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Original article

Synthesis and evaluation of 9-anilinothiazolo[5,4-b]quinoline derivatives as potential antitumorals

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Abstract

Five new 9-anilinothiazolo[5,4-b]quinoline derivatives (compounds **5, 7, 9, 10, 11**) have been prepared. Some of the compounds were prepared by coupling properly substituted anilines to the novel compound 9-chloro-2-(methylthio)thiazolo[5,4-b]quinoline. Of these, compound **7** (9-anilino-2-[[2-(*N,N*-diethylamino)ethyl]amino]thiazolo[5,4-b]quinoline) showed the best cytotoxic activity in several cell lines. All compounds demonstrated DNA binding in nanomolar range. Compound **7** inhibited the ^{14}C-thymidine incorporation into DNA. Results indicate that these derivatives deserve more considerations as potential antitumoral drugs.

Keywords: 9-anilinothiazolo[5,4-b]quinoline derivatives; Antitumoral; DNA intercalation; Cytotoxic activity

1. Introduction

Several tricyclic compounds have been studied in the search of better antitumoral agents. Particularly, 9-aminoacridines, 9-arylacridines, 9-alkylaminoacridines or 9-arylaminoacridines, with or without substituents at positions 3, 4, or/and 5, have been characterized extensively [1–4]. The activity of these compounds seems to be due to their interaction with DNA. One example is amsacrine **1** (*N*-[4-(9-acridylamino)-3-methoxy-phenyl]methanesulphonamide, *m*-AMSA), a potent antileukemic drug and a moderate DNA-intercalating agent [5,6] (Fig. 1).

Recently, a new approach to this kind of compounds was reported by Alvarez-Ibarra et al. [7] where benzene moiety in these acridines was isosterically replaced with thiazole. Several 9-hydroxy or 9-alkylaminothiazolo[5,4-b]quinoline derivatives were synthesized and evaluated in three cell lines (P-388, A-549, Ht-29) demonstrating good cytotoxic activity. This resulted in the formation of a new class of potential antitumor agents.

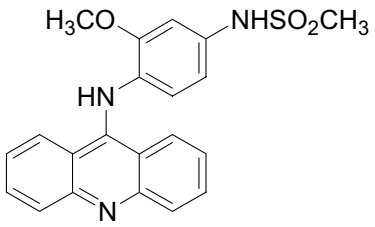

Fig. 1. *m*-AMSA structure **1**.

The present study describes the synthesis, the preliminary antitumoral evaluation, and the intercalating properties of thiazolo[5,4-b]quinoline derivatives that combine the general substitution pattern of acridine derivatives with thiazolo[5,4-b]quinoline template.

2. Chemistry

The synthesis of the novel compounds is outlined in Fig. 2. The preparation of **7** is based on the methodology used

Abbreviations: Ac_2O, acetic anhydride; AcOH, acetic acid; DMSO, dimethyl sulfoxide; EtOH, ethanol; H_2O_2, hydrogen peroxide; $KMnO_4$, potassium permanganate; KOH, potassium hydroxide; MeOH, methanol; Na_2SO_4, sodium sulfate; $NaHCO_3$, sodium bicarbonate; $POCl_3$, phosphorus oxychloride; PPA, polyphosphoric acid; Py, pyridine; $SOCl_2$, thionyl chloride.

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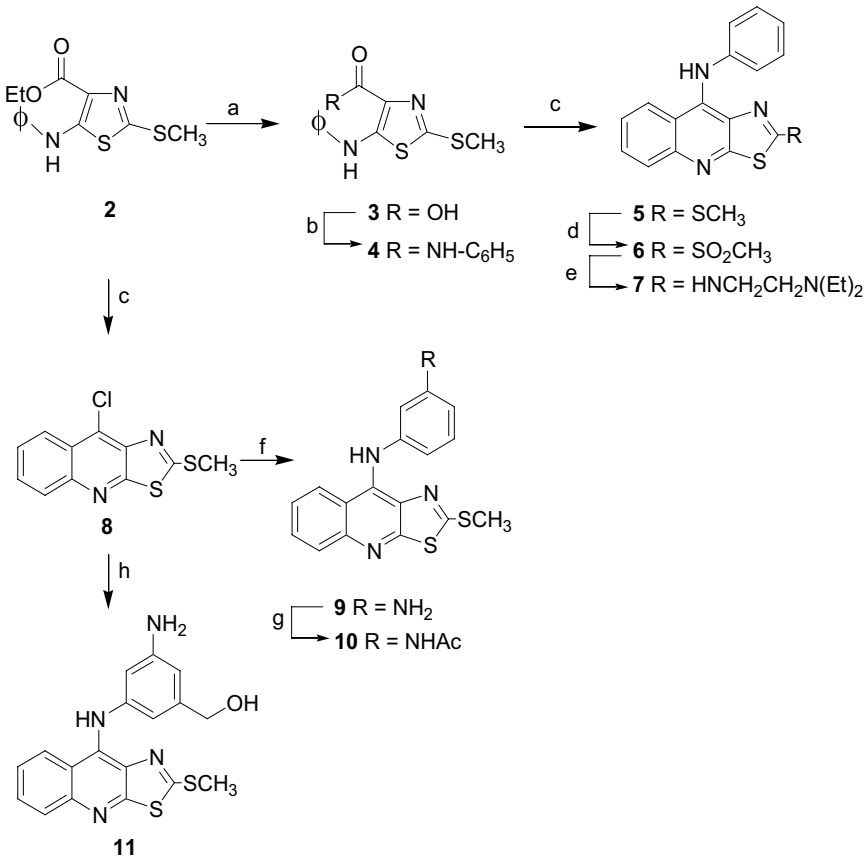


Fig. 2. (a) KOH/EtOH; (b) (1) $SOCl_2$/Py/C_6H_6, (2) aniline/C_6H_6; (c) $POCl_3$/PPA; (d) H_2O_2/AcOH; (e) $H_2NCH_2CH_2N(Et)_2$; (f) HCl/MeOH/*m*-phenylendiamine; (g) Ac_2O/Py; (h) HCl/MeOH/3,5-diaminobenzyl alcohol. All compounds were characterized by mp, spectroscopic data (IR, ^{1}H NMR, MS) and elemental analysis.

by Alvarez-Ibarra et al. [7], with some modifications. The sequence of reactions started from compound **2**. Basic hydrolysis of **2** afforded the carboxylic acid derivative **3**, which was treated with $SOCl_2$ and the acid chloride formed was treated in situ with aniline to give compound **4**. Cyclization of the amide **4** to thiazolo[5,4-b]quinoline derivative **5** was achieved by the treatment of **4** with $POCl_3$/PPA. When we tried to oxidize compound **5** with $KMnO_4$/AcOH, according to a reported procedure, we obtained the starting material. The desired compound was obtained in good yield by making react **5** with H_2O_2 in AcOH. The incorporation of the *N,N*-diethylethylendiamine group at position 2 of thiazolo[5,4-b]quinoline ring was achieved in a moderate yield. All attempts to increase this yield were unsuccessful. Alvarez-Ibarra et al. [7] reported quantitative yields for the same reaction. We presume that the anilino group at position 9 exerts some influence on this reaction. On the other hand, the preparation of the other 9-anilinothiazolo[5,4-b]quinoline derivatives was carried out by using a different approach. Thus, to prepare **9**, novel compound **8** (prepared by treating **2** with $POCl_3$/PPA at 136 °C for 4 h) and *m*-phenylendiamine were heated under reflux for 8 h, under acid catalysis. Several attempts under neutral or basic conditions were unsuccessful. Following this procedure, but using 3,5-diaminobenzyl alcohol instead, compound **11** was prepared. The compound **10** was obtained by treating **9** with Ac_2O/Py mixture at 25 °C.

3. Biological activity

3.1. Cells and cytotoxic assay

Cytotoxic assay and ^{14}C-thymidine incorporation into DNA were carried out using a reported procedure [8]. The results are shown in Tables 1 and 2, respectively.

3.2. Ethidium displacement

DNA intercalation was determined from the displacement of ethidium bromide from DNA [9]. The results are shown in Table 3.

4. Results and discussion

We selected compounds **12** and **13** as templates for this study. 9-Anilinoacridine **12** is a DNA intercalating agent [10] but it does not have antitumor activity. Compound **13** (AHMA) exhibits potent antitumor activity against L-1210 and HL-60 and various murine tumors both in vitro and in vivo [11] as well as intercalating properties (Fig. 3).

Compounds **14** and **15**, also used as templates, are reported by Alvarez-Ibarra et al. [7] as good antitumoral agents against P-388, A-549 and HT-29 cell lines. The intercalating

Table 1
Cytotoxic activity of compounds **5, 7, 9–11** and the reference compound *m*-AMSA in human cancer cell lines [a,b]

Compounds	MCF-7	HeLa	Calo	C-33	SW480	SW620	CHO	K-562
5	>200	>200	198.3 (15.8)	153.7 (7.7)	>200	>200	>200	>200
7	16.6 (2.7)	15.96 (0.3)	29.9 (1.5)	22.4 (4.2)	37.7 (0.8)	21.6 (1.8)	30.6 (11.1)	16.8 (0.5)
9	74.3 (19.2)	176.5 (30.3)	128.1 (25.4)	138.8 (23.9)	>200	>200	136.8 (18.2)	143.4 (11.5)
10	132.3 (28.5)	>200	>200	>200	>200	153.9 (28.6)	58.8 (2.7)	85.3 (10.2)
11	138.5 (23.4)	>200	106.4 (2.1)	>200	>200	183.9 (69.9)	111.4 (6.5)	143.4 (6.9)
m-AMSA	4.1 (1.2)	9.5 (0.6)	18.3 (1.5)	8.8 (0.5)	27.7 (2.0)	16.7 (2.8)	9.3 (2.7)	19.9 (0.8)

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

properties of these compounds have not been reported yet (Fig. 4).

On the basis of our hypothesis given above, we propose that compounds **5, 7, 9–11** (Fig. 2) should differ in their cytotoxic and intercalating properties, because the anilino group at position 9 enhances DNA intercalation, as it inserts itself on to the minor groove. The various substituents were chosen to modify the intercalation and the antitumoral properties. The effect of a diethylethylendiamine substituent at position 2 was also studied, because compound **15** is 12-fold more active than compound **14**.

At the present time, we have evaluated the cytotoxic activity of compounds **5, 7, 9–11**. The results, listed in the Table 1, show that compound **7** was more potent than compounds **5, 9–11** in all the cell lines.

We assayed ^{14}C-thymidine incorporation into DNA in the cancer cell lines to evaluate whether there is inhibition of cell growth (at the DNA synthesis level) by compound **7**. The results (Table 2) indicate that compound **7** inhibits DNA replication.

Comparison of cytotoxic activity data between compound **5** and **9** showed that the incorporation of an amino group to the anilino ring increased the activity and, in some cases, the additional incorporation of hydroxymethyl group showed enhanced activity. On the other hand, the acyl derivative of compound **9**, compound **10**, showed lower activity on some cell lines. It appears that the amino group plays significant role in enhancing the cytotoxic properties of the compounds.

The ethidium bromide displacement assay was also carried out to evaluate the DNA-binding affinity of compounds **5, 7, 9–11** (Table 3). All compounds were able to displace ethidium bromide from DNA.

The results are consistent with compound **7** binding to more sites than compound **9**, which suggests that compound **9** binds to a specific subset of DNA sites, while compound **7** may have a broader specificity. The higher specificity of compound **9** is accompanied by a higher affinity for these sites. In this case, it appears that the presence of the amino group increases the affinity while the acyl derivative, compound **7**, has lower affinity suggesting that the amino group favors binding.

5. Conclusions

The results indicated that compound **9** formed the tightest intercalation complex, but this did not correlate with its cytotoxic activity. On the other hand, compound **7** formed the weakest and most extensive complexes with DNA being highly cytotoxic. A similar trend has been observed in several studies. For instance, trying to establish a correlation between DNA-drug binding and cytotoxicity, it was observed that *m*-AMSA showed the weakest intercalation complexes, but its cytotoxic activity was higher than the other compounds. On the contrary, *o*-AMSA showed a higher affinity for DNA than *m*-AMSA but the former was inactive as a cytotoxic agent [12–14]. We know that the real target of many tricyclic compounds is the topoisomerase II and the intercalating ability of many compounds is not reflective of good antitumoral property. Further studies are required to propose a plausible action mechanism. Our current efforts are directed toward improving the antitumoral properties. We propose that 9-anilinothiazolo[5,4-b]quinoline derivatives are potential antitumorals.

6. Experimental protocols

6.1. Chemistry

Melting points were determined on a Fisher-Jones apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet FT-5SX spectrophotometer model. ^{1}H NMR spec-

Table 2
Inhibition of ^{14}C-thymidine incorporation into DNA by compound **7** and *m*-AMSA in human cancer cell lines

Cell line	MCF-7	HeLa	Calo	C-33	SW-480	SW-620	CHO	K-562
Compound **7**	83.14 (3.24)	78.84 (0.48)	45.64 (5.25)	44.43 (9.04)	55.63 (2.96)	59.35 (9.30)	80.44 (9.38)	73.28 (14.58)
m-AMSA	84.91 (3.13)	73.58 (6.70)	62.67 (7.39)	74.56 (11.36)	81.49 (5.20)	60.93 (16.79)	73.7 (6.81)	76.8 (9.38)

[a] Concentration of each compound (20 μM) in each experiment. The results are expressed as % of the inhibition of ^{14}C-thymidine incorporation in DNA in relation to the control (without compound).
[b] Values are means of three experiments, standard deviation is given in parentheses.

Table 3
Apparent constant of ethidium bromide displacement from DNA by compounds **5, 7, 9–11**

Compounds	Q [a]	Q_{max} [b]
5	13.62 (4.64)	0.2987 (0.03)
7	52.46 (14.08)	1.7428 (0.1769)
9	6.96 (2.095)	0.5861 (0.045)
10	18.447 (8.97)	1.0781 (0.1982)
11	22.64 (6.207)	1.2804 (0.1418)

[a] Concentration to give 50% quenching of fluorescence of bound ethidium (nM). Values are means of three experiments, standard deviation is given in parentheses.
[b] Maximum quenching.

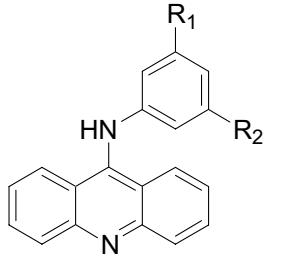
tra were recorded on a Varian VxR (300 MHz). Chemical shifts are reported in ppm (δ) and the signals are described as singlet (s), doublet (d), triplet (t), quartet (q) broad (br), broad singlet (br s) and multiplet (m). Coupling constants are reported in Hz. EI-MS was done on a JEOL JMS-AX505-HA apparatus. CI- and FAB-MS were done on a JEOL Sx102 apparatus. Silica gel plates ($Merck_{254}$) were used for analytical chromatography. Solvents and reagents were purchased from the commercial vendors in the appropriate grade and were used without further purification.

*6.1.1. 4-(Ethoxycarbonyl)-2-(methylthio)-5-(phenylamino) thiazole **2***

The compound **2** was prepared according to a procedure already described [7].

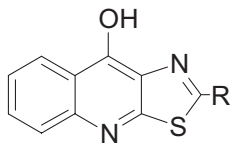
*6.1.2. 4-Carboxy-2-(methylthio)-5-(phenylamino)thiazole **3***

To a solution of compound **2** (4.3 g, 14.62 mmol) in EtOH (43 ml) was added an KOH aqueous solution (2.45 g, 3.5 ml).



12 $R_1 = R_2 = H$
13 $R_1 = NH_2$ $R_2 = CH_2OH$

Fig. 3. 9-Anilinoacridine structure **12**, AHMA structure **13**.



14 R = SCH_3
15 R = $NHCH_2CH_2N(CH_2CH_3)_2$

Fig. 4. 9-hydroxy-2-(methylthio)thiazolo[5,4-b]quinoline structure **14**, 2-[[2-(*N,N*-diethylamino)ethyl]amino]-9-hydroxy-thiazolo[5,4-b]quinoline structure **15**.

The reaction mixture was heated under reflux for 30 min. Normal work up after acidification, isolation and crystallization from EtOH afforded **3** as pink crystals. Yield: 3.51 g (90.23%). m.p.: 142–144 °C; IR (KBr): 3428, 3258, 3018, 1638, 1550, 1232 cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.7 (br s; 1H); 9.6 (s; 1H, D_2O exchangeable); 7.3 (dd; 2H, J = 7.5, 7.8 Hz); 7.2 (d; 2H; J = 8 Hz); 7.1 (dd; 1H, J = 7.5, 7.2 Hz); 2.6 (s; 3H); MS (EI): *m/z* 267 (M^++1, 21%); 266 (M^+, 56%); 221 (M^+-45, 100%).

*6.1.3. 4-(Phenylcarbamoyl)-2-(methylthio)-5-(phenylamino) thiazole **4***

Obtained as greenish crystals from **3** (3.03 g, 11.4 mmol) according to a procedure already described [7]. Yield: 2.98 g (76.8%). m.p.: 99–102 °C.; IR (KBr): 3364, 1646, 1594, 1560, 1526 cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.3 (s; 1H); 8.8 (s; 1H); 7.65 (dd; 2H, J = 9.0, 1.2 Hz); 7.3 (m; 4H); 7.2 (dd; 2H, J = 8.7, 1.0 Hz); 7.1 (t, 2H, J = 5 Hz); 2.7 (s; 3H); MS (EI): *m/z* 341 (M^+,100%); 248 (M^+-93, 25%); 215 (M^+-126, 58%).

*6.1.4. 9-Anilino-2-(methylthio)thiazolo[5,4-b]quinoline **5***

To compound **4** (1 g, 2.9 mmol) were added PPA (213 mg) and $POCl_3$ (0.9 ml). The reaction mixture was vigorously stirred at 130–135 °C for 4 h. The reaction mixture was cooled at room temperature, 1 ml of EtOH was carefully added and the mixture was concentrated at reduced pressure. The suspension was poured into water (20 ml) and neutralized with a saturated solution of $NaHCO_3$. The yellow–orange precipitate formed was collected and dried by vacuum filtration. The crude was washed several times with acetone to afford **5** as a dark yellow solid. Yield: 1 g (98.9%). m.p.: 226–229 °C; IR (KBr): 3353, 3243-2711, 1593, 1549, 1415 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.4 (d; 1H, J = 8.4 Hz); 7.9 (dd; 1H, J = 8.4, 0.9 Hz); 7.7 (dd; 1H, J = 8.4, 1.5 Hz); 7.5 (td; 1H, J = 7.8,1.5 Hz); 2.3 (s; 3H) thiazolo[5,4-b]quinoline protons; 10.5 (s,1H); 7.3 (dd; 1H, J = 7.8, 7.2 Hz); 7.1 (d; 2H, J = 7.2 Hz); 7.0 (dd; 1H, J = 7.5, 7.2 Hz) anilino ring protons; MS (CI): *m/z* 324 (M^++1, 100%); 323 (M^+, 83%).

*6.1.5. 9-Anilino-2-(methylsulphonyl)thiazolo[5,4-b]quinoline **6***

To a solution of **5** (200 mg) in glacial AcOH (6 ml) was slowly added 30% H_2O_2 solution (6 ml). The suspension was stirred at 30 °C for 3 h. After this time the reaction mixture was stirred overnight at 25 °C. The yellow solution was then poured into stirred cold water (50 ml). The yellow precipitate was collected, washed with cold water and dried by vacuum filtration to afford **6** as a light yellow solid. Yield: 180 mg (82.2%); m.p.: 212–214 °C; IR (KBr): 3373, 2921, 1577, 1313, 1140 cm^{-1}; ^1H NMR (Me_2CO-d_6): δ 8.0 (dd; 1H, J = 8.4, 0.6 Hz); 8.3 (dd; 1H, J = 8.7, 0.9 Hz); 7.8 (ddd; 1H, J = 9.0, 8.4, 1.2 Hz); 7.5 (ddd; 1H, J = 9.0, 8.1, 1.2 Hz) thiazolo[5,4-b]quinoline ring protons; 9.2 (br s; 1H); 7.3 (m; 3H); 7.2 (t; 2H, J = 6.9 Hz) anilino ring protons; 3.3 (s; 3H); MS (EI): *m/z* 355 (M^+, 100%).

*6.1.6. 9-Anilino-2-[[2-(*N,N-*diethylamino)ethyl]amino]thiazolo[5,4-b]quinoline **7***

To compound **6** (0.177 g, 0.5 mmol) was added *N,N*-diethylethylendiamine (0.214 ml, 3 mmol). The suspension was stirred at 140 °C for 20 min. The reaction mixture was then cooled to room temperature. $CHCl_3$ (30 ml) was then added. The $CHCl_3$ solution was successively extracted with 1 N NaOH solution (3 × 10 ml), NH_4Cl saturated solution (3 × 10 ml) and brine (3 × 10 ml), and dried over Na_2SO_4. The organic phase, after filtration, was evaporated under reduced pressure to a small volume. The precipitate formed was collected and dried under vacuum filtration to afford **7** as a white solid. Yield: 215 mg (55%); m.p.: 253–256 °C (dec); IR (KBr): 3211, 3003-2596, 1588-1519 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.5 (s; 1H); 8.2 (s; 1H); 8.0 (d; 1H, J = 9 Hz); 7.8 (d; 1H, J = 9.3 Hz); 7.5 (dd; 1H, J = 9.0, 6.0 Hz); 7.4 (dd; 1H, J = 9.0, 6.0 Hz); 7.1 (dd; 1H, J = 8.4, 7.2 Hz); 6.8 (m; 3H); 3.2 (m; 2H); 2.5 (dd, 2H, J = 7.2, 6.3 Hz); 2.3 (q; 4H; J = 7.2 Hz); 0.8 (t; 6H, J = 7.0 Hz); MS (EI): *m/z* 391 (M^+, 92 %); 389 (M^+-2, 100%).

*6.1.7. 9-Chloro-2-(methylthio)thiazolo[5,4-b]quinoline **8***

To compound **2** (2.94 g, 10 mmol) were added PPA (710 mg) and $POCl_3$ (3 ml). The reaction mixture was vigorously stirred at 136 °C for 4 h. The mixture was cooled to room temperature and EtOH (10 ml) was carefully added. The suspension was poured into water (20 ml) and neutralized with a $NaHCO_3$ saturated solution. The yellow precipitate formed was collected and dried by vacuum filtration. The crude was suspended in MeOH. The solid was collected and dried by vacuum filtration to afford **8** as a yellow solid. Yield: 1.2 g (60.3%); m.p.: 160 °C; IR (KBr): 3447, 1583, 1543, 1371, 1014, 751 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.3 (dd; 1H, J = 8.7, 0.9 Hz); 8.0 (dd; 1H, J = 7.2, 0.6 Hz); 7.7 (ddd; 1H, J = 9.0, 6.6, 1.8 Hz); 7.6 (ddd; 1H, J = 7.6, 1.5, 1.2 Hz); 2.88 (s, 3H); MS (EI): *m/z* 268 (M^++2, 41%); 266 (M^+, 100%); MS (FAB$^+$, High resolution): *m/z* 267 (M^++1); Anal. for $C_{11}H_8N_2ClS_2$: C, 49.53; H, 2.64; N, 10.50; S, 24.04. Found: C, 49.67; H, 2.46; N, 10.50; S, 23.52.

*6.1.8. 9-[(3-Aminophenyl)amino]-2-(methylthio)thiazolo [5,4-b]quinoline **9***

To compound **8** (0.266 g, 0.1 mmol) were added successively MeOH (15 ml) and two drops of HCl (36%). The mixture was stirred for ten minutes and *m*-phenylendiamine (80 mg) was then added. The stirred mixture was heated under reflux for 8 h. After cooling, the MeOH was evaporated under reduced pressure. The residue was suspended in EtOAc (25 ml), stirred several minutes and collected by vacuum filtration. The solid was again suspended in a 10% $NaHCO_3$ solution, stirred for 15 min, collected and dried by vacuum filtration. The solid residue was purified by crystallization from MeOH to afford **9** as yellow crystals. Yield: 200 mg (59.2%). m.p.: 178–183 °C, IR (KBr) 3447, 3347, 3301, 3186, 1593, 1550, 1490 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.1 (s; 1H); 8.0 (d; 1H, J = 9.0 Hz); 7.9 (d; 1H, J = 8.4 Hz); 7.6 (dd; 1H, J = 7.8, 7.5 Hz); 7.3 (dd, 1H, J = 9.0, 7.5 Hz); 7.0 (dd, 1H, J = 9.0, 7.2 Hz); 6.4 (m; 3H); 4.2 (br s; 2H); 2.6 (s; 3H); MS (EI): *m/z* 338 (M^+, 100); 322 (M^+-16, 16%); 305 (M^+-33, 53%).

*6.1.9. 9-[(3-Acetamidephenyl)amino]-2-(methylthio)thiazolo[5,4-b]quinoline **10***

To compound **9** (200 mg, 0.6 mmol) were successively added Ac_2O (2 ml) and pyridine (2 ml). The reaction mixture was stirred at room temperature for 14 h. The mixture was then poured into stirred water (30 ml). The solid formed was collected by vacuum filtration. The residue was purified by crystallization from MeOH to afford **10** as a yellow solid. Yield: 160 mg (71.4%); m.p.: 207–210 °C; IR (KBr): 3283, 2923, 1667, 1586, 1551 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.3 (d; 1H, J = 12 Hz); 7.9 (d; 1H, J = 12 Hz); 7.7 (ddd; 1H, J = 12.0, 10.0, 2.1 Hz); 7.5 (ddd; 1H, J = 12.6, 10.5, 2.1 Hz); 7.3-7.1 (m; 3H); 6.7 (d; 1H, J = 1.2 Hz); 2.4 (s; 3H); 1.2 (s; 3H); MS (EI): *m/z* 381 (M^++1, 21%); 380 (M^+, 100%); 365 (M^+-15, 9%); 337 (M^+-43, 11%).

*6.1.10. 9-[((3-Amino-5-(hydroxymethyl)phenyl)amino]-2-(methylthio)thiazolo[5,4-b]quinoline **11***

To compound **8** (0.133 g, 0.5 mmol) were successively added MeOH (7 ml), $CHCl_3$ (3.5 ml) and three drops of HCl (36%). The mixture was stirred for ten minutes. Meanwhile, to a solution of 3,5-diaminobenzyl alcohol dihydrochloride (315 mg, 1.5 mmol) in MeOH anhydrous (3 ml) was added $NaHCO_3$ anhydrous (200 mg). The suspension was stirred for ten minutes and the free base solution was transferred to the initial solution. The mixture reaction was heated under reflux for 48 h. After this time, the reaction mixture was concentrated under reduced pressure. The solid residue was suspended in water (10 ml) and a 10% $NaHCO_3$ solution was added to pH 8. The solid was collected and dried by vacuum filtration. The residue (150 mg) was dissolved in MeOH and coevaporated with silica gel (200 mg) in vacuo to dryness. The residue was put on the top of a silica gel column (3 × 30 cm), which was washed with $CHCl_3$/MeOH (99:1 v/v) to afford compound **11** as a light yellow solid. Yield: 105 mg (42.9%), m.p.: 220 °C (dec.); IR (KBr): 3384, 3357, 3328, 2922, 1607, 1584 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.9 (s; 1H, exchangeable with D_2O); 8.23 (d; 1H, J = 8.1 Hz); 7.87 (d; 1H, J = 7.5 Hz), 7.67 (ddd; 1H, J = 8.4, 6.8, 0.9); 7.43 (ddd; 1H, J = 7.7, 6.8, 1.2 Hz); 6.27 (s; 1H); 6.23 (s; 1H); 6.12 (s;1H), 5.0 (s; 1H, exchangeable with D_2O); 4.9 (s;1H, exchangeable with D_2O); 4.3 (s; 2H); 2.58 (s;3H); MS (EI): *m/z* 368 (M^+,100%); 335 (M^+-33,15%).

6.2. Cells and cytotoxic assay

Cells lines. The cell lines used here were: two human colorectal cancer cell lines (SW-480 and SW-620); three cervical cell lines (HeLa, C-33, Calo); one breast cancer cell line (MCF-7), one ovarian cell line (CHO), and one myelogenous leukemia human cell line (K-562) [9].

6.3. Ethidium displacement

DNA intercalation was determined from the displacement of ethidium bromide from DNA [10]. Sterile solutions of high molecular weight DNA from calf thymus (Gibco, BRL, New York, USA) in 0.1 M Tris–HCl buffer, 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% dimethylsulphoxide (DMSO), and the solution fluorescence intensity 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 to that determined in 100% aqueous buffers. The recorded fluorescence change was corrected for the dilution caused by serial additions of this solvent.

The compounds were tested in the 1–100 μM range, except when, at lower concentration, precipitation was detected as light dispersal in the solution (at 600 nm and 90°). The displacement curves were fitted by non-linear regression analysis to a rectangular hyperbola. Evidence of the direct interaction of the compounds with DNA was obtained from the differential fluorescence spectra of high molecular weight DNA, excited at 260 nm and recorded from 400 to 500 nm, in the absence and presence of the ligand. Two new peaks appeared at 420 and 440 nm and their intensity increased in a concentration dependent manner. Those peaks were not present in the spectra of the pure compound or pure DNA solutions.

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