV λ_{max} (ethanol) nm: 228, 238, 254, 270, 294, 306, 332, 346. ^{1}H-NMR (DMSO-d_6) δ: 10.15 (1H, s), 8.53 (1H, dd, J=7.94, 1.00 Hz), 8.16 (1H, d, J=5.45 Hz), 7.92 (1H, d, J=5.42 Hz), 7.82 (1H, d, J=8.47 Hz), 7.68 (1H, m, J=8.55, 7.18, 1.32 Hz), 7.46 (1H, dd, J=7.26, 7.77 Hz), 4.50 (2H, t, J=7.25 Hz), 3.23 (2H, t, J=7.89 Hz), 2.74 (6H, s), 2.19 (2H, tt, J=7.56 Hz). ^{13}C-NMR (DMSO-d_6) δ: 157.2 (s), 143.7 (s), 141.2 (s), 136.9 (s), 133.8 (s), 133.0 (s), 131.8 (d), 129.5 (d), 124.8 (d), 122.7 (d), 121.3 (d), 117.5 (s), 115.7 (d), 53.9 (t), 41.9 (q), 41.9 (q), 39.2 (t), 22.6 (t). MS m/z: 343 (M+1; free base). *Anal.* Calcd for $C_{18}H_{19}ClN_2OS_2$: C, 57.05; H, 5.05; N, 7.39. Found: C, 57.29; H, 5.21; N, 7.24.

Methyl 5-(3-(Dimethylamino)propyl(-6-oxo-5,6-dihydrothieno-[3′,2′:4,5]thieno[2,3-*c*]quinoline-9-carboxylate Hydrochloride (6b) Starting from **5b** (0.50 g, 1.1 mmol) and using the same procedure as above, after 15 min of irradiation followed by stirring with saturated ethanolic HCl (10 ml) compound **6b** (0.38 g, 75.4%) was obtained as light yellow crystals, mp 240—242 °C. IR (KBr) cm^{-1}: 2472—2948 (CH_2), 1705 (C=O), 1634 (C=O). UV λ_{max} (ethanol) nm: 228, 242, 282, 334, 348. ^{1}H-NMR (DMSO-d_6) δ: 8.75 (1H, s), 8.55 (1H, d, J=8.00 Hz), 7.82 (1H, d, J=8.65 Hz), 7.70 (1H, dd, J=7.33, 8.32 Hz), 7.45 (1H, dd, J=7.33, 7.63 Hz), 4.47 (2H, t, J=6.94 Hz), 3.93 (3H, s), 3.39 (2H, dt, J=5.05, 7.97 Hz), 3.23 (2H, tt, J=7.22 Hz), 2.76 (3H, s), 2.74 (3H, s). ^{13}C-NMR (DMSO-d_6) δ: 161.8 (s), 157.1 (s), 149.4 (s), 140.3 (s), 137.1 (s), 137.1 (s), 134.4 (s), 133.8 (s), 130.0 (d), 127.4 (d), 125.2 (d), 123.1 (d), 117.3 (s), 115.9 (d), 54.1 (t), 52.7 (q), 42.1 (q), 42.1 (q), 39.3 (t), 22.8 (t). MS m/z: 401 (M+1; free base). *Anal.* Calcd for $C_{20}H_{21}ClN_2O_3S_2$: C, 54.97; H, 4.84; N, 6.41. Found: C, 54.91; H, 4.90; N, 6.39.

Methyl 6-Oxo-5,6-Dihydrothieno[3′,2′:4,5]thieno[2,3-*c*]quinoline-9-carboxylate (6c) Starting from **5c** (0.53 g, 1.5 mmol) and using the same procedure as above, after 30 min of irradiation followed by recrystallization from DMF compound **6c** (0.36 g, 75.8%) was obtained as light brown crystals, mp >300 °C. IR (KBr) cm^{-1}: 1728 (C=O), 1667 (C=O). UV λ_{max} (methanol) nm: 208, 226, 240, 282, 332, 346. ^{1}H-NMR (DMSO-d_6) δ: 12.20 (1H, s), 8.85 (1H, s), 8.55 (1H, d, J=7.85 Hz), 7.61 (1H, m, J=8.17, 7.02, 1.06 Hz), 7.53 (1H, dd, J=8.18, 1.19 Hz), 7.40 (1H, m, J=7.48, 1.31 Hz), 3.95 (3H, s). ^{13}C-NMR (DMSO-d_6) δ: 161.9 (s), 157.6 (s), 140.7 (s), 137.5 (s), 137.5 (s), 137.1 (s), 135.3 (s), 134.8 (s), 129.5 (d), 127.4 (d), 124.4 (d), 122.9 (d), 116.5 (d), 116.1 (s), 52.7 (q). MS m/z: 316 (M+1). *Anal.* Calcd for $C_{15}H_9NO_3S_2$: C, 57.13; H, 2.88; N, 4.44. Found: C, 57.10; H, 2.98; N, 4.70.

6-Oxo-5,6-dihydrothieno[3′,2′:4,5]thieno[2,3-*c*]quinoline-9-carboxanilide (6d) From **5d** (0.50 g, 1.2 mmol) and using the same procedure as above, after 2 h of irradiation followed by recrystallization from dioxane compound **6d** (0.17 g, 40.0%) was obtained as light yellow crystals, mp >300 °C (lit.[20] mp >300 °C).

5-(3-(Dimethylamino)propyl(-6-oxo-5,6-dihydrothieno[3′,2′:4,5]-thieno[2,3-*c*]quinoline-9-carboxanilide Hydrochloride (7) To a cold solution of **6d** (1.00 g, 2.7 mmol) in anhydrous DMF (200 ml), sodium hydride as 60—65% oil dispersion (0.50 g, 13.0 mmol) was added in three portions.[31] After stirring under nitrogen for 15 min, a solution of 3-(dimethylamino)propyl chloride hydrochloride (1.12 g, 7.0 mmol) in DMF (50 ml) was added to the solution 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 column chromatography (neutral aluminium oxide) with toluene–ethanol mixture (9 : 1, v/v) followed by stirring the solid with saturated ethanolic HCl (10 ml) for 15 h to give **7** (0.12 g, 9.1%) as white crystals, mp 238—240 °C. IR (KBr) cm^{-1}: 2670—3059 (CH_2), 1660 (C=O), 1651 (C=O). UV λ_{max} (ethanol) nm: 230, 260, 328. ^{1}H-NMR (DMSO-d_6) δ: 10.59 (1H, s), 9.84 (1H, s), 7.57—7.37 (5H, m), 7.22 (1H, d, J=8.22 Hz), 7.05—6.92 (3H, m), 6.69 (1H, s), 3.10 (2H, t, J=7.20 Hz), 2.94 (2H, t, J=7.70 Hz), 2.55 (6H, s), 1.90 (2H, tt, J=7.40 Hz). ^{13}C-NMR (DMSO-d_6) δ: 161.3 (s), 156.5 (s), 146.1 (s), 142.9 (s), 140.1 (s), 139.3 (s), 137.8 (s), 134.0 (s), 131.3 (d), 131.3 (d), 131.1 (s), 129.1 (d), 125.5 (d), 123.9 (d), 123.9 (d), 122.9 (d), 122.5 (d), 122.2 (d), 118.4 (s), 116.0 (d), 55.2 (t), 42.1 (q), 42.1 (q), 33.0 (t), 23.7 (t). MS m/z: 462 (M+1; free base). *Anal.* Calcd for $C_{25}H_{24}ClN_3O_2S_2$: C, 60.29; H, 4.86; N, 8.44. Found: C, 60.14; H, 5.04; N, 8.42.

6-Oxo-5,6-dihydrothieno[3′,2′:4,5]thieno[2,3-*c*]quinoline-9-carboxylic Acid (8) A solution of NaOH (0.60 g, 15.0 mmol) in water (100 ml) was added to the solution of **7** (0.45 g, 1.4 mmol) in ethanol (50 ml). The resulting solution was refluxed for 1 h. Ethanol was removed under reduced pressure, the residue was dissolved in water and acidified with 5% HCl. The precipitate was filtered off, washed with water and recrystallized from DMSO–water mixture (1 : 2, v/v) to give **8** (0.32 g, 74.4%) as white crystals, mp >300 °C. IR (KBr) cm^{-1}: 1677 (C=O), 1631 (C=O). UV λ_{max} (methanol) nm: 226, 278, 316, 330, 346. ^{1}H-NMR (DMSO-d_6) δ: 13.60 (1H, s), 12.18 (1H, s), 8.76 (1H, s), 8.53 (1H, d, J=7.88 Hz), 7.60 (1H, dd, J=7.18 Hz), 7.53 (1H, d, J=7.18 Hz), 7.39 (1H, m, J=7.46, 1.22 Hz). ^{13}C-NMR (DMSO-d_6) δ: 163.0 (s), 157.6 (s), 140.8 (s), 139.3 (s), 137.5 (s), 137.5 (s), 135.3 (s), 135.3 (s), 129.5 (d), 126.8 (d), 124.3 (d), 122.8 (d), 116.5 (d), 116.1 (s). MS m/z: 302 (M+1). *Anal.* Calcd for $C_{14}H_7NO_3S_2$: C, 55.80; H, 2.34; N, 4.65. Found: C, 55.60; H, 2.11; N, 4.54.

6-Oxo-5,6-dihydrothieno[3′,2′:4,5]thieno[2,3-*c*]quinoline-9-carbonyl Chloride (9) A mixture of **8** (0.23 g, 0.8 mmol) and thionyl chloride (5 ml, 68.8 mmol) was refluxed for 4 h. Excess of thionyl chloride was removed under reduced pressure to give **9** (0.24 g, 100%) as yellow crystals, mp >300 °C. IR (KBr) cm^{-1}: 1741 (C=O), 1661 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 12.18 (1H, s), 8.86 (1H, s), 8.53 (1H, d, J=7.90 Hz), 7.60 (1H, dd, J=7.20 Hz), 7.52 (1H, d, J=7.20 Hz), 7.39 (1H, m, J=7.40, 1.20 Hz). ^{13}C-NMR ($CDCl_3$) δ: 157.6 (s), 156.7 (s) 140.8 (s), 139.3 (s), 137.8 (s), 137.5 (s), 135.3 (s), 135.1 (s), 129.5 (d), 126.8 (d), 124.3 (d), 122.8 (d), 116.5 (d), 116.1 (s). MS m/z: 320 (M+1). *Anal.* Calcd for $C_{14}H_6ClNO_2S_2$: C, 52.58; H, 1.89; N, 4.38. Found: C, 52.72; H, 1.97; N, 4.19.

***N*-[3-(Dimethylamino)propyl]-6-oxo-5,6-dihydrothieno[3′,2′:4,5]-thieno[2,3-*c*]quinoline-9-carboxamide Hydrochloride (10a)** Method C: A solution of **9** (0.21 g, 0.7 mmol) and [3-(dimethylamino)propyl]amine (2.5 ml, 19.8 mmol) in toluene (20 ml) was refluxed for 30 min. After cooling, the precipitate was filtered off and washed with chloroform. Recrystallization from DMSO–water mixture (1 : 2, v/v), followed by conversion into the hydrochloride salt by stirring it with saturated ethanolic HCl for 60 h, gave white crystals (0.18 g, 65.7%), mp 275—280 °C.

Method D: A solution of **6c** (0.10 g, 0.3 mmol) in [3-(*N*-dimethylamino)-propyl]amine (5 ml, 39.7 mmol) was stirred at room temperature under nitrogen for 96 h. The excess of amine was then removed by evaporation. The residue after evaporation was treated with ethanol in order to remove starting material, which was then filtered off (0.01 g). The filtrate was evaporated to dryness under reduced pressure. Recrystallization from DMSO–water mixture (1 : 2, v/v), followed by conversion into the hydrochloride salt by stirring it with saturated ethanolic HCl for 60 h, gave white crystals (0.04 g, 31.6%), mp 275—280 °C. IR (KBr) cm^{-1}: 2696—2956 (CH_2), 1645 (C=O). UV λ_{max} (ethanol) nm: 226, 242, 282, 318, 332, 348. ^{1}H-NMR (DMSO-d_6) δ: 12.17 (1H, s), 10.48 (1H, s), 9.30 (1H, t, J=5.70 Hz), 9.16 (1H, s), 8.48 (1H, dd, J=7.86 Hz), 7.60—7.52 (2H, m, J=7.98, 6.61, 1.67 Hz), 7.37 (1H, m, J=7.34, 6.65, 1.68 Hz), 3.42 (2H, dt, J=6.44 Hz), 3.19 (2H, dt, J=6.69 Hz), 2.79 (3H, s), 2.78 (3H, s), 2.02 (2H, tt, J=6.62 Hz). ^{13}C-NMR

(DMSO-d_6) δ: 161.4 (s), 157.6 (s), 146.7 (s), 144.6 (s), 141.1 (s), 137.7 (s), 135.3 (s), 134.0 (s), 129.5 (d), 126.8 (d), 123.8 (d), 122.6 (d), 116.7 (d), 116.2 (s), 54.7 (t), 42.2 (q), 42.2 (q), 36.6 (t), 24.4 (t). MS *m/z*: 386 (M+1; free base). *Anal.* Calcd for $C_{19}H_{20}ClN_3O_2S_2$: C, 54.08; H, 4.78; N, 9.96. Found: C, 53.96; H, 4.71; N, 10.05.

***N*-[3-(Dimethylamino)propyl]-6-oxo-5,6-dihydrothieno[3′,2′:4,5]-thieno[2,3-*c*]quinolin-9-carboxanilide Hydrochloride (10b)** The chloride **9** (0.24 g, 0.8 mmol) was converted into free base of **10b** by the method described earlier for the synthesis of **5a**. Free base was then converted into the hydrochloride salt by stirring it with saturated ethanolic HCl for 15 h. Resulting precipitate was filtered off, washed with absolute ethanol and dried in vacuo at 50 °C for 5 h to give **10b** (0.10 g, 9.1%) as white crystals, mp 280—282 °C. IR (KBr) cm^{-1}: 2361—2923 (CH_2), 1655 (C=O), 1631 (C=O). UV λ_{max} (ethanol) nm: 228, 242, 286, 318, 332, 348. ^{1}H-NMR (DMSO-d_6) δ: 12.12 (1H, s), 9.84 (1H, s), 7.69—7.48 (8H, m), 7.31 (1H, dd, J=7.52 Hz), 6.91 (1H, s), 3.95 (2H, t, J=7.21 Hz), 3.20 (2H, t, J=8.26 Hz), 2.79 (6H, s), 1.99 (2H, tt, J=8.21 Hz). ^{13}C-NMR (DMSO-d_6) δ: 161.3 (s), 157.5 (s), 147.4 (s), 143.1 (s), 141.7 (s), 140.3 (s), 137.5 (s), 134.8 (s), 134.1 (s), 130.4 (d), 130.4 (d), 129.5 (d), 129.5 (d), 129.4 (d), 129.2 (d), 124.9 (d), 122.8 (d), 122.4 (d), 116.7 (d), 115.8 (s), 54.3 (t), 47.6 (t), 42.3 (q), 42.3 (q), 22.5 (t). MS *m/z*: 462 (M+1; free base). *Anal.* Calcd for $C_{25}H_{24}ClN_3O_2S_2$: C, 60.29; H, 4.86; N, 8.44. Found: C, 59.99; H, 5.05; N, 8.24.

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, melanoma; Hep-2, laryngeal carcinoma) and normal human fibroblasts (WI-38) were tested for sensitivity on quinolones *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 quadriplicate in 96-microwell flat bottom plates at the concentrations of 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 (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} M). Compounds were dissolved in DMSO at the concentration 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 grown 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.[32,33] For this purpose the medium was discarded and MTT was added to each well at a concentration of 20 μg/40 μl. After 4 h of incubation at 37 °C the precipitates were dissolved in 160 μl of DMSO. The absorbance was measured on ELISA reader at 570 nm, and the percentage of growth was calculated. Each number was the mean of four parallel samples in three individual experiments. The cytotoxic effects of each compound were obtained as IC_{50} values, which represents the molar drug concentrations required to cause 50% inhibition.

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