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Synthesis, cytotoxic activity, DNA topoisomerase-II inhibition, molecular modeling and structure–activity relationship of 9-anilinothiazolo[5,4-*b*]quinoline derivatives

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ABSTRACT

Some novel 9-anilinothiazolo[5,4-*b*]quinoline derivatives were synthesized and their cytotoxic activities were examined. The inhibition of some of the most active compounds over human topoisomerase II (Topo II) activity was assessed with the kDNA decatenation assay. The novel compounds differ in the substituents attached to the anilino ring, a dialkylamino alkylamino group, a saturated heterocyclic moiety, a methylthio group at position 2 and a fluorine atom present or absent at 7-position. According to the data, compounds with a diethylaminopropylamino group and a chlorine atom at 4′-position of the anilino ring were the most cytotoxic. The molecular models of all compounds indicated a correlation between hydrophobicity and cytotoxic activity although the direction and magnitude of the dipole moment also had a significant influence on its cytotoxicity. The 2-dialkylaminoalkylamino substituent is flexible and is known to facilitate the crossing of cell membranes; thus, this last barrier may be a limiting step in the mechanisms mediating the cytotoxicity. On the other hand, the activity of 2-methylthio derivatives seems to rely more on the electronic effects brought about by the substitution of the aniline ring. The synthesis, cytotoxicity against cancer cell lines, in vitro inhibition of human topoisomerase II, molecular modeling and the preliminary analysis of structure–activity relationships are presented.

1. Introduction

Several compounds based on tricyclic templates have been studied in the search for better anticancer agents. Among these, 9-anilinoacridines have been extensively studied as potential antitumorals, and their capability of interacting with DNA and inhibiting the DNA topoisomerase II has been proposed as a mechanism of action. The stabilization of a enzyme-drug-DNA ternary complex covalent-intermediate has been shown to lead to DNA lesions, the interaction anilino ring-enzyme being one of the principal stabilizing factors.[1–3] One of these derivatives, amsacrine (Fig. 1, *m*-AMSA, **1a**), has been clinically used for leukemia and lymphoma treatment.[4,5] Structural modifications in the acridine nucleus have led to a novel agent, asulacrine **1b**,[6] which was capable of inhibiting the growth of some solid tumors. On the other hand, the isosteric replacement of a benzene moiety in the acridine template has

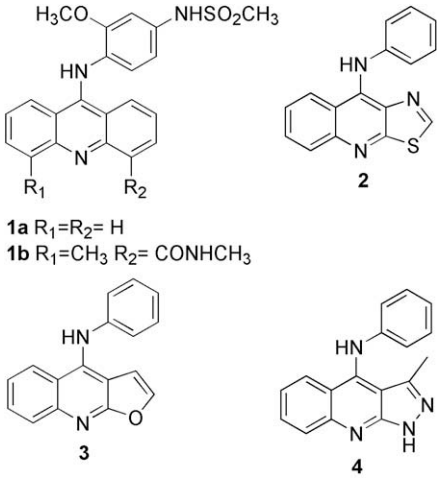

Figure 1. Chemical structures of amsacrine **1a**, asulacrine **1b** and some aniline-substituted tricyclic templates **2**–**4**.

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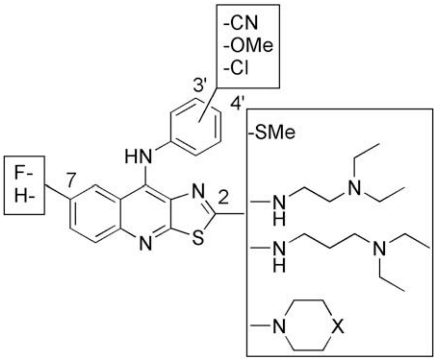


Figure 2. 9-Anilinothiazolo[5,4-*b*]quinoline template and the substituents considered in the present study.

led to compounds such as 9-anilinothiazolo[5,4-*b*]quinoline **2**,[7,8] 4-anilinofuro[2,3-*b*]quinoline **3**[9] and 4-anilinopyrazolo[3,4-*b*]quinoline **4**[10] derivatives which have shown good cytotoxic activity. The incorporation of a five- or six-membered heterocyclic ring fused to an anthracenedione or acridine nucleus usually increases the cytotoxic activity, thus enabling the compounds to overcome multidrug resistance of tumor cells.[11,12]

In our previous paper, the importance of the substitution pattern in the anilino ring of 2-methylthio derivatives of **2** was explored by introducing different substituents at positions 3′ and 4′ of the anilino ring as well as a diethylaminoethylamino chain at positions 2 and 9 of the tricyclic nucleus. Electron withdrawing groups (EWG) and hydrogen bond acceptors (HBA) attached to position 3′ of the anilino ring improved cytotoxic activity. Besides, the position of the diethylaminoethylamino chain had less influence on cytotoxicity than it did in the DNA binding affinity.[7,8]

In the present study, the effect of additional substituents on the anilino ring of the 9-anilinothiazoloquinoline system (Fig. 2, Scheme 1, series **7f–7h)** is presented. In those compounds previously identified as good to fair cytotoxics (with substituents at positions 3′ or 4′ of the anilino ring), the influence of a dialkylaminoalkylamino (diethylaminoethylamino or diethylaminopropylamino) chain at position 2 was studied (Scheme 1, series **10** and **11**). In particular, the chain length and flexibility of the dialkylaminoalkylamino chain was modified by incorporating a saturated heterocycle substituent at position 2, while keeping a nitrogen atom attached to the C-2 of the tricyclic system (Scheme 1, series **8**). Finally, fluorination of position 7 of the thiazoloquinoline system was included in some compounds (Scheme 1, series **7a**–**7e**) since it was reported to increase cytotoxic effects.[13] Cytotoxic activity as well as the inhibition of human topoisomerase II activity of the novel and some previously reported compounds were evaluated, and a preliminary study structure–activity relationship was carried out. The information obtained has rendered important clues to the understanding of the cytotoxic profile for these types of compounds.

2. Chemistry

The synthesis of the proposed compounds is depicted in Scheme 1. Preparation of intermediates for compounds **5a–5b** has already been described.[7,13] Compounds **6a** and **6b** were prepared by cyclization of **5a** and **5b** with $POCl_3$/PPA,[7] but lower yields were obtained for the fluorinated analogue (60% for **6a,** 35% for **6b**). Attempts to increase the yield (higher temperature and/or increment of $POCl_3$/PPA) were unsuccessful. Although compounds **8a–8c** were obtained from **6a** by direct condensation with the heterocyclic amine in good yields (over 80%), this method was not useful for preparing anilino-substituted compounds **8d–8f** or those of series **10** and **11**. The anilino group diminishes the susceptibility of the tricyclic ring to the nucleophilic attack, apparently by steric hindrance. Compounds **10a–10f**, **11a–11e** and **8d–8f** were obtained by the oxidation of the methylthio derivative to the respective sulfone, and subsequent condensation with the appropriate amine in moderate to good yields. We also tried to obtain compounds **8d–8f** by condensation of aniline with **8a–8c** by using different conditions, but the starting material was recovered each time. Compounds of series **7** were obtained by a method previously reported,[7] but in the case of fluorinated derivatives it was necessary to extend reaction time. All compounds were characterized by IR, ^{1}H NMR, MS and elemental analysis. Unequivocal assignment of all ^{1}H NMR signals was possible by NOESY experiments carried out with compound **10e**.

3. Biological

3.1. In vitro cytotoxicity

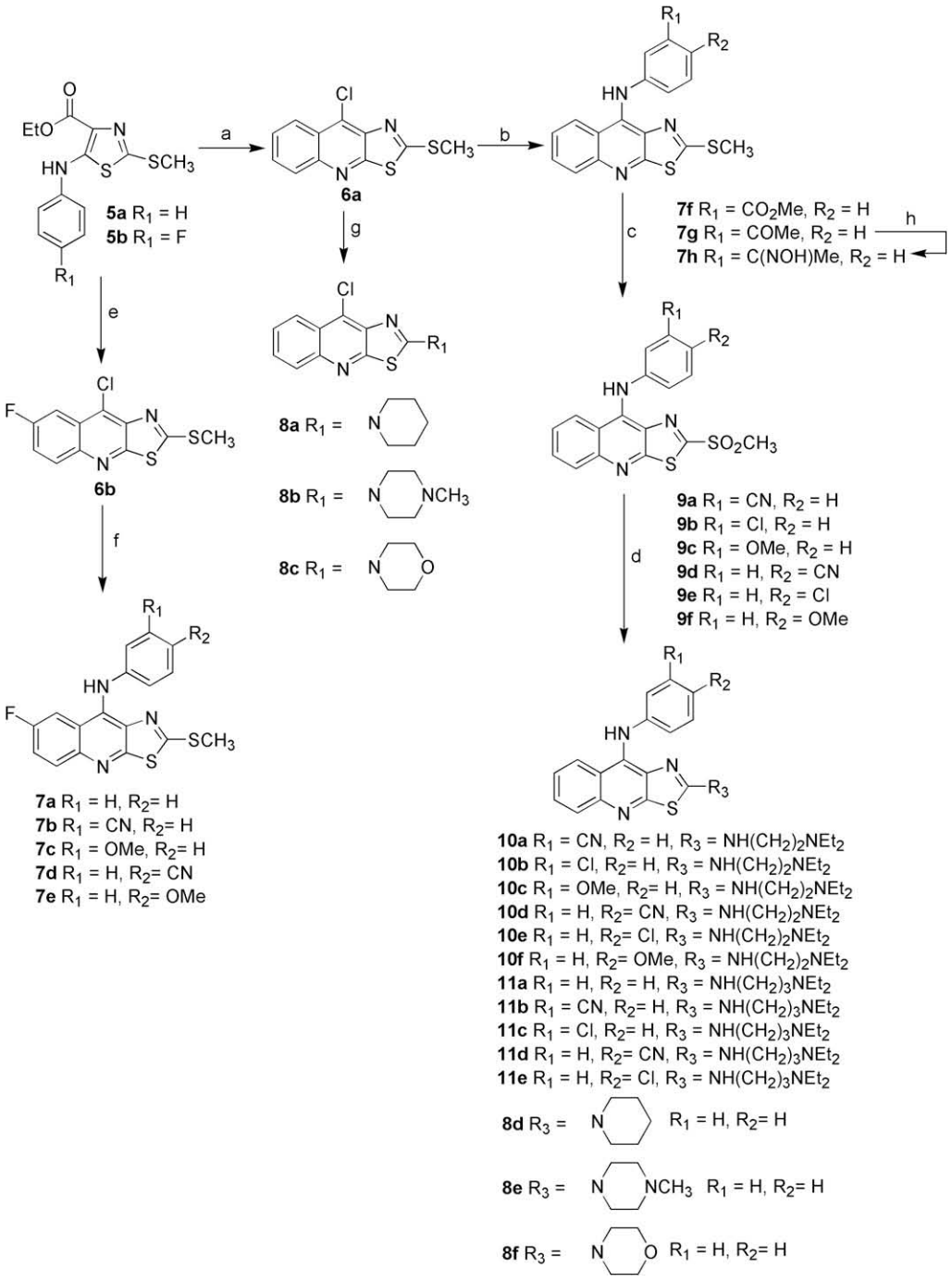
The results of the evaluation of cytotoxic activity of the newly prepared and some previously reported compounds[7,8] are shown in Table 1. 9-Anilino-7-fluoro-susbtituted derivatives were not as active as their non-fluorinated analogues (compare **7a–e** and **7i–m**), except for compound **7c,** which was more active than the non-fluorinated analogue **7k**. In general, the trend differs from the one found by Alvarez-Ibarra et al. for 9-hydroxythiazoloquinoline derivatives,[13] where a substitution with a fluorine atom in position 7 favored cytotoxicity.

Since, according to our previous studies, the presence of an electron withdrawing group at 3′-position favored cytotoxicity, additional substituents were incorporated at this position, that is, compounds **7f–7h**. As discussed in that preceding paper,[8] a putative hydrogen bond acceptor atom seems to be relevant to cytotoxicity of such a compound series. In the new compounds, only the oxime-substituted compound was cytotoxic. Thus, the cytotoxic activity may be influenced by the distance from the anilino ring to the putative hydrogen bond acceptor atom (distance to HBA, Figure 3), because the cyano derivative **7j** was cytotoxic, but a shorter or longer distance to HBA, as in **7f** and **7g**, eliminated the activity. In addition, the alignment of the cyano group possibly contributes to a better hydrogen bond interaction. Moro, Capranico et al. have proposed a model for the interaction of some intercalators with DNA and DNA topoisomerase II, based on docking studies.[14] Strong interactions were found between a hydroxyl group of Thr744 of topoisomerase II (hydrogen bond donor atom, HBD) and the sulfonamide moiety (HBA) of the *m*-amsacrine. A similar interaction may mediate anilinothiazolo[5,4-*b*]quinolines antitumor activity, and if so, the HBA group could be involved.

Compounds of series **8** that carry a six-member saturated nitrogen-containing heterocyclic substituent at 2-position showed low activity. A similar result was observed for 9-hydroxy-2-saturated thiazoloquinoline substituted compounds.[13] Thus, flexibility of the alkylamino chain at 2-position seems to have a positive influence on cytotoxic activity. In addition, compounds of series **10** and **11** showed similar cytotoxic activity, **11e** being the most active. Since the 'propylenediamine' substituted compounds in these series were more active than 'ethylenediamine' substituted compounds, the length of the alkylamino chain was relevant but not critical for cytotoxic activity. On the other hand, attachment of the substituents at any position of the anilino ring in these derivatives did not improve activity and in some cases it even reduced cytotoxicity.

3.2. Inhibition of human DNA topoisomerase II activity

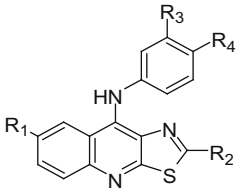
As reported in our previous paper[8], the cytotoxicity of several thiazolo[5,4-*b*]quinoline derivatives did not correlate with the



Scheme 1. Reactions and conditions (a) $POCl_3$/PPA, 130 °C, 4 h; (b) $H_2N–C_6H_4–R$, MeOH, reflux, 6 h; (c) H_2O_2/AcOH, rt, overnight; (d) method A amine, reflux 30 min; method B amine, DMF, rt, 3 h; (e) $POCl_3$/PPA, 150 °C, 4 h; (f) $H_2N–C_6H_4–R$, EtOH, reflux, 20 h; (g) amine, rt, 24 h; (h) NH_2OH, MeOH, reflux, 2 h.

in vitro DNA-intercalation ability of the compounds. In the case of acridines the mechanism of action seems to be related to the stabilization of the ternary complex between DNA, Topoisomerase II and the drug.[15] The decatenation of kDNA molecules was used to assay human Topoisomerase II (Topo II) activity. Only topoisomerase type II can resolve individual DNA molecules from the interlocked network of covalently closed minicircles composing the kDNA. The resulting individual molecules were analyzed by agarose-gel electrophoresis, and the activity was calculated from the percentage of kDNA converted to individual DNA molecules, as judged from densitometric analysis of ethidium bromide stained gels. In control lanes no residual kDNA was detected, this activity being considered 100%. In the initial screening, the most cytotoxic compounds were tested at a final concentration of 160 μM in the assay. For those showing significant activity the IC_{50} was calculated from dose–response curves in the range of concentration of 1–160 μM. The resulting curves were sigmoidal in most cases with Hill numbers ranging from 2 to 8. Apparent cooperativity binding may indicate the participation of more than one molecule of the compound in the enzyme–DNA–inhibitor complex or the existence of high and low affinity DNA subsites where the compound binds, yet not all of them lead to the inhibition of Topo II. The concentration values giving 50% inhibition (IC_{50}) were calculated by non-linear regression fits of the data to the following equation:

Table 1
In vitro cytotoxic activity of novel and already reported thiazolo[5,4-*b*]quinoline derivatives



	R_1	R_2	R_3	R_4	HeLa	SW480	SW620	K-562
7a	F	SMe	H	H	>80	>80	>80	>80
7b	F	SMe	CN	H	>80	>80	>80	55.36
7c	F	SMe	OMe	H	15.8	13.7	12.2	n.t
7d	F	SMe	H	CN	>80	>80	>80	n.t
7e	F	SMe	H	OMe	>80	>80	>80	54.91
7f	H	SMe	CO_2Et	H	>80	>80	>80	>80
7g	H	SMe	COMe	H	>80	>80	>80	>80
7h	H	SMe	OXM	H	34.2	37.3	39.4	32.8
7i[c]	H	SMe	H	H	>80	>80	>80	>80
7j[d]	H	SMe	CN	H	7.75	28.68	43.75	8.01
7k[d]	H	SMe	OMe	H	25.34	66.65	26.58	22.17
7l[d]	H	SMe	Cl	H	69.37	>80	>80	80.26
7m[d]	H	SMe	H	CN	>80	>80	>80	>80
7n[d]	H	SMe	H	OMe	>80	>80	>80	77.2
7o[d]	H	SMe	H	Cl	>80	>80	>80	79.45
7p[d]	H	SMe	NMe	H	46.22	>80	>80	46.85
7q[d]	H	SMe	(CO)E	H	21.69	13.6	19.72	12.54
7r[d]	H	SMe	CF_3	H	43.34	65.13	62.28	67.06
7s[d]	H	SMe	OH	H	>80	>80	>80	>80
7t[c]	H	SMe	NH_2	H	>80	>80	>80	>80
7u[c]	H	SMe	NH_2	[b]	>80	>80	>80	>80
7v[c]	H	SMe	NHAc	H	>80	>80	>80	>80
8a	H	PIP	[a]	[a]	>80	>80	>80	>80
8b	H	MPZ	[a]	[a]	>80	>80	>80	>80
8c	H	MOR	[a]	[a]	>80	>80	>80	>80
8d	H	PIP	H	H	>80	>80	>80	>80
8e	H	MPZ	H	H	60.7	41.8	>80	73.01
8f	H	MOR	H	H	>80	>80	>80	>80
10a	H	E	CN	H	13.33	13.10	14.62	12.48
10b	H	E	Cl	H	9.12	14.33	17.78	12.19
10c	H	E	OMe	H	19.2	11.5	20.0	23.5
10d	H	E	H	CN	15.18	14.18	16.49	8.36
10e	H	E	H	Cl	10.16	12.56	12.20	7.26
10f	H	E	H	OMe	13.60	12.04	16.38	10.87
10g[c]	H	E	H	H	15.96	37.7	21.6	16.8
11a	H	P	H	H	6.27	6.90	16.56	7.52
11b	H	P	CN	H	19.21	11.53	19.65	12.88
11c	H	P	Cl	H	7.46	7.91	10.17	9.84
11d	H	P	H	CN	24.18	22.70	29.15	12.88
11e	H	P	H	Cl	8.82	4.92	7.48	3.36
			Amsacrine[c]		9.5	27.7	16.7	19.9

IC_{50}, μM, compound concentration inhibiting 50% of cellular growth assessed by the MTT assay.
E = $NH(CH_2)_2NEt_2$ P = $NH(CH_2)_3NEt_2$, PIP = -1-piperidinyl, MPZ = -4-methylpiperazinyl, MOR = 1-morpholinyl, OXM = C(NOH)Me, n.t. not tested.
[a] Chlorine atom in position 9 instead of aniline ring.
[b] 5′-CH_2OH substituted.
[c] Taken from Ref. 7.
[d] Taken from Ref. 8.

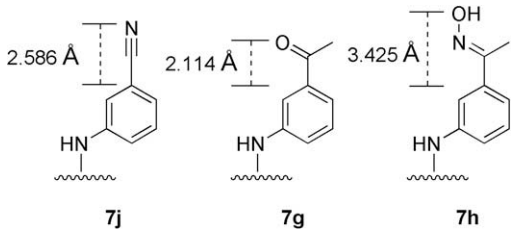


Figure 3. Distance from aniline ring to hydrogen bond acceptor atom (distance to HBA).

Table 2
Inhibitory activity of thiazolo[5,4-*b*]derivatives and *m*-amsacrine, as reference, against on topoisomerase II (IC_{50}, μM)

Compound	Activity	±se	Hill	±se
7l	21.20	0.61	8	1.52
7o	20.49	0.24	7	0.57
7p	12.27	0.28	3	0.15
7r	15.42	0.01	6	0.01
7s	64.34	5.13	7	1.86
7t	16.74	0.14	4	0.10
7u	18.04	1.66	2	0.48
7v	29.56	6.21	2	0.40
10g	168.90	6.07	1	0.06
11c	40.95	8.66	1	0.05
m-AMSA	9.94	0.51	4	0.56

$$\text{Activity } (\%) = \frac{100 IC_{50}^{n_H}}{IC_{50}^{n_H} + [I]^{n_H}}$$

where n_H is the cooperativity index and I the concentration of added compound. Non-linear regression was performed with the Sigmastat 2.0 statistical package (Jendel Sci. Inc. San Rafael, Ca.).

There was no correlation between in vitro Topo II inhibition (Table 2) and the cytotoxicity of the compounds (Table 1). Compound **7p** was as nearly a good Topo II inhibitor as *m*-AMSA, but it exhibited low cytotoxicity, while compound **11c** was highly cytotoxic, but, as an inhibitor of Topo II, it was four times less potent than *m*-AMSA. Thus, the thiazolo[5,4-*b*]quinoline derivatives may not be acting by the same mechanism as *m*-AMSA, may be differentially modified by cell metabolism, or may present large differences in their ability to penetrate the cell.

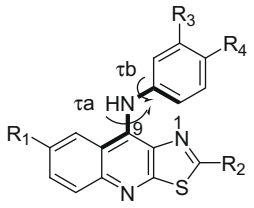
4. Molecular modeling

The cytotoxic activity of these compounds must be related to their molecular structure, and molecular modeling of the isolated molecules may reveal some of the factors involved. Molecular models were obtained with SPARTAN′04[16] software using the semi-empirical AM1 method. A systematic search protocol (torsion angles τa and τb) was used to obtain the minimal energy conformers. Table 3 shows some of the calculated molecular parameters. Optimized geometries of selected compounds are shown in Figure 4. Substituents attached to the phenyl ring linked to the amino group had a greater effect on the phenyl conformation than those attached to 2-position of the tricyclic nucleus system. The results of the conformational analysis show an oblique orientation of the anilino ring in relation to the tricyclic nucleus. In accordance with the models, the analysis of the NOESY spectrum of **10e** showed a long-range interaction between the protons of the anilino ring and those of alkylamino chain.

Because DNA–intercalator complex can be stabilized through frontier molecular orbital interactions, LUMO energy values of the compounds were calculated. All compounds appear to behave as electron acceptors since their LUMO energies were negative. In contrast, the DNA base pairs have been shown to act as electron donors.[17] This type of interaction should be very important for fluorinated compounds **7a–7e** because their LUMO energy values were the lowest. In the case of alkylamino derivatives, a cationic species can be formed due to the basic properties of the side chain, which can interact the phosphate backbone, facilitating DNA intercalation. This last interaction has been shown to occur in similar compounds[18] and was suggested by us based on previous results.[8]

The dipole moment describes the charge distribution in a molecule and somehow summarizes electronic effects involved in molecular recognition. With DNA intercalators, a direct correlation between their dipole moment values and cytotoxicity has been

Table 3
Selected parameters calculated for thiazolo[5,4-*b*]quinoline derivatives



	R_1	R_2	R_3	R_4	E HOMO (eV)	E LUMO (eV)	Dipole (Debye)	PSA (Å^2)	Log*P*	τa (°)
7a	F	SMe	H	H	−8.2812	−1.2490	2.7750	25.4798	5.33	−60.9
7b	F	SMe	CN	H	−8.5381	−1.4596	2.9112	40.94	5.37	−66.0
7c	F	SMe	OMe	H	−8.2981	−1.2551	2.9116	32.6213	5.21	−62.4
7d	F	SMe	H	CN	−8.5720	−1.5062	4.3856	40.9403	5.37	−67.2
7e	F	SMe	H	OMe	−8.1733	−1.2143	2.5061	32.6746	5.21	−58.4
7f	H	SMe	CO_2Et	H	−8.3488	−1.2317	1.5972	44.009	5.33	−64.5
7g	H	SMe	COMe	H	−8.3493	−1.2319	1.8153	38.628	4.49	−63.4
7h	H	SMe	OXM	H	−8.2219	−1.1237	2.2472	59.969	4.88	−62.6
7i	H	SMe	H	H	−8.1929	−1.0978	2.6069	25.4955	5.18	−61.8
7j	H	SMe	CN	H	−8.4384	−1.3072	1.4718	40.9621	5.21	−66.3
7k	H	SMe	OMe	H	−8.2095	−1.1038	3.0656	32.6417	5.05	−63.2
7l	H	SMe	Cl	H	−8.3269	−1.2085	1.0670	25.5319	5.73	−64.6
7m	H	SMe	H	CN	−8.4712	−1.3560	3.0940	40.9473	5.21	−67.1
7n	H	SMe	H	OMe	−8.1371	−1.0973	1.8501	32.6991	5.05	−60.0
7o	H	SMe	H	Cl	−8.3010	−1.2130	1.7710	25.5309	5.73	−64.3
7p	H	SMe	NMe	H	−8.4467	−1.3666	3.9320	37.5510	4.67	−67.9
7q	H	SMe	(CO)E	H	−8.6759	−1.5141	1.9465	53.1684	4.99	−70.9
7r	H	SMe	CF_3	H	−8.8215	−1.5828	4.1788	25.8454	6.10	−79.0
7s	H	SMe	OH	H	−8.5419	−1.3882	4.2182	45.2594	4.79	−74.0
7t	H	SMe	NH_2	H	−8.4487	−1.3750	3.4153	50.388	4.37	−70.0
7u	H	SMe	NH_2	[b]	−8.4069	−1.1691	5.0351	67.5379	3.8	−70.0
7v	H	SMe	NHAc	H	−8.5198	−1.4433	1.045	50.2311	4.08	−68.0
8a	H	PIP	[a]	[a]	−8.5277	−0.9740	2.1853	16.5785	6.13	n/a
8b	H	MPZ	[a]	[a]	−8.5574	−1.0003	1.9366	19.1469	4.82	n/a
8c	H	MOR	[a]	[a]	−8.6512	−1.0832	0.4513	24.4935	4.66	n/a
8d	H	PIP	H	H	−8.1366	−0.8559	2.8936	27.3391	6.52	−67.0
8e	H	MPZ	H	H	−8.1507	−0.8753	2.4780	29.895	5.21	−66.3
8f	H	MOR	H	H	−8.2160	−0.9483	1.1818	35.2189	5.06	−65.2
10a	H	E	CN	H	−8.4021	−1.0062	3.7587	54.4904	5.38	−75.1
10b	H	E	Cl	H	−8.2583	−0.9071	3.2760	39.0601	5.9	−71.1
10c	H	E	OMe	H	−8.1415	−0.8290	4.4488	46.2024	5.22	−68.7
10d	H	E	H	CN	−8.3940	−1.0392	6.0452	54.4736	5.38	−72.7
10e	H	E	H	Cl	−8.2344	−0.9171	3.8532	39.0872	5.9	−71.3
10f	H	E	H	OMe	−8.0092	−0.7954	3.4852	46.2707	5.22	−67.1
10g	H	E	H	H	−8.1317	−0.8131	3.7866	39.0819	5.35	−69.3
11a	H	P	H	H	−8.0845	−0.7612	4.1858	36.7885	5.63	−69.9
11b	H	P	CN	H	−8.3473	−0.9520	4.6902	52.1346	5.66	−75.2
11c	H	P	Cl	H	−8.2280	−0.8615	4.0702	34.2395	6.18	−72.9
11d	H	P	H	CN	−8.3434	−0.9862	6.9274	52.1784	5.66	−73.1
11e	H	P	H	Cl	−8.1920	−0.8659	4.3978	36.7761	6.18	−72.4
		Amsacrine			−8.2450	−1.3367	1.7783		3.11	−114

E = $NH(CH_2)_2NEt_2$ P = $NH(CH_2)_3NEt_2$, PIP = -1-piperidinyl, MPZ = -4-methylpiperazinyl, MOR = 1-morpholinyl, OXM = C(NOH)Me, n/a = not applicable.
[a] Chlorine atom in position 9 instead of aniline ring.
[b] 5′-CH_2OH substituted.

found.[19–21] In the present study, the dipole moment vector and magnitude were calculated.

A cyano group or chlorine atom increased the dipole moment magnitude more when placed at 4′-position than when placed at 3′-position. The opposite trend is observed for the methoxy group. For compounds with a dialkylaminoalkylamino substituent, the incorporation of an electron withdrawing group (EWG) notably increased the dipole moment magnitude. The replacement of the methylthio group at 2-position by a dialkylaminoalkylamino residue also resulted in a higher dipole moment, and the incorporation of a saturated heterocycle reduced its magnitude slightly. However, the variations in the direction of the dipole moment may be of greater importance, and based on the dipole moment direction, the compounds can be grouped as indicated in Table 4 and Figure 5. From the data, the presence of EWG in the anilino ring and incorporation of a fluorine atom at position 7 cause changes in the dipole moment direction. It is worthy of note that **7g** and **7f** lack EWG at position 4′ but belong to group D, since their dipole vectors resemble the **7m** dipole instead of the one of **7j**.

5. Structure–activity relationship

5.1. Cytotoxic activity

In Table 4, the compounds with a dialkylaminoalkyl group, regardless of their dipole orientation, are the most active; in the case of 2-methyltio derivatives (series 7), however, only the

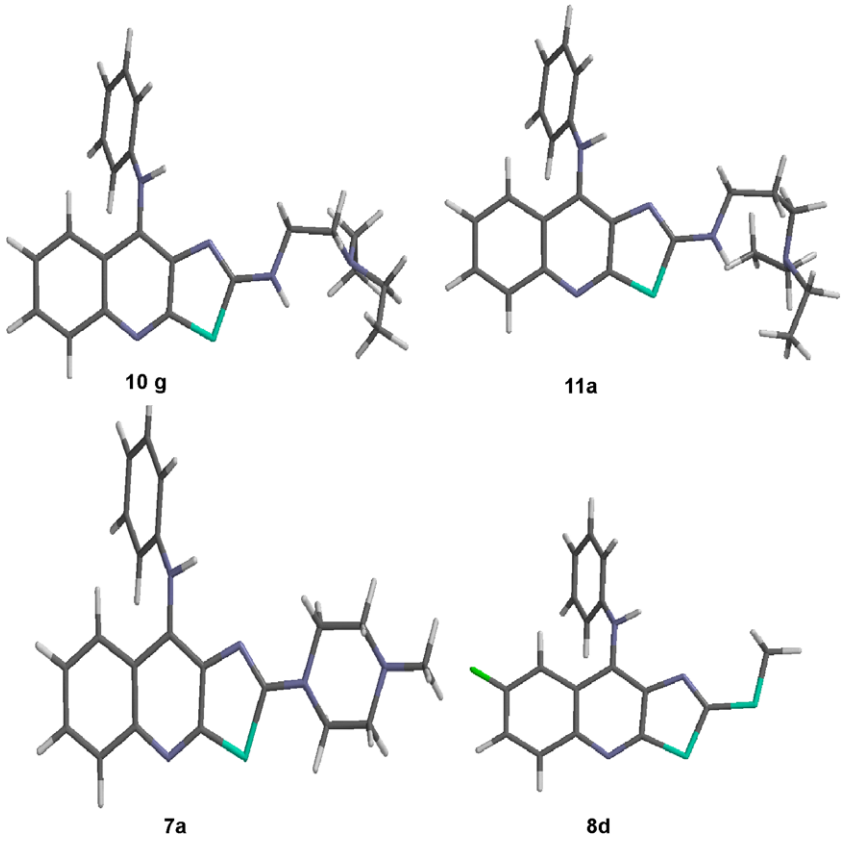
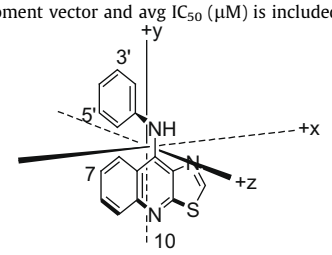


Figure 4. Optimized geometries for selected compounds.

Table 4
Analyzed compounds grouped according to direction of dipole moment vector and avg IC_{50} (μM) is included (HeLa cell line)



Group A (position 10)	Avg IC_{50}	Group B (position 7)	Avg IC_{50}	Group C (position 3′)	Avg IC_{50}	Group D (position 4′)	Avg IC_{50}
7h, **7i**, **7k**, **7l**, **7n**, **7o**, **7s**, **7t**, **7u**, **7v**	>80[a]	**7a**, **7c**, **7e**	>80[b]	**7j**, **7q**, **7r**	24.2	**7b**, **7d**, **7f**, **7g**, **7m**	>80
		8a, **8b**, **8d**, **8e**, **8f**	>80[c]			**8c**	>80
		10b, **10c**, **10e**, **10f**, **10g**	13.6			**10a**, **10d**	14.2
		11a, **11c**, **11e**	7.52			**11b**, **11d**	21.7

[a] Except **7h** IC_{50} = 34.2, **7k,** IC_{50} = 25.34.
[b] Except **7c** IC_{50} = 15.8.
[c] Except **8e** IC_{50} = 60.7.

compounds with the dipole orientation through 3′-position and an EWG are active. The comparison of compound **7j** with **10a** and **11d** indicates a greater influence of the alkyl chain on the dipole orientation, and while compounds **10a**, **10d**, **11b** and **11d** show no cell line selectivity at all, compound **7j** is very active only against HeLa and K-562 cell lines. Moreover, the incorporation of a fluorine atom in **7j** severely modifies its dipole orientation (relative to compound **7b**) and diminishes its cytotoxic activity. There is possibly a low density region in the putative interaction site, which is only accessed through a substituent with a high charge density. This proposal would explain the low activity of compound **7l**, which, in spite of its chlorine atom at 3′-position, has a different dipole orientation relative to **7j**.

The influence of the dipole moment on cytotoxicity is clear because the 2-methylthio compounds of group D (series 7) have no cytotoxic activity while the same kind of compounds of groups A and C are cytotoxic. Nevertheless, other factors ought to be considered since in 3′-substituted compounds of group A, a higher dipole moment magnitude is associated with higher cytotoxicity (**7h**, **7k**), but the opposite trend was found for compounds in group C (**7j**,**7q**) (Fig. 6). Furthermore, compounds of group D (**10d** and **11d**) with higher dipole moment are among the less active

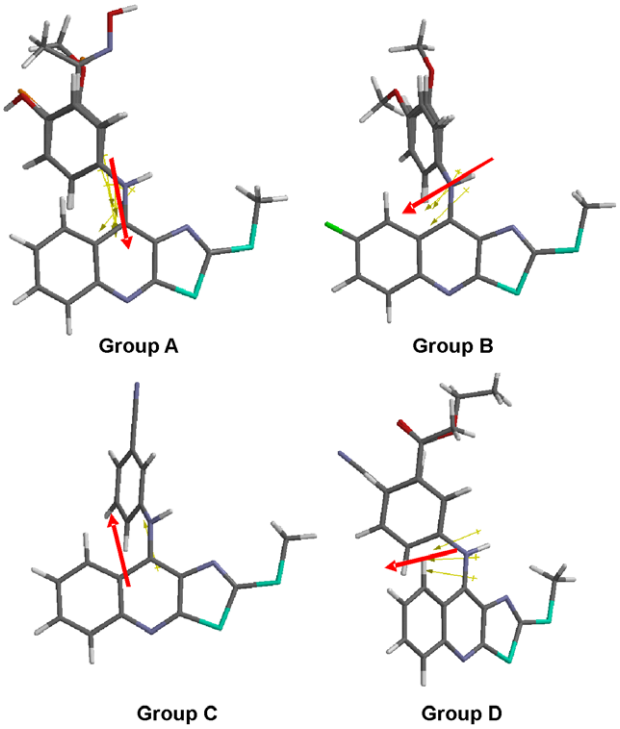


Figure 5. Analyzed compounds grouped according to the direction of the dipole vector. Red arrow indicates dipole orientation.

compounds of the alkylamino series, and hence, other electronic factors influence cytotoxicity. In any case, hydrophobic factors remain among the most relevant.

Some QSAR equations were derived, but in this case compounds with IC_{50} above 80 μM were excluded from the SAR analysis. Compounds **7j**, **7q**, **8e** and **11e** did not fit into the first model. This can possibly be explained by the different direction of the dipole moment toward the nitrogen atom of the central ring instead of pointing towards the anilino ring as in the remaining compounds (see Fig. 4). When these compounds were excluded, the equation found

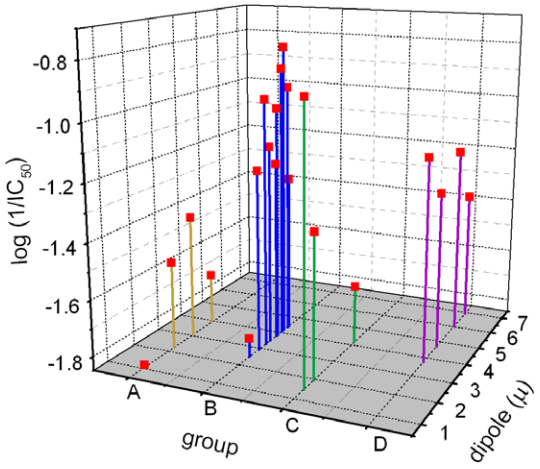


Figure 6. Relationships between dipole moments and cytotoxic activity in HeLa cell line. Groups are those included in Table 4.

for K-562 cell line was: $\log(1/IC_{50}) = 1.14576 * \text{LUMO} + 0.13084 * \text{-dipole moment} - 0.59538$ ($n = 19$; $r^2 = 0.850$; $F = 45.583$). Interestingly, with this subset of compounds, two subgroups become evident, one including alkylamino substituted compounds and the other comprising methylthio substituted compounds (Fig. 7). A similar tendency was observed with data from the other cell lines.

For the compounds with a dialkylaminoalkylamino group, the hydrophobic factors appear to be important since compounds with a higher log*P* were among the most active. To develop a 2D-QSAR, several descriptors representative of size (polar surface area, polar volume, etc.), electronic and lipophilic characteristics were used to characterize the compounds, but only one satisfactory QSAR equation was found for compounds of series **10** and **11** (HeLa cell line): $\log(1/IC_{50}) = -0.01964\ \text{PSA} - 0.19409$ ($n = 10$; $r^2 = 0.740$; $F = 24.541$), **10c** and **10g** as outliers. Where PSA is the Polar Surface Area descriptor. Thus, one important factor for the activity of these compounds lies in their ability to pass through biological membranes because this parameter strongly correlates with membrane permeability.[22] Therefore, the better activity of series **11** could be

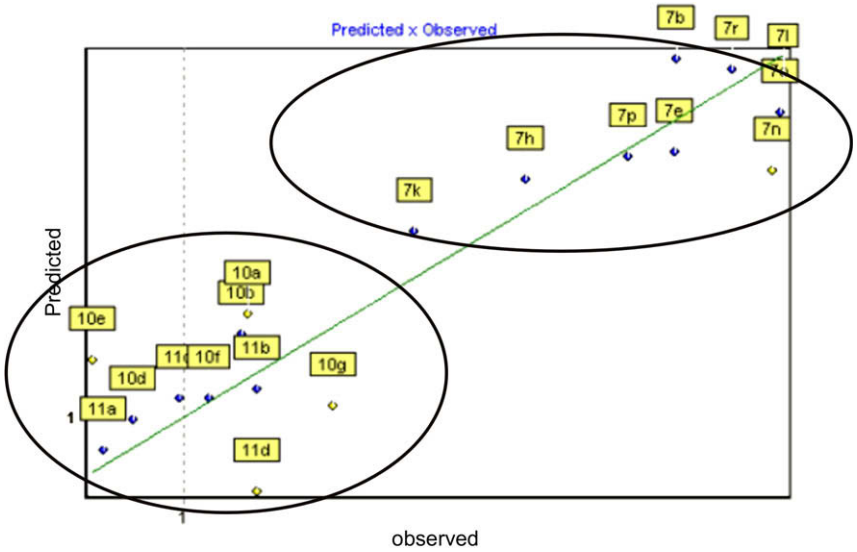


Figure 7. Predicted activities, calculated by equation A, versus observed activities. Lower subset includes alkylamino substituted compounds, upper, methylthio substituted compounds.

attributed to higher ability to cross cell membranes and not necessarily to the better binding to DNA.

5.2. Topoisomerase II inhibition

For the inhibition of human Topo II, the IC_{50} value correlated only with the LUMO parameter [$\log(1/IC_{50}) = -0.99025 * \text{LUMO} - 2.46625$; $n = 07$; $r^2 = 0.810$; $F = 21.233$, **8r**, **8s** and **8t** as outliers]. As discussed before, the LUMO parameter is relevant to the DNA intercalation and appears to be important in the formation of the Topoisomerase II-DNA-inhibitor ternary complex, at least for these compounds. An unusual feature of the Topo II inhibition curves is the presence of positive cooperativity, with Hill numbers approaching 4. Therefore, four or more molecules of the compound seem to be required to produce the inhibition. This result does not necessarily reflect the presence of 4 inhibitor molecules at the ternary complex but may result from high-affinity-intercalation of the inhibitor to DNA sites, which are poor substrates of the Topoisomerase II.

Taking all data together, the 2-alkylamino compounds (series **10** and **11**) have different structural requirements for cytotoxic activity as compared with 2-methylthio derivatives (series **7**). For the first group of compounds the capacity to pass through biological membranes is more important and for the latter the nature of the substituent in position 3′ becomes predominant. Possibly this last factor affects the binding of these compounds to a putative receptor. Even in series **7,** two different behaviors were observed, one class of compounds with dipole moment oriented to the anilino ring and the other class with dipole moment oriented to the tricyclic template. Perhaps compounds of series **10** and **11** have a different mode of binding to DNA or to topoisomerase II, from those of series **7**. Studies on the determination of mechanism of action of both classes of compounds are in course.

6. Conclusions

Novel 9-anilinothiazolo[5,4-*b*]quinoline derivatives with a different substitution pattern in the anilino ring and in the tricyclic template were synthesized and their cytotoxic activity, tested in vitro. Incorporation of a saturated heterocyclic ring and of a fluorine atom in positions 2 and 7 of the tricyclic template, respectively, resulted in a depletion of activity. A flexible dialkylaminoalkylamino residue in position 2 improves activity; its length is important but not critical for cytotoxicity. For 2-methylthio derivatives, the nature of the substituent in position 3′ is relevant for cytotoxicity, apparently through the formation of hydrogen bonds with its in vivo molecular target, the direction of dipole moment being likely to orient the compound into the target molecule, thus enhancing or weakening the binding.

7. 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 (70–230 mesh, Merck). Melting points were determined on a Fisher–Jones apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet FT-5SX spectrophotometer model. ^1H and ^{13}C NMR spectra were recorded on a Varian VxR-300S spectrometer (300 and 75.5 MHz, respectively). 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 Hertz. EI-MS were carried out on a JEOL JMS-AX505-HA apparatus. FAB-MS were carried out on a JEOL Sx102 apparatus. Compounds **5a–b** and **6a–b** were prepared according to procedures already described.[7,8,13]

7.1. General preparation of 2-(methylthio)-9-anilinothiazolo[5,4-*b*]quinolines (7a–h)

This method has already been described.[7,8] Briefly, to the compound **6a** or **6b** (0.5 mmol) were successively added methanol or ethanol for fluorinated analogues (5 mL) and two drops of HCl (36%). The cream-colored suspension formed was stirred for ten minutes. Meanwhile, a mixture of the aniline with the desired substitution pattern (0.7 mmol) in 5 mL of methanol or ethanol was prepared. This mixture was added to the suspension of **6a** or **6b** and heated to reflux for 6–18 h. After cooling, the solvent 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.

7.1.1. 7-Fluoro-9-(phenylamino)-2-(methylthio)-9-anilinothiazolo[5,4-*b*]quinoline (7a)

Yellow solid; 138 mg (81.5%); mp 187–190 °C. IR (KBr, cm^{-1}): 3423 (NH), 1624, 1597, 1545, 1525, 1493, (aromatic); ^1H NMR (DMSO-d_6, δ): 2.33 (s, 3H) SCH_3; 7.15 (d, $J = 7.6$ Hz, 2H), H-2′, H-6′; 7.32 (t, $J = 8.0$ Hz, 2H), H-3′, H-5′; 7.09 (t, $J = 7.6$ Hz, 1H) H-4′; 7.99 (dd, $J = 9.2$, 5.2 Hz, 1H) H-5; 7.71 (ddd, $J = 10.8$, 9.6, 2.8 Hz, 1H) H-6; 8.27 (dd, $J = 11.2$, 2.1 Hz, 1H) H-8; 9.70 (br), NHAr. MS (FAB, *m/z*): 342 (M^++1, 100%), 341 (M^+, 41%) Anal. Calcd for $C_{17}H_{12}FN_3S_2$: C, 59.80; H, 3.54; N, 12.31; S, 18.78. Found: C, 59.65; H, 3.79; N, 12.16; S, 18.93.

7.1.2. 9-[[(3-Cyano)phenyl]amino]-7-fluoro-2-(methylthio)-9-anilinothiazolo[5,4-*b*]quinoline (7b)

Yellow solid; 153 mg (81%); mp 252–253 °C. IR (KBr, cm^{-1}): 3430 (NH), 2222 (CN), 1603, 1586, 1543, 1511, 1479, (aromatic); ^1H NMR (DMSO-d_6, δ): 2.31 (s, 3H) SCH_3; 7.65 (s, 1H), H-2′; 7.53 (m, 3H), H-4′, H-5′, H-6′; 8.01 (dd, $J = 9.6$, 5.6 Hz, 1H) H-5; 7.57 (dd, $J = 9.2$, 2.0 Hz, 1H) H-6; 8.49 (dd, $J = 10.4$, 2.1 Hz, 1H) H-8; 10.42 (br), NHAr. MS (FAB, *m/z*): 367 (M^++1, 100%), 366 (M^+, 17%) Anal. Calcd for $C_{18}H_{11}FN_4S_2$: C, 59.00; H, 3.03; N, 15.29; S, 17.50. Found: C, 59.25; H, 3.18; N, 15.04; S, 17.35.

7.1.3. 9-[[(3-Methoxy)phenyl]amino]-7-fluoro-2-(methylthio)-9-anilinothiazolo[5,4-*b*]quinoline (7c)

Yellow solid; 131 mg (70%); mp 193–195 °C. IR (KBr, cm^{-1}): 3424 (NH), 3028 (OMe), 1599, 1578, 1549, 1510, 1486, (aromatic); ^1H NMR (DMSO-d_6, δ): 2.35 (s, 3H) SCH_3; 3.61 (s, 3H) OCH_3, 6.74 (m, 3H), H-2′, H-4′, H-6′; 7.25 (t, $J = 8.7$ Hz, 1H), H-5′; 7.75 (ddd, $J = 10.5$, 7.8, 2.7 Hz, 1H) H-6; 7.97 (dd, J =9.2, 5.1 Hz, 1H) H-5; 8.27 (dd, $J = 10.4$, 2.1 Hz, 1H) H-8; 10.13 (br), NHAr. MS (FAB, *m/z*): 372 (M^++1, 100%), 371 (M^+, 17%)%) Anal. Calcd for $C_{18}H_{14}FN_3OS_2$: C, 58.20; H, 3.80; N, 11.31; S, 17.26. Found: C, 58.38; H, 3.87; N, 11.23; S, 17.09.

7.1.4. 9-[[(4-Cyano)phenyl]amino]-7-fluoro-2-(methylthio)-9-anilinothiazolo[5,4-*b*]quinoline (7d)

Yellow solid; 111 mg (75%); mp 235–238 °C. IR (KBr, cm^{-1}): 3422 (NH), 2220 (CN), 1600, 1575, 1534, 1511, 1496, (aromatic); ^1H NMR (DMSO-d_6, δ): 2.49 (s, 3H) SCH_3; 7.2 (d, $J = 8.1$ Hz, 2H), H-2′, H-6′; 7.66 (d, $J = 8.1$ Hz, 2H), H-3′, H-5′; 7.72 (dd, $J = 9.6$, 7.5 Hz, 1H) H-6; 8.07 (d, $J = 9.2$, 1H) H-5; 8.15 (d, $J = 9.0$ Hz, 1H) H-8; 9.90 (br), NHAr. MS (FAB, *m/z*): 367 (M^++1, 100%), 366 (M^+, 28%) Anal. Calcd for $C_{18}H_{11}FN_4S_2$: C, 59.00; H, 3.03; N, 15.29; S, 17.50. Found: C, 59.13; H, 3.10; N, 15.16; S, 17.43.

7.1.5. 9-[[(4-Methoxy)phenyl]amino]-7-fluoro-2-(methylthio)-9-anilinothiazolo[5,4-*b*]quinoline (7e)

Yellow solid; (72%); mp 203–205 °C. IR (KBr, cm^{-1}): 3444 (NH), 3026 (OCH_3), 1602, 1577, 1545, 1504, 1444, (aromatic); ^1H NMR (DMSO-d_6, δ): 2.30 (s, 3H) SCH_3; 3.77 (s, 3H) OCH_3, 6.96 (d, *J* = *8.8* Hz, 2H), H-3′, H-5′; 7.22 (d, *J* = 8.1, 2H), H-2′, H-6′; 7.78 (ddd, *J* = 9.6, 8.0, 2.8 Hz, 1H) H-6; 7.98 (dd, *J* = *9.2,* 5.6 Hz, 1H) H-5; 8.41 (dd, *J* = 11.2, 2.4 Hz, 1H) H-8; 10.11 (br), NHAr. MS (FAB, *m/z*): 372 (M^++1, 100%), 371 (M^+, 28%) Anal. Calcd for $C_{18}H_{14}FN_3OS_2$: C, 58.20; H, 3.80; N, 11.31; S, 17.26. Found: C, 58.41; H, 3.91; N, 11.20; S, 17.05.

7.1.6. 9-[[(3-Ethoxycarbonyl)phenyl]amino]-2-(methylthio)-thiazolo[5,4-*b*]quinoline (7f)

Yellow solid; (70%); mp 173–5 °C. IR (KBr, cm^{-1}): 3411 (NH), 3129, 3056, 2971 (CH), 1713 (C═O), 1621, 1570, 1545, 1507 (aromatic); ^1H NMR (DMSO-d_6, δ): 1.30 (t, *J* = 6.9 Hz, 3H) CH_3; 2.21 (s, 3H) SCH_3; 4.30 (q, *J* = 6.9 Hz, 2H) OCH_2; 7.52 (t, *J* = 6.9 Hz, 1H) H-5′; 7.55 (dt, *J* = 6.9, 1.5 Hz, 1H) H-6′; 7.68 (ddd, *J* = 8.7, 6.3, 1.2, 1H) H-7; 7.70 (dt, *J* = 6.9, 1.5 Hz, 1H) H-4′; 7.89 (t, *J* = 1.5 Hz, 1H) H-2′; 7.92 (ddd, *J* = 8.7, 6.3, 1.2 Hz, 1H) H-6; 7.99 (dd, *J* = 8.7, 1.2, 1H) H-5; 8.70 (d, *J* = 8.4 Hz, 1H) H-8; 10.7 (br, 1H) NH; MS (FAB, *m/z*): 396 (M^++1, 100%) Anal. Calcd for $C_{20}H_{17}N_3O_2S_2$: C, 60.74; H, 4.33; N, 10.62; S, 16.22. Found: C, 60.52; H, 4.54; N, 10.46; S, 16.39.

7.1.7. 1-(3-{[2-(Methylthio)thiazolo[5,4-*b*]quinolin-9-yl]-amino}phenyl)ethanone (7g)

Yellow solid; (70%); mp 185–7 °C. IR (KBr, cm^{-1}): 3411 (NH), 3129, 3122, 3059 (CH), 1681 (C═O), 1621, 1570, 1550, 1504 (aromatic); ^1H NMR (DMSO-d_6, δ): 2.24 (s, 3H) $COCH_3$; 2.55 (s, 3H) SCH_3; 7.52 (t, *J* = 6.9 Hz, 1H) H-5′; 7.55 (dt, *J* = 6.9, 1.5 Hz, 1H) H-6′; 7.67 (ddd, *J* = 8.7, 6.3, 1.2, 1H) H-7; 7.78 (dt, *J* = 6.9, 1.5 Hz, 1H) H-4′; 7.86 (t, *J* = 1.5 Hz, 1H) H-2′; 7.91 (ddd, *J* = 8.7, 6.3, 1.2 Hz, 1H) H-6; 7.99 (dd, *J* = 8.7, 1.2, 1H) H-5; 8.58 (d, *J* = 8.4 Hz, 1H) H-8; 10.66 (br, 1H) NH; MS (FAB, *m/z*): 366 (M^++1, 100%) Anal. Calcd for $C_{19}H_{15}N_3OS_2$: C, 62.44; H, 4.14; N, 11.50; S, 17.55. Found: C, 62.63; H, 4.33; N, 11.42; S, 17.23.

7.1.8. (1*E*)-1-(3-{[2-(Methylthio)thiazolo[5,4-*b*]quinolin-9-yl]amino}phenyl)ethanone oxime (7h)

To a suspension of 110 mg (0.3 mmol) of compound **7f** in 10 mL of methanol 50 mg of hydroxylamine chlorhydrate in 5 mL of methanol were added and heated to reflux for 1 h. After cooling, the solvent 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, giving 100 mg (87%) of title compound as a yellow solid, mp 201–3 °C. IR (KBr, cm^{-1}): 3411 (NH), 3227, 3053 (CH), 1617 (C═N), 1585, 1551, 1496, (aromatic); ^1H NMR (DMSO-d_6, δ): 2.10 (s, 3H) $C(NOH)CH_3$; 2.41 (s, 3H) SCH_3; 7.08 (m, 1H) H-5′; 7.28 (m, 2H) H-4′, H-6′; 7.39 (t, *J* = 1.5 Hz, 1H) H-2′; 7.51 (ddd, *J* = 8.7, 6.3, 1.2, 1H) H-7; 7.71 (ddd, *J* = 8.7, 6.3, 1.2 Hz, 1H) H-6; 7.92 (dd, *J* = 8.7, 1.2, 1H) H-5; 8.37 (d, *J* = 8.4 Hz, 1H) H-8; 9.36 (br, 1H) NH; 11.12 (s, 1H) OH; MS (FAB, *m/z*): 366 (M^++1, 100%) Anal. Calcd for $C_{19}H_{16}N_4OS_2$: C, 59.98; H, 4.24; N, 14.73; S, 16.85. Found: C, 59.81; H, 4.37; N, 14.70; S, 16.95.

7.2. Preparation of compounds 8a–c

A solution of **6a** (133 mg, 0.5 mmol) in 2 mL of the corresponding amine was stirred for 24 h at room temperature. After that time, 10 mL of a saturated aqueous solution of NH_4Cl was added, the greenish suspension was extracted with dichloromethane (3 × 5 mL) and the organic extracts were collected, washed with a $NaHCO_3$ solution (3 × 5 mL) and water (3 × 5 mL) and dried over Na_2SO_4. After concentration under reduced pressure, the pale yellow crude product was purified by crystallization from methanol or hexane.

7.2.1. 9-Chloro-2-(1-piperidinyl)thiazolo[5,4-*b*]quinoline (8a)

Pale yellow solid; 130 mg (80%); mp 165–168 °C (hexane). IR (KBr, cm^{-1}): 2937, 2850 (CH), 1601, 1543, (aromatic); ^1H NMR ($CDCl_3$, δ): 1.76 (s, 6H) H-3a, H-4a, H-6a; 3.76 (s, 4H) H-2a, H-5a, 7.60 (m, 2H), H-6, H-7; 8.0 (dd, *J* = 7.2,. 1.8 Hz, 1H), H-5; 8.25 (dd, *J* = 7.5, 1.8 Hz, 1H) H-8. MS (EI, *m/z*): 305 (M^++2, 37%), 303 (M^+, 100%) Anal. Calcd for $C_{15}H_{14}ClN_3S$: C, 59.30; H, 4.64; N, 13.83; S, 10.55. Found: C, 59.45; H, 4.89; N, 13.70; S, 10.28.

7.2.2. 9-Chloro-2-[1-[(4-methyl)piperazinyl]]thiazolo[5,4-*b*]-quinoline (8b)

Pale yellow solid; 130 mg (80%); mp 165–168 °C (methanol). IR (KBr, cm^{-1}): 2932, 2841, 2789 (CH), 1601, 1548, (aromatic); ^1H NMR ($CDCl_3$, δ): 2.42 (s, 3H) NCH_3; 2.64 (t, *J* = 4.8 Hz, 4H) H-3a, H-5a; 3.86 (t, *J* = 4.5 Hz, 4H), H-2a, H-6a; 7.61 (m, 2H), H-6, H-7; 8.0 (dd, *J* = 7.8,. 1.8 Hz, 1H), H-5; 8.25 (dd, *J* = 7.5, 1.8 Hz, 1H) H-8. MS (EI, *m/z*): 320 (M^++2, 10%), 318 (M^+, 30%), 70 (100%) Anal. Calcd for $C_{15}H_{15}ClN_4S$: C, 56.51; H, 4.74; N, 17.57; S, 10.06. Found: C, 56.51; H, 4.74; N, 17.57; S, 10.06.

7.2.3. 9-Chloro-2-(1-morphonyl)thiazolo[5,4-*b*]quinoline (8c)

Pale yellow solid; 130 mg (80%); mp 165–168 °C (hexane). IR (KBr, cm^{-1}): 2967, 2937, 2880 (CH), 1600, 1548, (aromatic); ^1H NMR ($CDCl_3$, δ): 3.80 (s, 4H) H-2a, H-6a; 3.87 (s, 4H) H-3a, H-5a; 7.64 (m, 2H), H-6, H-7; 8.04 (d, *J* = 7.2, 1H), H-5; 8.27 (d, *J* = 7.5, 1H) H-8. MS (EI, *m/z*): 307 (M^++2, 37%), 305 (M^+, 100%) Anal. Calcd for $C_{14}H_{12}ClN_3OS$: C, 54.99; H, 3.96; N, 13.74; S, 10.49. Found: C, 54.84; H, 4.10; N, 13.61; S, 10.66.

7.3. General preparation of 2-methylsulphanyl derivatives 9a–f

A suspension of 0.5 mmol of the 2-(methylthio)-9-anilinothiazolo[5,4-*b*]quinoline derivative with the desired substitution pattern in the aniline ring, in 10 mL of a mixture of H_2O_2 30%-acetic acid (1:1) was stirred overnight at room temperature. 20 mL of cold water were then added and the orange precipitate of the 2-methylsulphanyl derivative was collected by filtration, dried by suction and used without further purification.

7.4. General preparation of compounds 10a–10f, 11a–11e and 8d–8f

Method A: A solution of the 2-methylsulphanyl derivative (0.4 mmol) in 0.5 mL of the corresponding amine was heated to reflux for 30 min. After that time, 30 mL of dichloromethane were added and the yellowish solution was washed with a NaOH 2 N (3 × 5 mL), saturated NH_4Cl solution (3 × 5 mL) and water (3 × 5 mL) and dried over Na_2SO_4. After concentration under reduced pressure, the pale yellow crude was purified by column chromatography (silica gel, mixture of CH_2Cl_2/MeOH/NH_4OH 98:2:0.1 as eluent) or crystallization from methanol for compounds **8d–f**. *Method B*: An alternative procedure for compounds **10a–f** and **11a–e** was as follows: To a solution of the 2-methylsulphanyl derivative in 1 mL of DMF 1.5 equiv of the aliphatic amine were added and stirred overnight at room temperature; the dark solution was poured into a 10 mL of a well stirred brine solution and left at room temperature overnight. The precipitate formed was filtered, dried by suction and treated with hot hexane. This procedure gave similar yields as method A.

7.4.1. 9-[[(3-Cyano)phenyl]amino]-2-[2-(*N*,*N*-diethylamino)] ethylaminothiazolo[5,4-*b*]quinoline (10a)

Pale yellow solid; (69%); mp 110–111 °C. IR (KBr, cm^{-1}) 3311 (NH), 2966, 2810 (CH); 2227 (CN); 1600, 1560, 1476 (aromatic);

^{1}H NMR ($CDCl_3$, δ): 1.02 (t, J = 7.2 Hz, 6H) 2-CH_3; 2.55 (q, J = 7.2 Hz, 4H) CH_2; 2.68 (t, J = 6.9 Hz, 2H) CH_2; 3.48 (q, J = 6.9 Hz, 2H) $NHCH_2$; 6.44 (br, 1H) -NH-; 7.00 (s, 1H) –NHAr; 7.04 (d, J = 8.1 Hz, 1H) H-6′; 7.08 (s, 1H) H-2′; 7.18 (d, J = 8.1 Hz, 1H) H-4; ′7.26 (t, J = 8.1, 1H) H-5′; 7.37 (ddd; J = 8.7, 6.9, 1.2 Hz; 1H) H-7; 7.60 (ddd; J = 8.7, 6.9, 1.5 Hz; 1H) H-6; 7.67 (d, J = 8.7 Hz, 1H) H-5; 8.03 (d, J = 8.7 Hz, 1H) H-8; MS (FAB, *m/z*): 417 (M^++1, 100%) Anal. Calcd for $C_{23}H_{24}N_6S$: C, 66.32; H, 5.81; N, 20.18; S, 7.70. Found: C, 66.17; H, 6.00; N, 20.96; S, 7.51.

7.4.2. 9-[[(3-Chloro)phenyl]amino]-2-[2-(*N*,*N*-diethylamino)] ethylaminothiazolo[5,4-*b*]quinoline (10b)

Yellow solid; (70%); mp 109–110 °C. IR (KBr, cm^{-1}) 3411 (NH), 2966, 2919, 2849 (CH); 1549, 1561, 1475 (aromatic); ^{1}H NMR (DMSO-d_6, δ): 1.10 (t, J = 7.2 Hz, 6H) 2-CH_3; 2.97 (q, J = 7.2 Hz, 4H) CH_2; 3.10 (t, J = 6.9 Hz, 2H) CH_2; 3.59 (q, J = 6.9 Hz, 2H) $NHCH_2$; 6.78 (d, J = 8.1 Hz, 1H) H-6′; 6.90 (d, J = 8.1 Hz, 1H) H-4′; 7.20 (t, J = 8.1, 1H) H-5′; 7.30 (s, 1H) H-2′; 7.51 (ddd; J = 8.7, 6.9, 1.2 Hz, 1H) H-7; 7.64 (ddd, J = 8.7, 6.9, 1.5 Hz, 1H) H-6; 7.90 (d, J = 8.7 Hz, 1H) H-5; 8.22 (d, J = 8.7 Hz, 1H) H-8; 8.97 (br, t, J = 6.9, 1H) –NH–; 9.22 (s, 1H) –NHAr–; MS (FAB, *m/z*): 426 (M^++1, 40%), 86 (M-339, 100%) Anal. Calcd for $C_{22}H_{24}ClN_5S$: C, 62.03; H, 5.68; N, 20.69; S, 7.49. Found: C, 62.17; H, 5.81; N, 20.52; S, 7.35.

7.4.3. 9-[[(3-Methoxy)phenyl]amino]-2-[2-(*N*,*N*-diethylamino)] ethylaminothiazolo[5,4-*b*]quinoline (10c)

Brownish solid; (50%); mp 118–120 °C. IR (KBr, cm^{-1}): 3120 (NH), 3053, 2966 2850 (CH), 1595, 1571, 1543, 1503, 1476, (aromatic), ^{1}H NMR (DMSO-d_6, δ): 0.88 (t, J = 7.2 Hz, 6H) 2-CH_3; 2.41 (q, J = 7.2 Hz, 4H) CH_2; 2.45 (t, J = 6.9 Hz, 2H) CH_2; 3.23 (q, J = 6.9 Hz, 2H) $NHCH_2$; 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, 1H) H-5; 8.53 (d, J = 8.4 Hz) H-8; 8.13 (br, t, J = 6.9, 1H) –NH–; 8.29 (s, 1H) –NHAr–; MS (EI, *m/z*): 421 (M^+, 15%), 322 (M-99, 80%), 86 (M-335, 100%) Anal. Calcd for $C_{23}H_{27}N_5OS$: C, 65.53; H, 6.46; N, 16.61; S, 7.61. Found: C, 65.41; H, 6.52; N, 16.67; S, 7.76.

7.4.4. 9-[[(4-Cyano)phenyl]amino]-2-[2-(*N*,*N*-diethylamino)] ethylaminothiazolo[5,4-*b*]quinoline (10d)

Pale yellow solid; (61%); mp 114–115 °C. IR (KBr, cm^{-1}) 3320 (NH); 2967, (CH) 2216 (CN), 1602, 1580, 1560, 1511 (aromatic); ^{1}H NMR (DMSO-d_6, δ):1.10 (t, J = 7.2 Hz, 6H) 2-CH_3; 2.97 (q, J = 7.2 Hz, 4H) CH_2; 3.10 (t, J = 6.9 Hz, 2H) CH_2; 3.59 (q, J = 6.9 Hz, 2H) $NHCH_2$; 6.84 (d, J = 8.4 Hz, 2H) H-2′, H-6′; 7.55 (m, 3H) H-3′, H-5′, H-7; 7.64 (ddd, J = 7.2, 6.9, 1.2 Hz, 1H) H-6; 7.91 (d, J = 7.8 Hz, 1H) H-5; 8.04 (d, J = 8.4 Hz, 1H) H-8; 8.50 (br, 1H) –NH– 9.3 (s, 1H) –NH– MS (FAB, *m/z*) 430 (M^++1, 50%) 86 (M-344, 100%) Anal. Calcd for $C_{23}H_{24}N_6S$: C, 66.32; H, 5.81; N, 20.18; S, 7.70. Found: C, 66.47; H, 5.93; N, 20.10; S, 7.50.

7.4.5. 9-[[(4-Chloro)phenyl]amino]-2-[2-(*N*,*N*-diethylamino)] ethylaminothiazolo[5,4-*b*]quinoline (10e)

Yellow solid; (63%); mp 113–114 °C. IR (KBr, cm^{-1}) 3410 (NH), 2965, 2809, (CH); 1600, 1586, 1566 (aromatic); ^{1}H NMR (DMSO-d_6, δ): 1.08 (t, J = 7.2 Hz, 6H) 2-CH_3; 2.94 (q, J = 7.2 Hz, 4H) CH_2; 3.08 (t, J = 6.9 Hz, 2H) CH_2; 3.58 (q, J = 6.9 Hz, 2H) $NHCH_2$; 6.82 (d, J = 8.7 Hz, 2H) H-2′, H-6′; 7.21 (d, J = 8.7 Hz, 2H) H-3′, H-5′; 7.48 (ddd, J = 8.7, 6.9, 1.2 Hz, 1H) H-7; 7.60 (ddd, J = 8.7, 6.9, 1.5 Hz, 1H) H-6; 7.88 (d, J = 8.7 Hz, 1H) H-5; 8.16 (d, J = 8.7 Hz, 1H) H-8; 8.4 (br, t, J = 6.9, 1H) –NH–; 8.91 (s, 1H) –NHAr–; MS (FAB, *m/z*): 426 (M^++1, 40%), 86 (M-339, 100%) Anal. Calcd for $C_{22}H_{24}ClN_5S$: C, 62.03; H, 5.68; N, 20.69; S, 7.49. Found: C, 62.21; H, 5.73; N, 20.64; S, 7.30.

7.4.6. 9-[[(4-Methoxy)phenyl]amino]-2-[2-(*N*,*N*-diethylamino)] ethylaminothiazolo[5,4-*b*]quinoline (10f)

Yellow solid; (65%); mp 110–112 °C. IR (KBr, cm^{-1}) 3179 (NH) 2966, 2827, (CH); 1599, 1563, 1508, 1495, 1469 (aromatic). 1236 (OCH_3); ^{1}H NMR (DMSO-d_6, δ): 0.88 (t, J = 7.2 Hz, 6H) 2-CH_3; 2.41 (q, J = 7.2 Hz, 4H) CH_2; 2.45 (t, J = 6.9 Hz, 2H) CH_2; 3.23 (q, J = 6.9 Hz, 2H) $NHCH_2$; 3.68 (s, 3H) OCH_3; 6.80 (m, 4H) H-2', H-6′, H-3′, H-5′; 7.36 (ddd, J = 8.7, 6.9, 0.9 Hz, 1H) H-7; 7.52 (ddd, J = 8.7, 6.9, 0.9 Hz, 1H) H-6; 7.79 (dd, J = 8.7, 0.9 Hz, 1H) H-5; 8.06 (dd, J = 8.7, 0.9 Hz, 1H) H-8; 8.13 (br, t, J = 6.9, 1H) –NH–; 8.29 (s, 1H) –NHAr–; MS (EI, *m/z*): 421 (M^+, 15%), 322 (M-99, 80%), 86 (M-335, 100%) Anal. Calcd for $C_{23}H_{27}N_5OS$: C, 65.53; H, 6.46; N, 16.61; S, 7.61. Found: C, 65.66; H, 6.49; N, 16.59; S, 7.47.

7.4.7. 9-(Phenylamino)-2-[3-(*N*,*N*-diethylamino)]propylamino-thiazolo[5,4-*b*]quinoline (11a)

Yellow solid; 62 mg (59%); mp 72–75 °C. IR (KBr, cm^{-1}) 3233, 1562, 1493 (–NH–); 2967,2818 (CH); 1597 (aromatic). ^{1}H NMR (DMSO-d_6, δ): 0.9 (t, J = 7.2 Hz, 6H) 2-CH_3; 1.6 (q, J = 7.0 Hz, 2H) –CH_2–; 2.4 (m, 6H) 3-CH_2–; 3.2 (sa, 2H) –CH_2–; 6.8 (d, J = 7.2 Hz, 3H) H-2′, H-4′, H-6'; 7.1 (ddd, J = 8.4,6.9,1.8 Hz, 2H) H-3′, H-5′; 7.4 (ddd, J = 8.4,6.9,1.5 Hz, 1H) H-7; 7.5 (ddd, J = 8.4,6.9,1.5 Hz, 1H) H-6; 7.9 (dd, J = 8.7,0.9 Hz, 1H) H-5; 8.0 (dd, J = 8.7,0.9 Hz, 1H) H-8; 8.3 (t, J = 5.4 Hz, 1H) –NH–; 8.5(s, 1H) –NH– MS (EI, *m/z*) 405 (M^+ 100%); 376 (M^+-29, 15%); 319 (M^+-89, 56%); 306 (M^+-99, 53%); Anal. Calcd for $C_{23}H_{27}N_5S$: C, 68.11; H, 6.71; N, 17.27; S, 7.91. Found: C, 68.15; H, 6.67; N, 17.31; S, 7.87.

7.4.8. 9-[[(3-Cyano)phenyl]amino]-2-[3-(*N*,*N*-diethylamino)] propylaminothiazolo[5,4-*b*]quinoline (11b)

Yellow solid; 68 mg (57%); mp 73–76 °C. IR (KBr, cm^{-1}) 3209 (–NH–); 2960, 2815 (CH), 2277 (CN); 1599, 1562,1494 (aromatic); ^{1}H NMR (DMSO-d_6, δ): 0.9 (t, J = 7.2 Hz, 3H) 2-CH_3; 1.6 (q, J = 7.2 Hz; 2H) –CH_2–; 2.4 (m, 6H) 3-CH_2–; 3.2 (q, J = 7.2 Hz, 2H) –CH_2–; 7.1 (d, J = 7.5 Hz, 2H) H-2′, H-6′; 7.2 (d, J = 7.5 Hz, 1H) H-4′; 7.3 (ddd, J = 8.4, 7.8, 0.9 Hz, 1H) H-5′; 7.5 (ddd, J = 8.1,6.9,1.2 Hz, 1H) H-7; 7.6 (ddd, J = 8.4,6.9,1.2 Hz, 1H) H-6; 7.9 (d, J = 7.5 Hz, 1H) H-5; 8.1 (d, J = 7.8 Hz, 1H) H-8; 8.5 (br) –NH–; 9.0 (br) –NH– MS (EI, *m/z*) 430 (M^+ 100%); 401 (M^+-29, 12%); 344(M^+-86, 30%); 330 (M^+-100, 27%). Anal. Calcd for $C_{24}H_{26}N_6S$: C, 66.95; H, 6.09; N, 19.52; S, 7.45. Found: C, 66.74; H, 6.39; N, 19.33; S, 7.54.

7.4.9. 9-[[(3-Chloro)phenyl]amino]-2-[3-(*N*,*N*-diethylamino)] propylaminothiazolo[5,4-*b*]quinoline (11c)

Yellow solid; 45 mg (55.0%); mp 55–58 °C. IR (KBr, cm^{-1}) 2966, 1375 (–CH); 1594, 1561, 1497 (aromatic); ^{1}H NMR (DMSO-d_6, δ): 0.9 (t, J = 7.2 Hz, 6H) 3-CH_3; 1.6 (q, J = 6.9 Hz, 2H) –CH_2–; 2.4 (m, 6H) 3-CH_2–; 3.3 (c, J = 6.6 Hz, 2H) –CH_2–; 6.7 (d, J = 9.0 Hz, 1H) H-6′; 6.8 (d, J = 6.3 Hz, 2H) H-2′, H-4′; 7.1 (t, J = 8.1 Hz, 1H) H-5′; 7.5 (t, J = 7.5 Hz, 1H) H-7; 7.6 (t, J = 7.5 Hz, 1H) H-6; 7.9 (t, J = 8.4 Hz, 1H) H-5; 8.1 (d, J = 8.4 Hz, 1H) H-8; 8.4 (br) –NH–; 8.8 (s, 1H) –NH– MS (EI, *m/z*) 438 (M^+, 100%); 410 (M^+-29, 8%); 353 (M^+-57, 28%). Anal. Calcd for $C_{23}H_{26}ClN_5S$: C, 62.78; H, 5.96; N, 15.92; S, 7.29. Found: C, 62.78; H, 5.85; N, 15.81; S, 7.29.

7.4.10. 9-[[(4-Cyano)phenyl]amino]-2-[3-(*N*,*N*-diethylamino)] propylaminothiazolo[5,4-*b*]quinoline (11d)

Yellow solid; 92 mg (71%); mp 75–78 °C IR (KBr, cm^{-1}) 3316 (R–NH–R); 2960, 1466, 1378 (CH); 2217 (CN); 1603, 1561, 1512, 1466 (aromatic); ^{1}H NMR (DMSO-d_6, δ):0.9 (t, J = 7.2 Hz, 6H) 2-CH_3; 1.6 (q, J = 6.9 Hz, 2H) –CH_2–; 2.4 (m, 6H) 3-CH_2–; 3.2 (sa, 2H) –CH_2–; 6.8 (d, J = 8.4 Hz, 2H) H-2', H-6′; 7.5 (d, J = 8.7 Hz, 3H) H-3′, H-5′, H-7; 7.6 (ddd; J = 7.2, 6.9, 1.2 Hz, 1H) H-6; 7.9 (d, J = 7.8 Hz, 1H) H-5; 8.0 (d, J = 8.4 Hz, 1H) H-8; 8.5 (br, 1H) –NH– 9.3 (s, 1H) –NH– MS (EI, *m/z*) 430 (M^+, 71%) 401 (M^+-29, 9%) 344 (M^+-57,

31%); 330 (M^+-14, 31%) Anal. Calcd for $C_{24}H_{26}N_6S$: C, 66.95; H, 6.09; N, 19.52; S, 7.45. Found: C, 66.84; H, 6.28; N, 19.44; S, 7.44.

7.4.11. 9-[[(4-Chloro)phenyl]amino]-2-[3-(*N*,*N*-diethylamino)] propylaminothiazolo[5,4-*b*]quinoline (11e)

Yellow solid; 75 mg (61%); mp 120–123 °C IR (KBr, cm^{-1}) 2965, 2809, (CH); 1600, 1586, 1566 (aromatic); ^1H NMR (DMSO-d_6, δ): 0.92 (t, J = 7.2 Hz, 6H) 2-CH_3; 1.6 (q, J = 6.9 Hz, 2H) –CH_2–; 2.4 (m, 6H) 3-CH_2–; 3.2 (c, J = 6.6 Hz, 2H) –CH_2–; 6.8 (d, J = 8.7 Hz, 2H) H-2', H-6′; 7.1 (d, J = 8.7 Hz, 2H) H-3′, H-5′; 7.4 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H) H-7; 7.6 (ddd, J = 8.1, 6.9, 1.5 Hz, 1H) H-6; 7.9 (d, J = 7.5 Hz, 1H) H-5; 8.1 (d, J = 7.8 Hz, 1H) H-8; 8.4 (br, 1H) –NH–; 8.7 (s, 1H) –NH–; MS (EI, m/z): 439 (M^+, 75%) 410 (M^+-29, 11%); 367 (M^+-43, 13%); 353 (M^+-14, 40%). Anal. Calcd for $C_{23}H_{26}ClN_5S$: C, 62.78; H, 5.96; N, 15.92; S, 7.29. Found: C, 62.73; H, 5.90; N, 15.86; S, 7.34.

7.4.12. 9-Phenylamino-2-(1-piperidinyl)thiazolo[5,4-*b*]quinoline (8d)

Yellow solid; (84%); mp 200–203 °C (methanol). IR (KBr, cm^{-1}) 3440 (NH), 3223, 2953, 2851 (C–H); 1600, 1494 (aromatic); ^1H NMR ($CDCl_3$, δ): 1.71 (s, 6H) H-3a, H-4a, H-6a; 3.64 (s, 4H) H-2a, H-5a, 7.02 (d, J = 8 Hz, 2H) H-2′, H-6′; 7.07 (t, J = 7.2 Hz, 1H) H-7; 7.24 (m, 3H) H-3′, H-4', H-5′; 7.54 (td, J = 7.6, 1.2 Hz, 1H) H-6; 7.68 (dd, J = 8.4, 0.8 Hz, 1H) H-5; 8.04 (d, J = 8.4 Hz, 1H) H-8; MS (EI, m/z): 390 (M^+, 100%) Anal. Calcd for $C_{21}H_{20}N_4S$: C, 69.97; H, 5.59; N, 15.54; S, 8.90. Found: C, 69.78; H, 5.53; N, 15.76; S, 8.93.

7.4.13. 9-Phenylamino-2-[1-[(4-methyl)piperazinyl]]thiazolo[5,4-*b*]quinoline (8e)

Pale yellow solid; (97%); mp 150–152 °C (methanol). IR (KBr, cm^{-1}): 3374 (NH), 2936, 2847, 2799 (CH), 1599, 1494, (aromatic); ^1H NMR ($CDCl_3$, δ): 2.37 (s, 3H) NCH_3; 2.55 (t, J = 4.8 Hz, 4H) H-3a, H-5a; 3.71 (t, J = 5.2 Hz, 4H), H-2a, H-6a; 6.94 (t, J = 7.6, 2H) H-2′, H-6′; 6.99 (t, J = 7.2 Hz, 1H) H-7; 7.24 (m, 3H) H-3', H-4′, H-5′; 7.53 (td, J = 7.6, 1.6 Hz, 1H) H-6; 7.71 (dd, J = 8.8, 1.8 Hz, 1H) H-5; 7.97 (dd, J = 8.4, 0.8 Hz, 1H) H-8, MS (EI, m/z): 375 (M^+, 30%), 70 (100%) Anal. Calcd for $C_{21}H_{21}N_5S$: C, 67.17; H, 5.64; N, 18.65; S, 8.54. Found: C, 67.34; H, 5.87; N, 18.42; S, 8.31.

7.4.14. 9-Phenylamino-2-(1-morphonyl)thiazolo[5,4-*b*]quinoline (8f)

Pale yellow solid; (80%); mp 205–208 °C (methanol–water). IR (KBr, cm^{-1}): 3330(NH) 2956, 2904, 2859 (CH), 1600, 1548, (aromatic); 1318 (C–O) ^1H NMR ($CDCl_3$, δ): 3.66 (t, J = 4.4 Hz, 4H) H-2a, H-6a; 3.83 (t, J = 4.4 Hz, 4H) H-3a, H-5a; 7.05 (d, J = 8 Hz, 2H) H-2′, H-6'; 7.11 (t, J = 7.6, 1H) H-7; 7.28 (m, 3H) H-3′, H-4′, H-5′; 7.40 (sa, 1H) NH; 7.57 (td, J = 7.6, 1.2 Hz, 1H) H-6; 7.71 (dd, J = 8.8, 1.2 Hz, 1H) H-5; 8.06 (d, J = 8.4 Hz, 1H) H-8. MS (EI, m/z): 362 (M^+, 100%) Anal. Calcd for $C_{20}H_{18}N_4OS$: C, 66.28; H, 5.01; N, 15.46; S, 8.85. Found: C, 66.53; H, 5.23; N, 15.24; S, 8.60.

7.5. Cytotoxic assay[23]

The effects of the compounds were determined in one cervical cell line (HeLa), two human colorectal cancer cell lines (SW480 and SW620) and one myelogenous leukemia human cell line (K-562). The cytotoxic assays were carried out according to the microculture MTT method. Cells were harvested at 4.5 to 5.0 × 104 cells/mL/well and inoculated in 24 well microtiter plates. The culture cells were then 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) were added. 1 mL of DMSO was added 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 DUR-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.

7.6. Decatenation assay for topoisomerase II activity[24]

DNA topoisomerase activity was monitored with the κDNA decatenation assay using human DNA topoisomerase II, and the DNA topoisomerase II determination kit from Topogen™. Briefly, κDNA from *Crithidia fasciculata,* at a concentration of 5 μg/mL, was incubated in 30 mM Tris–HCl, pH 7.6 containing 60 mM NaCl, 8 mM $MgCl_2$, 15 mM 2-mercaptoethanol, 3 mM ATP, 4 units of human DNA topoisomerase II, 8% (v/v) dimethyl sulphoxide, and with or without the indicated concentration of the inhibitor in a final volume of 20 μL. Reactions were allowed to proceed for 30 min at 37 °C, terminated by the addition of 2 μL of 10% SDS and treated with proteinase K for 15 min at 37 °C. The samples were mixed with 2.2 μL of loading dye (0.025% w/v bromophenol blue in 50% glycerol) and extracted with 25 μL of chloroform/isoamyl alcohol 24:1. Phase separation was promoted by rapid centrifugation in a microfuge, and the DNA in the aqueous layer was analyzed by electrophoresis in a standard agarose gel with 0.5 μg/mL of ethidium bromide. The gel was destained for 30 min and photographed under UV light using a Bio-Rad FlourS™ imaging system. The relative amounts of decatenated κDNA were measured using the Bio-Rad's Quantity-one™ software or the ImageJ software.[25] Both programs essentially rendered the same results. The activity was calculated as the density of the circular-decatenated plus linear DNA bands as a percentage of the total DNA. Activity in a control reaction, in the absence of inhibitors, was taken as 100%.

7.7. Molecular modeling procedure

All calculations were performed with SPARTAN'04 software.[16] The molecules were built by assembling standard fragments, and the resulting geometries were optimized by molecular mechanics. Conformational analysis of the compounds by Systematic Search protocol around rotable bonds was performed using the MMFF94 force field. The most frequent conformer for each compound was selected and geometry optimization and calculation of their electronic properties were carried out with semiempirical AM1 method.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.03.052.

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