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Synthesis, cytotoxic evaluation, and DNA binding of novel thiazolo[5,4-*b*]quinoline derivatives

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Abstract—A series of novel alkylamino and 9-anilinothiazolo[5,4-*b*]quinolines were synthesized as potential antitumoral agents. The in vitro cytotoxicity of these compounds was evaluated on several cell lines. The inclusion of electron-withdrawn/acceptor hydrogen-bond groups at position 3′ of the anilino ring and the presence of an alkylamino chain on the tricyclic framework (regardless of its position) seem to be structural features relevant to cytotoxic activity.

1. Introduction

The acridine derivatives are a group of compounds believed to express their antitumor activity through an interaction with DNA.[1–4] The acridine intercalation properties are responsible for their high affinity for DNA. However, intercalation ability does not ensure antitumor activity, and a requirement for specific interaction with other targets, such as DNA topoisomerase,[5] has frequently been proposed. Besides acridine derivatives, other fused compounds, such as thiazolo[5,4-*b*]quinolines,[6] furo[2,3-*b*]quinolines,[7] and pyrazolo[3,4-*d*]pyrimidines,[8] have been studied. Recently, the cytotoxic activity and intercalation properties of some derivatives of 9-anilinothiazolo[5,4-*b*]quinoline have been reported.[9] The chemical structure of these compounds accommodates the general substitution pattern of acridine derivatives on a thiazoloquinoline template **1** (Fig. 1). Our results revealed an increase in cytotoxic activity with the presence of a diethylethylenediamino group at the 2 position of a thiazoloquinoline nucleus **2**, but noteworthy, the AHMA **7** analogue, compound **3**, showed low activity. In order to gain a better understanding of the relationship between the substitution pattern and cytotoxicity and to acquire insight into the contribution of the diethylethylendiamino group, additional compounds with different substituents were prepared. Here, the synthesis, in vitro cytotoxicity, and DNA intercalating properties of novel thiazoloquinoline derivatives are reported.

2. Chemistry

The synthesis of the compounds is illustrated in Scheme 1. The preparation of compounds **8** and **9** has already been described.[6,9] From compound **9**, the synthesis of compounds **10a**–**11c** was achieved in good yield under acidic conditions. Attempts, however, under neutral and basic conditions were unsuccessful and resulted in low yield or decomposition.

Keywords: Thiazolo[5,4-*b*]quinoline derivatives; Antitumoral; Cytotoxic activity; Structure–activity relationship; DNA binding.
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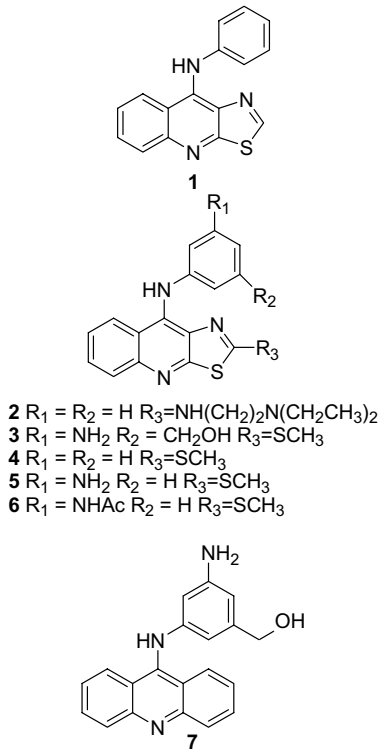


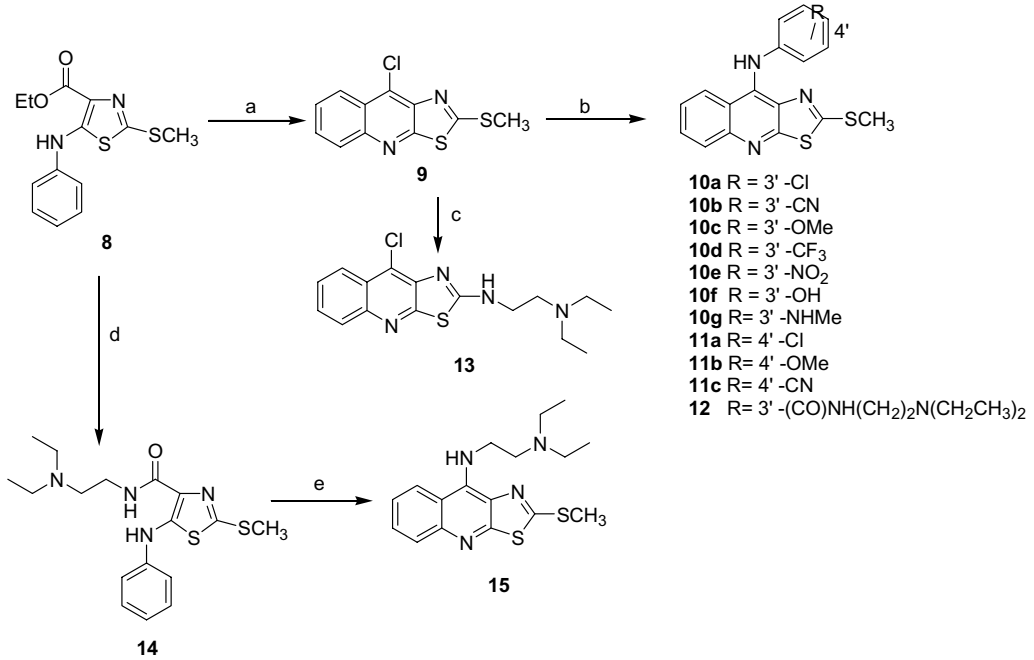
Figure 1. Structures of tricyclic compounds.

Preparation of **15** was attempted by a direct condensation of **9** with 2-(*N*,*N*-diethyl)ethylenediamine in MeOH as solvent. Though one principal product was obtained, the spectral data showed the absence of the methylthio group (^{1}H NMR), the presence of one chlorine atom (MS-FAB), and the preservation of the thiazoloquinoline ring (^{13}C NMR). Therefore, it was concluded that novel compound **13** and not **15** was formed during the reaction. The yield of this reaction was increased by direct condensation of the amine with **9** at room temperature (25 °C). The preparation of **15** was carried out by basic hydrolysis of compound **8**, the coupling of the carbonyldiimidazole derivative of **8** with 2-(*N*,*N*-diethylethylenediamino)ethylamine, compound **14**, and the cyclization of the last compound with $POCl_3$/PPA to afford **15** in low yield.

Notably, aromatic amines react at position 9 of the thiazoloquinoline system while the aliphatic amines do so at position 2. This behavior may be explained with the HSAB principle[10] since aliphatic amines are hard bases which should react with the harder region of the molecule (thiazole ring), while aromatic amines are medium-soft bases and should react with the softer region (the central ring of tricyclic system), as illustrated in Fig. 2. An alternative explanation could be a steric hindrance of the sulfur atom at position 2 to react with aromatic amines; such hindrance does not exist with aliphatic amines.

All thiazolo[5,4-*b*]quinoline derivatives **10**–**15** were characterized by ^{1}H and ^{13}C NMR and elemental analysis. NOESY experiments of compounds **10a**, **10b** and **10f** led to the unequivocal assignment of the aromatic protons for all compounds. The assignment was based on the long range interaction between the H-8 proton and the one present at the amino group linking both aromatic rings (Fig. 3). The data are summarized in Table 1.

An interesting interaction between the H-2′ and H-6′ protons and the methylthio group protons was observed



Scheme 1. Reactions and conditions: (a) $POCl_3$/PPA, 130 °C, 4 h; (b) NH_2–C_6H_4–R in MeOH/HCl, reflux, 6 h; (c) $H_2NCH_2CH_2N(CH_2CH_3)_2$, rt, 24 h., (d) 1— KOH/EtOH; 2— 1,1-carbonyldiimidazole in CH_2Cl_2, reflux, 1 h; 3— $H_2NCH_2CH_2N(CH_2CH_3)_2$ in CH_2Cl_2, reflux, 4 h; (e) $POCl_3$/PPA, 120 °C, 30 min.

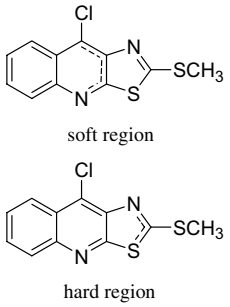


Figure 2. Hard and soft regions in the thiazolo[5,4-*b*]quinoline ring.

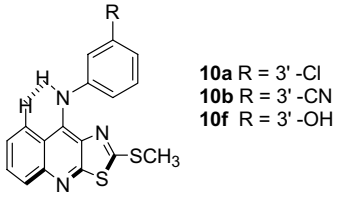


Figure 3. Spatial interaction expected between H-8 and NH.

in the same experiment. This fact suggests that the anilino ring is not orthogonal to the tricyclic skeleton, but oblique. A similar spatial arrangement has been observed for several 9-anilinoacridines and has been ascribed to a high degree of conjugation between C9 and *N*-anilino atoms.[11,12] Another interesting interaction was observed for protons H-2′ and H-6′ and the anilino proton Ar–N*H*–Ar. In the case of compounds **10a**, **10f**, and **10g** a strong interaction between H-2′ and Ar–N*H*–Ar was observed but not with H-6′, while protons H-2′ and H-6′ in compound **10b** interacted with Ar–N*H*–Ar. Therefore, compounds **10a**, **10f**, and **10g** present one preferred conformation, while compound **10b** exists in at least two. The arrangements are illustrated in Figure 4.

Clearly, the substituents attached to the anilino ring influence the spatial arrangement of this ring, possibly through an electronic repulsion between the anilino ring and the nitrogen thiazole atom because an electron-releasing group (compounds **10a**, **10f**, and **10g**) increases the electron density on the anilino ring while an electron-withdrawing group (compound **10b**) diminishes it. The different chemical shifts for the methylthio group found in the compounds support this proposal. This phenomenon was observed only in the 3′-substituted compounds.

The assignment of the ^{13}C NMR signals (Table 2) was carried out by HETCOR and HMBC experiments. All

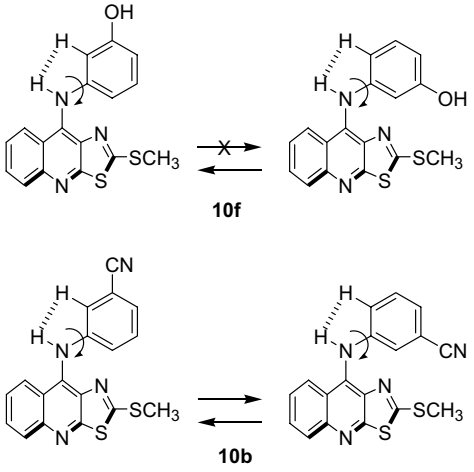


Figure 4. Possible conformers in 3′-substituted 9-anilinothiazolo[5,4-*b*]quinolines.

Table 1. ^{1}H NMR chemical shifts (ppm) of all novel thiazolo[5,4-*b*]quinoline derivatives synthesized[a]

	10a	10b	10c	10d	10e	10f	10g	11a	11b	11c	12	13	15
SMe	2.42	2.30	2.38	2.33	2.36	2.38	2.52	2.32	2.28	2.44	2.68		2.77
H-5	7.94	8.00	7.97	8.00	7.97	7.97	7.89	7.94	7.95	8.01	7.81	8.00	7.73
H-6	7.79	7.89	7.84	7.87	7.84	7.90	7.70	7.91	7.90	7.84	7.62	7.63	7.61
H-7	7.58	7.68	7.60	7.65	7.65	7.63	7.47	7.67	7.64	7.64	7.29	7.58	7.41
H-8	8.45	8.64	8.53	8.61	8.47	8.59	8.28	8.68	8.66	8.50	7.99	8.25	8.26
H-2′	7.18	7.68	6.74	7.55	7.96	6.71	6.37	7.31	7.28	7.21	7.70		
H-3′								7.43	6.98	7.70			
H-4′	7.09	7.56	6.74	7.51	7.88	6.72	6.32				7.64		
H-5′	7.30	7.56	7.25	7.58	7.58	7.17	7.02	7.43	6.98	7.70	7.31		
H-6′	7.06	7.56	6.74	7.43	7.53	6.67	6.37	7.31	7.28	7.21	7.07		
NHAr	9.87	10.5	10.2	10.2	10.23	10.5	9.22	10.66	10.6	10.4	5.20		
–CONH–											8.06		
NH												6.78	7.51
–NHCH$_2$–											3.69	3.58	4.52
–CH$_2$N–											2.97	2.78	2.52
–NCH$_2$–											2.88	2.61	2.50
–CH$_3$											1.21	1.07	0.92
Others			OC*H*$_3$ 3.71			OH 9.59	NHC*H*$_3$ 2.62 N*H*CH$_3$ 3.39		OC*H*$_3$ 3.77				

[a] All spectra were carried out in DMSO-d_6, except for **12**, **13**, and **15**, which were carried out in $CDCl_3$.

methine carbon signals were assigned by HETCOR experiments, and quaternary carbon atom signals were assigned by HMBC experiments. These experimental data are in agreement with the theoretical assignment carried out by Álvarez-Ibarra et al.[6]

3. Biological

3.1. DNA binding (ethidium bromide displacement)

Apparent DNA intercalation constants were determined by a conventional method based on quenching of the fluorescence of the ethidium bromide–DNA complex, as previously described.[9] According to the data presented in Table 3, the compounds carrying an ethylenediamino group (**12**, **13**, and **15**) displace ethidium bromide from more sites (higher Q_{max}). Comparison of the binding affinity of **13** and **15** with **2** indicates that the presence of the anilino group is unfavorable. In addition, the position of the ethylenediamino group alters the maximum quenching and binding affinity, which was higher for **15** than for **12** and **13**. On the other hand, the compounds with a 3′-anilino substituted group (**10b**, **10c**) have higher binding affinity than compounds 4′-anilino substituted (**11b**, **11c**); this roughly correlates with the cytotoxic data. The ratio Q_{max}/Q_{50} can be considered as an apparent efficiency index because compounds with higher ratios present a better combination of binding strength and displacement ability. Accordingly, the well-known DNA intercalating compound AHMA, used here as a reference, performed better than any other of the compounds studied, and only compound **15** was nearly as good. On the opposite site, compounds **11b** and **10b** showed efficiencies almost as low as the compounds reported in a previous study;[9] nevertheless, most of the substituent patterns tested here favoured DNA intercalation.

Table 3. Apparent constants for ethidium bromide displacement from DNA by thiazolo[5,4-*b*]quinoline derivatives and AHMA, as reference

Compound	Q_{max}[a]	Q_{50}[b]	Q_{max}/Q_{50}
10a	2.60 (0.32)	1.29 (0.36)	2.0
10b	7.84 (0.45)	11.54 (3.6)	0.68
10c	6.65 (0.58)	3.71 (1.8)	1.79
10d	1.62 (0.2)	0.53 (0.22)	3.08
10f	16.45 (1.5)	7.64 (1.7)	2.15
10g	9.89 (1.8)	4.18 (1.9)	2.37
11a	1.84 (0.5)	2.19 (2.3)	0.84
11b	3.73 (0.69)	9.48 (5.7)	0.39
11c	12.81 (2.4)	14.26 (8.2)	0.90
12	23.08 (1.4)	16.57 (5.4)	1.39
13	26.24 (1.03)	11.1 (3.3)	2.36
15	46.00 (1.12)	9.44 (2.5)	4.87
AHMA	47.79 (1.36)	7.36 (2.01)	6.49
2[c]	24.31 (0.18)	52.46 (14.1)	0.46
3[c]	17.86 (0.14)	22.64 (6.2)	0.79
4[c]	4.17 (0.03)	13.62 (4.6)	0.31
5[c]	8.18 (0.05)	6.96 (2.1)	1.17
6[c]	15.04 (0.19)	18.45 (8.9)	0.82

[a] Maximum quenching.
[b] Concentration to give 50 % quenching of fluorescence of bound ethidium bromide (mM). Values are means of three experiments; standard deviation given in parentheses.
[c] Data taken from Ref. 9.

3.2. In vitro cytotoxicity

The results of evaluation of cytotoxic activity of thiazolo[5,4-*b*]quinolines **10a**–**f**, **11a**–**c**, **12**, **13**, and **15** as well as **2**–**6**[9] against the proliferation of two cervical cell lines (HeLa, C-33), two colorectal cancer cell lines (SW480, SW620), and one leukemic cell line (K-562) in vitro are shown in Table 4.

In general, the most active compounds were **2**, **10b**, **12**, **13**, and **15,** four of which (**2**, **12**, **13**, and **15)** carry the

Table 2. ^{13}C NMR chemical shifts (ppm) of novel thiazolo[5,4-*b*]quinoline derivatives synthesized

No. C	**10a**	**10b**	**10c**	**10d**	**10e**	**10f**	**10g**	**11a**	**11b**	**11c**	**12**	**13**	**15**
SMe	14.5	14.6	14.5	14.3	14.6	14.8	14.6	14.6	15.3	14.8	15.2		15.4
C-2	162.9	163.8	162.5	163.2	163.8	161.6	162.1	162	161.3	165	167.2	166	162.8
C-3[a]	138.4	138.6	137.5	140.7	143	141.3	143.3	134.9	133.7	137.6	137	142.8	140.8
C-4[a]	143.5	142.7	144.0	141.0	143.2	142.0	146	142.5	139.7	143.5	143.3	144.8	146.5
C-5	125.9	125.4	128.1	129.2	127.6	123.1	127.5	126.1	127.8	130.7	124.5	127.7	128.1
C-6	130.2	130.6	129.1	131.4	130.7	131.4	129.4	132.1	133.1	132	129.1	128.1	129.2
C-7	124.8	125.1	124.1	125.3	125.3	124.9	124.2	125.4	125.9	125.5	124.1	126.4	124.4
C-8	123.9	124.0	123.9	124.5	124.1	124.5	124	124.5	125.1	124.4	128.8	123.7	122.3
C-8[a]	119	119.0	119.6	118.5	119.1	117.6	119.2	122	117.1	120	118.9	125.4	117.2
C-9	159.5	159.0	161.6	142.2	158.8	157.5	161	155.4	158.3	159	166.4	158.7	160.9
C-9[a]	131.7	131.7	132.1	131.5	131.9	130.9	132	130.6	130.2	133.5	134.8	125.4	146.5
C-1′	144	143.4	146.4	141.7	147.7	150.1	149	139.6	145	146.7	147.2		
C-2′	119.9	124.7	106.5	119.4	115.5	111.9	106	117.4	121.8	120.3	119.4		
C-3′	132.4	110.9	159.3	129.4	138.5	156.4	138.6	128.1	114.4	132.4	135		
C-4′	121.1	126.2	107.6	120.1	117.1	114.6	110.6	129.1	155	103	121.5		
C-5′	129.5	129.4	128.8	126.7	129.2	128.9	128.7	128.1	114.4	132.4	129		
C-6′	122.3	126.3	113.2	123.6	115.5	110.8	107.6	117.4	121.8	120.3	123		
–$NHCH_2$											36.2	41.3	41.2
–CH_2N											52.3	51.1	55.0
–NCH_2											47.7	46.8	49.3
–CH_3											10.0	10.7	9.24
Others		CN	OMe	CF_3			$NHCH_3$		OCH_3	CN	C=O		
		118.8	54.9	124.3			30.4		56.1	119.6	161.1		

Table 4. In vitro cytotoxic activity of compounds **2**–**6** and **10a**–**15**[a,b]

Compound	HeLa	C-33	SW480	SW620	K-562
10a	69.37 (20.92)	129.33 (16.59)	110.69 (14.64)	129.73 (24.04)	80.26 (8.36)
10b	7.75 (1.13)	15.08 (10.36)	28.68 (16.88)	43.75 (15.47)	8.01 (0.65)
10c	25.34 (10.86)	75.41 (2.30)	66.65 (19.93)	26.58 (1.60)	22.17 (11.33)
10d	43.34 (11.80)	59.73 (3.46)	65.13 (5.29)	62.28 (5.43)	67.06 (22.08)
10e	152.21 (29.77)	>200	>200	160.82 (1.02)	>200
10f	>200	146.12 (10.35)	146.95 (59.51)	146.34 (5.17)	173.34 (3.98)
10g	46.22 (11.55)	133.98 (56.67)	101.38 (0.04)	118.69 (25.70)	46.85 (16.93)
11a	123.86 (78.19)	>200	>200	145.95 (14.07)	79.45 (4.10)
11b	>200	>200	>200	110.8 (15.51)	77.2 (25.16)
11c	140.02 (51.82)	>200	>200	>200	120.01 (28.70)
12	21.69 (3.35)	19.95 (5.59)	13.6 (3.44)	19.72 (0.48)	12.54 (3.03)
13	12.86 (2.34)	9.35 (1.96)	19.48 (9.81)	15.22 (2.34)	9.28 (0.65)
15	12.97 (3.83)	9.06 (1.86)	27.97 (9.22)	16.87 (2.19)	7.85 (1.85)
2[c]	15.9 (0.30)	22.4 (4.20)	37.7 (0.80)	21.6 (1.80)	16.8 (0.50)
3[c]	>200	>200	>200	183.9 (69.90)	143.4 (6.90)
4[c]	>200	153.7 (7.70)	>200	>200	>200
5[c]	176.5 (30.30)	138.8 (23.90)	>200	>200	143.4 (11.50)
6[c]	>200	>200	>200	153.9 (28.60)	85.3 (10.20)
Amsacrine[c]	9.50 (0.60)	8.80 (0.50)	27.70 (2.00)	16.70 (2.80)	19.90 (0.80)

[a] IC_{50}, μM, compound concentration inhibiting 50% of cellular growth assessed by the MTT assay.
[b] Values are means of three experiments, standard deviation given in parentheses.
[c] Data taken from Ref. 9.

2-(*N*,*N*-diethyl)ethylenediamino moiety, although the group is attached to different positions and in **2** position is present twice. To detect if part of the cytotoxicity can be explained by the intercalating properties, a Log Log multiple linear correlation analysis of the $-\mathrm{Log}\,IC_{50}$ in Table 4 was carried out for every cell line, against the $\mathrm{Log}\,Q_{max}$ and the $-\mathrm{Log}\,Q_{50}$ in Table 4. A significant correlation ($p < 0.05$) was found for only Q_{max} and the C33 cells, but even in this case, less than 25% of the cytotoxicity can be explained by the maximal intercalation ability (adjusted R squared was 0.21) with a negative correlation. Therefore, if the intercalation of these compounds into DNA is related to their cytotoxicity, only a subset of the DNA binding sites seems to be relevant.

As already mentioned the presence of an alkylamine residue seems to improve cytotoxic activity, since compounds **12**, **13**, and **15** were as potent as the reference compound, amsacrine. It is interesting to note that the change of position of the alkylamino residue has no significant effect on cytotoxic activity.

The 9-anilinothiazolo[5,4-*b*]quinoline derivatives previously reported had only electron-releasing groups and showed poor cytotoxic activity.[9] Then in this study the effect of electron-withdrawn groups was analyzed. It was observed, in a general way, that the incorporation of electron-withdrawn groups improves cytotoxic activity. This is in opposition to the 9-anilinoacridine derivatives, where the incorporation of electron-releasing groups is critical for good cytotoxic activity. The only exception was compound **10e** (3′-nitro derivative), but this compound had solubility problems, this possibly being the cause of its poor activity. Therefore, if the 9-anilinothiazolo[5,4-*b*]quinoline derivatives share a common mechanism of action with the 9-anilinoacridines, at least their binding modes should differ significantly because the structural requirements for cytotoxic activity are not the same.

It is also important to notice that compounds **10a** (3′-Cl), **10b** (3′-CN), and **10c** (3′-OMe) were more potent than compounds **11a** (4′-Cl), **11b** (4′-OMe), and **11c** (4′-CN); thus, the substitution pattern in the anilino ring is critical for biological activity.

Compound **10b** is as potent as the reference compound amsacrine despite the lack of an alkylamino residue. Perhaps, in addition to its electron-withdrawn properties, the nitrile group acts as an acceptor in the formation of a hydrogen bond. The great difference in activity between compounds **10c** (3′-OMe) and **10f** (3′-OH) is in agreement with this last proposal since, in the first case, the oxygen atom can act only as a hydrogen-bond acceptor, while in the second case it acts as a donor.

In light of these results, we propose the following requirements for good cytotoxic activity of 9-anilinothiazolo[5,4-*b*]quinolines: cytotoxic activity is improved by the incorporation of an alkylamino residue, but the location of the alkylamino residue is not critical. Incorporation of electron-withdrawing groups into the anilino ring enhances cytotoxic activity in comparison with that of electron-releasing groups. The substitution pattern of the anilino ring is critical for good cytotoxic activity, position 3′ being the most favorable. The presence of hydrogen-bond acceptors may also be relevant to this activity.

4. Conclusions

A new series of 9-anilinothiazolo[5,4-*b*]quinoline derivatives was synthesized and characterized. The preliminary antitumor studies showed that some derivatives exhibited significant cytotoxic activity in vitro. In line with the data reported here, the presence of electron-withdrawing or a hydrogen-bond acceptor in the anilino ring

and the incorporation of an alkylamino substituents improve the cytotoxic activity of thiazolo[5,4-*b*]quinolines.

5. Experimental

All starting materials were commercially available research-grade chemicals and used without further purification. Reactions were monitored by analytical TLC on precoated silica gel 60 F_{254} plates (Aldrich). Column chromatography was carried out on silica gel 60 (Merck). Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet FT-5SX spectrophotometer model. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VxR-300S spectrometer (300 and 75.5 MHz). Chemical shifts are reported in ppm (δ) and the signals are described as singlet (s), doublet (d), triplet (t), quartet (q), broad (br), and multiplet (m), coupling constants are reported in Hz. EI-MS were carried out on a JEOL JMS-AX505-HA apparatus. FAB-MS were carried out on a JEOL Sx102 apparatus. Compounds **9** and **10** were prepared according to procedures already described.[10,11]

5.1. General preparation of 2-(methylthio)-9-anilinothiazolo[5,4-*b*]quinolines (10a–f, 11a–c, 12)

To compound **9** (133 mg, 0.5 mmol) were added successively methanol (5 ml) and two drops of HCl (36%). The mixture was stirred for 10 min. Meanwhile, a mixture of the aniline with the desired substitution pattern (0.7 mmol) in 5 ml of methanol was prepared. This mixture was added to the mixture of **9** in methanol/HCl and heated to reflux for 6 h. After cooling, methanol was evaporated under reduced pressure, the residue was suspended in 10 ml of water and a 10% $NaHCO_3$ solution was added to render pH 8. The solid was collected by vacuum filtration and washed with cold acetone. In the case of compound **12** crystallization from ethanol–water was used for purification.

5.1.1. 9-[[(3-Chloro)phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (10a). Yellow solid; 133 mg (74.5%); mp 185–187 °C. IR (KBr, cm^{-1}): 3108 (NH), 3050 (C–H), 1572, 1548, 1516, 1477, 1430, (aromatic), 1266 (C–S); ^{1}H NMR (DMSO-d_6, δ): 2.42 (s, 3H) SCH_3; 7.06 (m, 1H), H-6′; 7.09 (m, 1H), H-4′; 7.18 (t, J = 2.1 Hz, 1H) H-2′; 7.30 (t, J = 8.1 Hz, 1H) H-5′; 7.58 (ddd, J = 8.1, 6.6, 2.1 Hz, 1H) H-7, 7.79 (ddd, J = 8.1, 6.6, 1.2 Hz, 1H) H-6; 7.94 (dd, J = 8.4, 0.9 Hz 1H) H-5; 8.45 (d, J = 8.4 Hz, 1H) H-8; 9.87 (br), NHAr; MS (FAB, *m*/*z*): 360 (M^++3, 43.5%), 358 (M^++1, 100%), 357(M^+, 25%), 324 (M^+−33, 2.8%). Anal. Calcd for $C_{17}H_{12}ClN_3S_2$: C, 57.05; H, 3.38; N, 11.74; S, 17.92. Found: C, 56.97; H, 3.39; N 11.59; S, 17.55.

5.1.2. 9-[[(3-Cyano)phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (10b). Yellow solid; 136 mg (72%); mp 210–212 °C. IR (KBr, cm^{-1}): 3245 (NH), 2231 (CN), 1624, 1573, 1550, 1498, 1473, 1433 (aromatic), 1286 (C–S); ^{1}H NMR (DMSO-d_6, δ): 2.30 (s, 3H) SCH_3; 7.56 (m, 3H) H-4′, H-5′, H-6′; 7.68 (ddd, J = 8.4, 6.9, 1.2 Hz) H-7; 7.86 (t, J = 1.5 Hz) H-2′; 7.89 (ddd, J = 8.4, 6.9, 1.2 Hz) H-6; 8.00 (dd, J = 8.7, 0.9 Hz) H-5; 8.64 (d, J = 8.4 Hz) H-8; 10.5 (br) NHAr; ^{13}C NMR (DMSO-d_6): 14.6, 110.9, 118.8, 119.0, 124.0, 124.6, 125.1, 125.4, 126.2, 126.3, 129.4, 130.6, 131.7, 138.5, 142.7, 143.3, 159.0, 163.3; MS (FAB, *m*/*z*): 349 (M^++1, 100%), 348 (M^+, 55%), 315 (M-33, 5.8%). Anal. Calcd for $C_{18}H_{12}N_4S_2$: C, 62.05; H, 3.47; N, 16.08; S, 18.40. Found: C, 62.40; H, 3.45; N, 16.01; S, 18.31.

5.1.3. 9-[[(3-Methoxy)phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (10c). Yellow solid; 132 mg (75%); mp 175–179 °C. IR (KBr, cm^{-1}): 3120 (NH), 3053, 2966 (CH), 2850 (O–CH), 1595, 1571, 1543, 1503, 1476, (aromatic), 1300 (C–S), 1266 (ArO); ^{1}H NMR (DMSO-d_6, δ): 2.38 (s, 3H) SCH_3; 3.71 (s, 3H) OCH_3; 6.74 (m, 3H) H-2′, H-4′, H-6′; 7.25 (t, J = 8.7 Hz) H-5′; 7.60 (ddd, J = 8.4, 6.6, 1.2 Hz) H-7; 7.84 (ddd, J = 8.7, 6.6, 1.2 Hz) H-6; 7.97 (dd, J = 8.7, 0.9 Hz) H-5; 8.53 (d, J = 8.4 Hz) H-8; 10.2 (br) NHAr. MS (EI, *m*/*z*): 354 (M^++1, 23%), 353 (M^+, 100%), 352 (M^+-1, 11%), 338 (M^+-15, 6%), 320 (M^+-33, 21%); Anal. Calcd for $C_{18}H_{15}N_3OS_2$: C, 61.16; H, 4.28; N, 11.89; S, 18.14. Found: C, 61.23; H.4.22; N, 11.69; S, 18.03.

5.1.4. 9-[[(3-Trifluoromethyl)phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (10d). Yellow solid; 130 mg (67%); mp 168–170 °C. IR (KBr, cm^{-1}): 3124 (NH), 3054 (C–H), 1598, 1522, 1503, 1472, 1451 (aromatic), 1290 (C–S); ^{1}H NMR (DMSO-d_6, δ): 2.33 (s, 3H) SCH_3; 7.43 (d J = 7.8 Hz, 1H), H-6′; 7.51 (dd J = 8.4, 2.1 Hz, 1H), H-4′; 7.55 (dd, J = 2.1, 2.1 Hz, 1H) H-2′; 7.58 (t, J = 7.8 Hz, 1H) H-5′; 7.65 (ddd J = 8.4, 6.9, 0.9 Hz, 1H) H-7, 7.87 (ddd, J = 8.1, 6.9, 0.9 Hz, 1H) H-6; 8.00 (dd, J = 8.1, 0.9 Hz, 1H) H-5; 8.61 (dd, J = 8.1, 0.9 Hz, 1H) H-8; 10.2 (br), NHAr. MS (FAB, *m*/*z*): 392 (M^++1, 100%), 391 (M^+, 15%), 376 (M^+-15, 2%), 358 (M^+-33, 2%). Anal. Calcd for $C_{18}H_{12}F_3N_3S_2$: C, 55.23; H, 3.09; F, 14.56; N, 10.73; S, 16.38. Found: C, 54.74; H, 2.95; N, 10.84; S, 16.62.

5.1.5. 9-[[(3-Nitro)phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (10e). Yellow solid; 140 mg (76%); mp 201–204 °C. IR (KBr, cm^{-1}): 3000 (NH), 2990 (C–H), 1595, 1571, 1543, 1503, 1476, (aromatic), 1518 (NO_2), 1348 (N–O), 1300 (C–S); ^{1}H NMR (DMSO-d_6, δ): 2.36 (s, 3H) SCH_3; 7.53 (ddd, J = 8.1, 2.1, 2.1 Hz, 1H), H-6′; 7.58 (t, J = 8.1 Hz, 1H), H-5′; 7.65 (ddd, J = 8.4, 6.9, 1.2 Hz, 1H) H-7; 7.84 (ddd, J = 8.4, 6.9, 1.2 Hz, 1H) H-6; 7.88 (ddd J = 8.1, 2.1, 2.1 Hz, 1H) H-4′, 7.96 (t J = 2.1 Hz, 1H) H-4′; 7.97 (d, J = 8.1 Hz, 1H) H-5; 8.47 (d, J = 8.1 Hz, 1H) H-8; 10.23 (br), NHAr. MS (EI, *m*/*z*): 369 (M^++1, 23%), 368 (M^+, 100%), 367(M^+-1, 2.5%), 353 (M^+-15, 2.8%), 338 (M^+-30, 32%), 335 (M^+-33, 7.5%); Anal. Calcd for $C_{17}H_{12}N_4O_2S_2$: C, 55.42; H, 3.28; N, 15.21; O, 8.68; S, 17.41.Found: C, 55.70; H, 3.38; N, 15.10; O, 8.73; S, 17.09.

5.1.6. 9-[[(3-Hydroxy)phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (10f). Yellow solid; 124 mg (73%); mp 227–230 °C. IR (KBr, cm^{-1}): 3147 (NH), 2775 (O–CH), 1607, 1573, 1549, 1497, 1453, (aromatic), 1268 (C–S), 1222 (ArO); ^{1}H NMR (DMSO-d_6, δ): 2.38 (s, 3H) SCH_3; 6.67 (ddd, J = 8.1, 1.5, 1.5 Hz, 1H), H-4′; 6.71

(t, $J = 1.2$ Hz, 1H), H-2′; 6.72 (m, 1H) H-6′; 7.17 (t, $J = 8.7$ Hz) H-5′; 7.63 (ddd, $J = 8.4$, 6.6, 1.2 Hz) H-7; 7.90 (ddd, $J = 8.7$, 6.9, 0.9 Hz) H-6; 7.97 (dd, $J = 8.4$, 1.2 Hz) H-5; 8.59 (d, $J = 8.4$ Hz) H-8; 9.59 (s) OH; 10.2 (br) NHAr. MS (EI, *m*/*z*): 340 (M^++1, 23%), 339 (M^+, 100%), 338 (M^+-1, 11%), 338 (M^+-15, 5%), 320 (M^+-33, 32%); Anal. Calcd for $C_{17}H_{13}N_3OS_2$: C, 60.15; H, 3.86; N, 12.38; 0, 4.71; S, 18.89. Found: C, 60.31; H, 3.56; N, 12.18; S, 18.49.

5.1.7. 9-[[(3-*N*-Methylamino)phenyl]amino]-2-(methylthio)-thiazolo[5,4-*b*]quinoline (10g). Yellow solid; 131 mg (74.5%); mp 173–175 °C. IR (KBr, cm^{-1}): 3414 (NH), 2921 (C–H), 1588, 1549, 1494, 1464 (aromatic), 1298 (C–S); ^{1}H NMR (DMSO-d_6, δ): 2.52 (s, 3H) SCH_3; 2.62 (s, 3H) NHC<u>H</u>$_3$; 6.32 (d, $J = 8.4$ Hz) H-4′; 6.37 (m, 2H) H-2′, H-6′; 7.02 (t, $J = 8.4$ Hz, 1H) H-5′; 7.47 (ddd, $J = 8.4$, 6.9, 1.2 Hz) H-7; 7.70 (ddd, $J = 8.1$, 6.9, 1.2 Hz) H-6; 7.89 (dd, $J = 8.4$, 1.2 Hz) H-5; 8.28 (dd, $J = 8.7$, 0.9 Hz) H-8; 9.22 (s) NH. MS (FAB, *m*/*z*): 353 (M^++1, 100%), 352 (M^+, 61%), 351 (M^+-1, 12.8%), 337 (M^+-33, 2%), 319 (M^+-33, 2%); Anal. Calcd for $C_{18}H_{16}N_4S_2$ C,61.34; H, 4.58: N, 15.90; S, 18.19. Found: C, 61.54; H, 4.62; N, 15.79; S, 18.05.

5.1.8. 9-[[(4-Chloro)phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (11a). Yellow solid; 125 mg (70%); mp 186–187 °C. IR (KBr, cm^{-1}): 3196 (NH), 3104, 2989 (C–H), 1627, 1573, 1540, 1486, 1467 (aromatic) 1295 (C–S); ^{1}H NMR (DMSO-d_6, δ): 2.32 (s, 3H) SCH_3; 7.31 (d, $J = 8.4$ Hz, 2H) H-2′, H-6′; 7.43 (d, $J = 8.8$ Hz, 2H) H-3′, H-5′; 7.67 (t, $J = 7.6$ Hz, 1H) H-7; 7.91 (t, $J = 7.6$ Hz, 1H) H-6; 7.94 (d, $J = 8.4$, 1H) H-5; 8.68 (d, $J = 8.4$ Hz, 1H) H-8; 10.66 (s,1H) NH. MS (FAB, *m*/*z*): 360 (M^++3, 43.5 %), 358 ((M^++1), 100%), 357 (M^+, 25%), 342 (M^+-15, 5%), 324 (M^+-33, 2%); Anal. Calcd for $C_{17}H_{12}ClN_3S$: C, 57.05; H, 3.38; N, 11.74; S, 17.92. Found: C,57.01; H, 3.37; N, 11.62; S, 17.57.

5.1.9. 9-[[(4-Methoxy)phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (11b). Yellow solid; 120 mg (68%); mp 205–207 °C. IR (KBr, cm^{-1}): 3196 (NH), 3200 (NH), 3062, 3028 (C–H), 2835 (O–CH), 1608, 1573, 1546, 1510, 1475 (aromatic), 1295 (C–S); ^{1}H NMR (DMSO-d_6, δ): 2.28 (s, 3H) SCH_3; 3.77 (s, 3H) OCH_3; 6.98 (d, $J = 8.4$ Hz, 2H) H-3′, H-5′; 7.28 (d, $J = 8.4$ Hz, 2H) H-2′, H-6′; 7.64 (t, $J = 7.6$ Hz, 1H) H-7; 7.90 (t, $J = 7.6$ Hz, 1H) H-6; 7.95 (d, $J = 8.4$, 1H) H-5; 8.66 (d, $J = 8.4$ Hz, 1H) H-8; 10.68 (s,1H) NH; MS (FAB, *m*/*z*): 355 (M^++2, 32%), 354 (M^++1, 100%), 353 (M^+, 23%), 338 (M^+-15, 7%); Anal. Calcd for $C_{18}H_{15}N_3OS_2$: C, 61.16; H, 4.28; N, 11.89; S, 18.14. Found: C, 61.25; H, 4.24; N, 11.66; S, 18.02.

5.1.10. 9-[[(4-Cyano)phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (11c). Yellow solid; 118 mg (68%); mp 178–180 °C. IR (KBr, cm^{-1}): 3423 (NH), 2222 (CN), 1622, 1573, 1532, 1497, 1424 (aromatic), 1274 (C–S); ^{1}H NMR (DMSO-d_6, δ): 2.44 (s, 3H), SCH_3; 7.21 (d, $J = 8.8$ Hz, 2H) H-2′, H-6′; 7.64 (t, $J = 7.2$ Hz, 1H) H-7; 7.70 (d, $J = 8.8$ Hz, 2H) H-3′, H-5′; 7.84 (t, $J = 7.2$ Hz, 1H) H-6; 8.01 (d, $J = 8.4$ Hz, 1H) H-5; 8.50 (d, $J = 8.4$ Hz, 1H) H-8; 10.41 (br, 1H) NH. MS (FAB, *m*/*z*): 349 (M^++1, 100%), 348 (M^+, 72%); Anal. Calcd for $C_{18}H_{12}N_4S_2$: C, 62.05; H, 3.47; N, 16.08; S, 18.40. Found: C, 61.97; H, 3.49; N, 15.98; S, 18.29.

5.1.11. 9-[[[3-[2-(*N*,*N*-Diethylamino)ethyl]carbamoyl]phenyl]amino]-2-(methylthio)thiazolo[5,4-*b*]quinoline (12). White solid; 118 mg (68%); mp 178–180 °C. IR (KBr, cm^{-1}): 3250 (NH), 3057, 2964, 2926, 2812 (C–H), 1581 (C═N), 1550, 1521, 1467 (aromatic), 1288 (C–S); ^{1}H NMR ($CDCl_3$,, δ): 1.21 (t, $J = 7.2$ Hz, 6H) CH_3; 2.68 (s, 3H), SCH_3, 2.88 (c, $J = 7.2$ Hz, 4H) –NCH_2–; 2.97 (t, $J = 5.1$ Hz, 2H) –CH_2N–; 3.69 (td, $J = 5.7$, 5.1 Hz, 2H) NHCH_2–; 5.20 (br, 1H), NH; 7.07 (dd, $J = 7.8$, 2.1 Hz, 1H) H-6′; 7.29 (ddd, 8.7, 6.9, 1.2 Hz,1H) H-7; 7.31 (t, $J = 7.8$ Hz, 1H) H-5′; 7.62 (ddd, $J = 8.7$, 6.6, 1.2 Hz, 1H) H-6; 7.64 (dd, $J = 7.2$, 1.8 Hz), H-4′; 7.70 (t, $J = 1.8$ Hz) H-2′; 7.81 (dd, $J = 8.4$, 0.9 Hz) H-5; 7.99 (dd, $J = 8.4$, 0.9 Hz, 1H) H-8; 8.07 (br, 1H) CON<u>H</u>. MS (EI, *m*/*z*): 466 (M^++1, 3%), 465 (M^+, 8%), 450 (M^+-15, 4%), 393 (M^+-72, 9%), 366 (M^+-99, 23%), 350 (M^+-115, 10%), 100 (M^+-365, 10%), 86 (M^+-379, 100%); Anal. Calcd for $C_{24}H_{27}N_5OS_2$: C, 61.91; H, 5.84; N, 15.04; S, 13.77. Found. C, 61.74; H, 5.97; N, 14.97; S, 13.91.

5.1.12. 9-Chloro-2-[[2-(*N*,*N*-diethylamino)ethyl]amino]-thiazolo[5,4-*b*]quinoline (13). A solution of 9 (500 mg, 1.88 mol) in 0.3 ml of *N*,*N*-diethylethylenediamine was stirred for 48 h at room temperature. To the brownish solution, dichloromethane (30 ml) was added and the resulting solution was successively extracted with a saturated aqueous solution of NH_4Cl (3×10 ml), water (3×10 ml) and dried over Na_2SO_4. After concentration under reduced pressure, the pale yellow crude product was purified by column chromatography (dichloromethane/methanol/NH_4OH 99:1:0.1) to give **13** as white crystals. **13**: 475 mg (75%), IR (KBr, cm^{-1}): 3202 (N–H), 2968, 2968, 2815(CH_2, CH_3); ^{1}H NMR ($CDCl_3$, δ): 1.07 (t, $J = 7.2$, 6H) $2CH_3$; 2.61 (c, $J = 7.2$, 4H) $2CH_2$; 2.78 (t, $J = 6.0$, 2H) CH_2; 3.58 (ta, 2H) CH_2; 6.78 (br, 1H) NH; 7.63 (ddd, $J = 8.1$, 6.9, 1.8, 1H) H-6; 7.58 (ddd $J = 7.8$, 6.6, 1.5, 1H, H-7); 8.00 (dd, $J = 8.1$, 1.5, 1H, H-5); 8.25 (dd, $J = 8.1$, 1.2, 1H) H-8. MS (FAB, *m*/*z*): 337(M^++3, 35%), 335 (M^++1, 100%), 333 (M^+-1, 24%), 262 (M^+-72, 44%), 100 (M^+-234, 33.5%), 86 (M^+-248, 67%); Anal. Calcd for $C_{16}H_{19}ClN_4S$: C, 57.39; H 5.72; N, 16.73; S, 9.58. Found: C, 57.73; H, 5.67; N, 16.63; S, 9.68.

5.1.13. 5-(Phenylamino)-4-[[2-(*N*,*N*-diethylamino)ethyl]carbamoyl]-2-(methylthio)thiazole (14). The carboxylic acid used as precursor of amide **14** was obtained with high yields (90%) by saponification of ester **8**, by a procedure already described.[10,11] To a suspension of the 1,1′-carbonyldimidazole (2.5 mmol) in 5 ml of dichloromethane, 500 mg (1.87 mmol) of the carboxylic acid was added. The mixture was stirred and heated to reflux for 1 h. After reflux, a solution of 0.2 ml of *N*,*N*-diethylethylenediamine in 5 ml of dichloromethane was added to the reaction mixture and heated to reflux for 4 h. After cooling, 20 ml of dichloromethane was added. The solution was washed with a 10% $NaHCO_3$ solution (3×10 ml), a saturated aqueous solution of NH_4Cl (3×10 ml), water

(3×10 ml), and dried over Na_2SO_4. After concentration of the solution under reduced pressure, the pale yellow crude oil product was purified by column chromatography (dichloromethane/methanol/NH_4OH 99:1:0.1) to give **14** as a colorless oil. **14**: 515 mg (75%); IR (KBr, cm^{-1}): 3401(N–H), 2966, 2926, 2811 (CH_2, CH_3), 1633 (C=O), 1589, 1533, 1526, 1496 (aromatic); ^1H NMR ($CDCl_3$, δ): 1.07 (t, $J = 7.1$, 6H) $2CH_3$; 2.60 (t, $J = 7.2$, 4H), $2CH_2$; 2.63 (s, 3H) SCH_3; 2.70 (c, $J = 7.2$, 2H) CH_2; 3.5 (dt, 2H) CH_2; 7.1 (dd, $J = 7.2$, 1.2, 1H) H-4′; 7.32 (ddd, $J = 8.7$, 7.5, 1.8, 2H) H-3′, H-5′; 7.15 (dd, $J = 8.7$, 1.2, 2H) H-2′, H-6′; 7.45 (t, 1H) CONH; 10.29 (s, 1H) NH; MS (FAB, *m/z*): 365 (M^++1, 100%), 364 (M^+, 47.5%), 292 (M^+-72, 35%), 249 (M-115, 22.5%).

5.1.14. 9-[[2-(*N*,*N*-diethylamino)]ethyl]amino]-2-(methylthio)-thiazolo[5,4-*b*]quinoline (15). Compound **15** was prepared from **14** with a procedure already described.[6,9] The crude product was purified by column chromatography (CH_2Cl_2/CH_3OH/NH_4OH 99:1:0.1) to render a yellowish oil, which was treated with hexane to give **15** as white crystals (30%). **15**: mp 94–98 °C, IR (KBr, cm^{-1}): 3308 (N–H), 2970 (C–H), 1554, 1531, 1497, 1470(aromatic), 1267; ^1H NMR ($CDCl_3$, δ): 0.92 (t, $J = 7.2$, 6H) $2CH_3$; 2.50 (c, 4H) $2CH_2$; 2.52 (t, $J = 7.2$, 2H) CH_2; 2.77 (s, 3H) SCH_3; 2.70 (t, $J = 7.2$, 2H) CH_2; 4.58 (dt, 2H) CH_2; 7.51 (t, $J = 6.3$, 1H) NH; 7.41 (ddd, $J = 8.4$, 6.9, 1.5, 1H) H-7; 7.61 (ddd $J = 6.9$, 6.0, 1.5, 1H); H-6 7.73 (dd, $J = 8.4$, 0.9 Hz, 1H) H-5; 8.26 (dd, $J = 8.7$, 0.6, 1H) H-8; MS (CI, *m/z*): 347 (M^++1, 39%), 346 (M^+, 24.5%) 274 (M^+-72, 4.5%), 260 (M^+-86, 4.5%), 100 (M^+-247, 10%), 86 (M^+-261, 100%). Anal. Calcd for $C_{17}H_{22}N_4S_2$: C, 57.39; H, 5.72; N, 16.73; S, 9.58. Found: C, 57.33; H, 5.77; N, 16.70; S, 9.49.

5.2. Cytotoxic assay

The effects of the compounds were determined in two cervical cell lines (HeLa, C-33), two human colorectal cancer cell lines (SW480 and SW620), and one myelogenous leukemia human cell line (K-562). The cytotoxic assays were performed according to the microculture MTT method. Cells were harvested at $4.5–5.0 \times 10^4$ cells/ml/well and inoculated in 24 well microtiter plates. Then the culture cells were inoculated free and with the compounds (which were dissolved in DMSO and added in a volume maximum of 2 ml/ml/well). After 72 h incubation, 100 mg/ml of MTT (in PBS, pH 7.2) was added. Adding 1 ml of DMSO to each well, followed by gentle shaking, dissolved the formazan dye. After centrifugation the extinction coefficient was measured at 540 nm using a Beckman photometer model DU^R-64. Cell growth inhibition was determined by the formula % cell growth inhibition = (1 − absorbance of treated cells/ absorbance of untreated cells) × 100. The assays were carried out in three independent experiments in quadruplicate.

5.3. DNA affinity and intercalation

DNA intercalation was determined from the displacement of ethidium bromide from DNA.[9] Sterile solutions of high molecular weight DNA from calf thymus (Gibco, BRL, New York, USA) in 0.1 M Tris–HCl buffer at pH 7.4, 0.15 M NaCl, and 5 mM ethidium bromide (ultrapure from Gibco, BRL, New York, USA) were mixed with serial additions of the compounds to be tested dissolved in 100% dimethylsulfoxide (DMSO), and the fluorescence intensity of the solution was recorded at 584 nm with an excitation light of 546 nm. The DMSO concentration never exceeded 8%. The effect of this amount of DMSO was small and had no effect on the shape of emission or excitation fluorescence spectra of a DNA–ethidium bromide complex as compared with that determined in 100% aqueous buffers. The recorded fluorescence change was corrected for the dilution caused by serial additions of this solvent.

The concentration of the compounds tested varied in the range of 1–100 μM depending on their respective solubility. The precipitation of the compound from the solution was detected from an increase in 600 nm light dispersal at 90 degrees in a Shimadzu RF5000U fluorescence spectrophotometer. The displacement curves were fitted by nonlinear regression analysis to a rectangular hyperbola.

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