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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro anticancer activity of novel thiazacridine derivatives

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Abstract Acridine derivatives represent a well-known class of anticancer agents that generally interfere with DNA synthesis and inhibit topoisomerase II. A series of eight new 3-acridin-9-ylmethyl-thiazolidine-2,4-dione and 3-acridin-9-ylmethyl-5-arylidene-thiazolidine-2,4-dione derivatives were synthesized. All the compounds were evaluated for their cell antiproliferation activity with the 3-(4,5-dimethyl-2-thiozolyl)-2,5-diphenyl-2*H*-tetrazolium bromide, MTT assay. The antiproliferative effects of the synthesized compounds were tested against several tumoral cell lines, namely SF-295 (central nervous system), HCT-8 (colon carcinoma), and MDA-MB-435 (melanoma) cells using doxorubicin as a positive control. Among the synthesized compounds, 3-acridin-9-ylmethyl-5-acridin-9-ylm ethylene-thiazolidine-2,4-dione, 3-acridin-9-ylmethyl-5-(4-methoxy-benzylidene)-thiazolidine-2,4-dione, and 3-acridin-9-ylmethyl-5-(4-bromo-benzylidene)-thiazolidine-2,4-dione exhibited the most potent anticancer activity against the HCT-8 and MDA-MB-435 cell lines. After a detailed analysis of the structure of the thiazacridine molecules, we revealed the main possible interactions using the compound 3-acridin-9-ylmethyl-5-acridin-9-ylmethylene-thiazolidine-2,4-dione as an example. The benefits of these compounds, regardless of the pharmacological target are the presence of two aromatic rings (pi systems), significant planarity (intercalating ability) and the presence of three hydrogen-bond acceptors, two of which are stronger (oxygen atoms) than the other (sulfur atom).

Keywords Thiazacridine MTT assay anticancer · Molecular modeling

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Introduction

Until the early 1980s, intense research of drug discovery programs for cancer resulted in efficient medicinals, but with high toxicity due to the lack of selectivity (Brana *et al.*, 2001; Demeunynck *et al.*, 2001; Martinez and Chacon-Garcia, 2005; Cummings and Smyth, 1993; Georghiou, 1977). The design and synthesis of small molecules that bind selectively to and cleave nucleic acids are still major challenges in this field. These synthetic nucleases have important applications as tools in molecular biology and as potential therapeutic agents for the treatment of cancer (Fernandez *et al.*, 2007). Acridines and their derivatives are well-known probes for nucleic acids as well as being relevant in the field of drug development to establish new chemotherapeutic agents (Ghosh *et al.*, 2010). The acridine ring, one such nuclease, is a potential anticancer chromophore, which has a long history of treatment of human diseases, particularly parasitic infections, cancer and Alzheimer's disease (Demeunynck *et al.*, 2001; Denny, 2002; Chatellier and Lacomblez, 1990).

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Most acridine derivatives interact with DNA as intercalators. They represent a well-known class of multi-targeted anticancer agents that generally interfere with DNA synthesis, due to their ability to intercalate into DNA base pair and leading to cell cycle arrest and apoptosis. Treatment of human melanoma cell line (A375) with 9-phenyl acridine derivatives led to lowering of mitochondrial potential, upregulation of bax, release of cytochrome *C*, and activation of caspase 3 (Ghosh *et al.*, 2012).

Some bis-acridines bind to DNA through intercalation between consecutive nucleotides. The act of intercalation induces local structural changes to the DNA, including the unwinding of the double helix and lengthening of the DNA strand (Ferguson and Denny, 2007; Ghosh *et al.*, 2012; Sondhi *et al.*, 2010). Also, acridines are able to stabilize the DNA-topoisomerase I and II cleavable complex, and form the so-called 'ternary complex' which involves DNA, the intercalated compound, and topoisomerase (Chilin *et al.*, 2009; Vispe *et al.*, 2007). These enzymes can manipulate DNA by changing the number of topological links between two strands of the same or different DNA molecules (Champoux, 2001) and are involved in many cellular processes, such as replication, recombination, transcription, and chromosome segregation (Corbett and Osheroff, 1993; Wang, 1996; Nitiss, 1998).

The aim of this study was to evaluate the antiproliferative effect of eight novel thiazacridine derivatives against tumor cell lines.

Results and discussion

Chemistry

Thiazacridine derivatives were prepared by the method summarized in Scheme 1. Initially compound **3** was synthesized by the N-alkylation reaction of thiazolidine-2,4-dione, compound **1**, with 9-bromomethyl-acridine, compound **2**, in the presence of sodium hydroxide. The Michael reaction of 3-acridin-9-ylmethyl-thiazolidine-2, 4-dione, compound **3**, with different cyanoacrylates, compounds **4** and **6a-f**, was carried out in the presence of piperidine, with ethanol as a solvent (Leite *et al.*, 2007; Mourao *et al.*, 2005; Pitta *et al.*, 2004, 2007; Pigatto *et al.*, 2011; Uchoa *et al.*, 2011). The presence of the arylidene proton peak in the synthesized derivatives, **5** and **7a–f**, in proton nuclear magnetic resonance (^{1}H NMR) confirms completion of the nucleophilic addition reaction. It is also confirmed by MS data, which contains ions at *m*/*z* 193 and 235 in positive ESI-MS2 spectrum indicates the presence of the acridine and thiazolidine moiety, respectively (Fig. 1).

Biological activity

MTT assay

Compounds **3**, **5**, and **7a–f** were tested for anticancer activity in a cell toxicity assay against three human cancer cell lines consisting of central nervous system (SF-295), colon carcinoma (HCT-8), and melanoma (MDA-MB-435), which were obtained from the National Cancer Institute (Bethesda, MD, USA).

The cells were maintained in RPMI 1640 medium supplemented with 10 % fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5 % CO_2. The cells were grown in complete medium 1 day before each experiment.

The cell survival were quantified by measuring the ability of living cells to reduce the yellow dye 3-(4, 5-dimethyl-2-thiozolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a purple formazan product (Uchoa *et al.*, 2011; Denizot and Lang, 1986; Mosmann, 1983). For the experiments, cells were seeded in 96-well plates (0.7×10^4 cells per well). After 24 h, when the cells reached confluence, the thiazacridine compounds (final concentration of 25 μg/mL) were dissolved in DMSO (0.1 %) and added to each well and incubated for 72 h. DMSO (0.1 %) and doxorubicin (0.3 μg/mL) were used as negative and positive controls, respectively. Thereafter, the plates were centrifuged, and the medium was replaced with fresh medium (150 μL) containing 0.5 mg/mL MTT. Three hours later, the formazan product was dissolved in 150 μL DMSO, and the absorbance was measured using a multiplate reader (Spectra Count, Packard, Ontario, Canada) at 595 nm. The effect of the compounds was expressed as the percentage of inhibition of cellular growth (%GI), obtained from the absorbance of negative control (A_{NC}) and cells treated (A_T), according to the following formula: $\%GI = 100 \times (1 - A_T/A_{NC})$.

The potential effects on cell viability of the thiazacridine compounds are presented in Table 1. An activity scale was utilized to rank the cytotoxic potential of the tested samples against each of the cell lines: inactive samples (IS, 0–35 % inhibition), samples with low activity (LA, 36–55 % inhibition), moderate activity (MA, 56–85 % inhibition) and high activity (HA, 86–100 % inhibition).

The experiments were analyzed using the GraphPad Prism program, and the data are reported as the mean of two assays completed in triplicate.

Determination of IC_{50}

Drugs concentration that inhibited cell survival by 50 % compared with control cells (IC_{50}) were determined using

Scheme 1 Synthetic pathways of thiazacridines

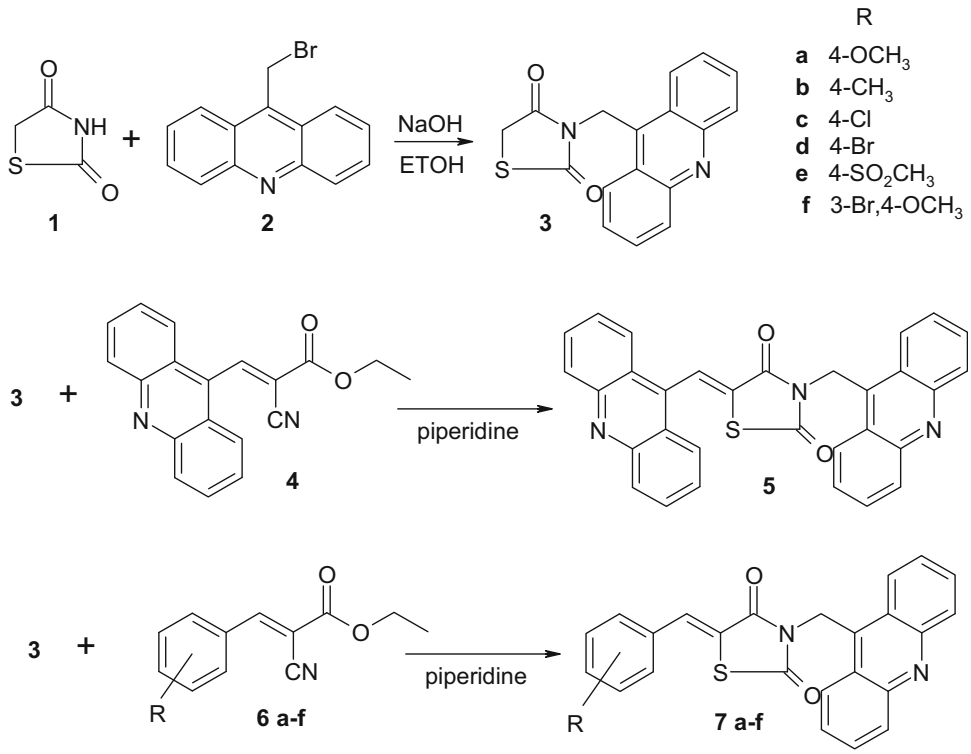
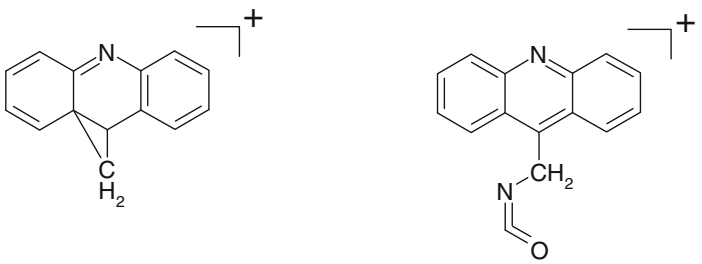


Fig. 1 Specific fragmentation in positive ESI-MS2 for 3-acridin-9-ylmethyl-5-arylidene-thiazolidine-2, 4-diones



M + H = $C_{14}H_{11}N$ = m/z = 193 M +H = $C_{15}H_{11}N_2O$ = m/z 235

Table 1 Evaluation of cytotoxicity towards tumor cells (% cell inhibition) for the synthesized compounds **3**, **5**, and **7**(**a**–**f**)

Compounds	SF-295	HCT-8	MDA-MB-435
3	32.8 (IS)	42.0 (LA)	0.00 (IS)
5	76.7 (MA)	92.4 (HA)	95.9 (HA)
7a	59.5 (MA)	86.7 (HA)	84.2 (MA)
7b	29.9 (IS)	51.3 (LA)	31.2 (IS)
7c	31.3 (IS)	37.8 (LA)	0.00 (IS)
7d	62.2 (MA)	96.6 (HA)	85.3 (MA)
7e	44.3 (LA)	64.3 (MA)	12.9 (IS)
7f	48.0 (LA)	72.5 (MA)	53.0 (LA)
Dox	91.1 (HA)	95.2 (HA)	93.6 (HA)

Date represent two experiments performed in triplicate. Doxorubicin (dox) was used as a positive control

Inactive samples (IS, 0–35 %), low activity (LA, 36–55 %)

Moderate activity (MA, 56–85 %) and high activity (HA, 86–100 %)

also MTT assay, as described above. The compounds were tested against four human cancer cell lines: HL-60 (promyelocytic leukemia), MDA-MB-435 (melanoma), HCT-8 (colon) and SF-295 (glyoblastoma) as presented in Table 2, all obtained from the National Cancer Institute (Bethesda, MD, USA). The cells were plated in 96-well plates (0.7×10^4 cells/well for adherent cells and 0.3×10^5 cells/well for suspended cells) and compounds (0.049–25 μg/mL) dissolved in DMSO were added to each plate well.

In this report, we show the effect of eight acridine derivatives on cell survival. The structures of the synthesized compounds were confirmed by spectral data and elemental analysis and they were in full agreement with the proposed structures. The extent of inhibition of carcinoma cell lines by these compounds is schematically presented in Fig. 2. Among all the tested compounds, **3** and **7c** exhibited

Table 2 Cytotoxicity activity of compounds for cancer cell lines

Compounds	Cell lines			
	HL-60	SF-295	HCT-8	MDA-MB-435
3	>25	>25	>25	>25
5	>25	4.44	4.45	7.01
		3.37–5.85	3.57–5.55	5.50–8.92
7a	>25	>25	19.26	>25
			15.11–24.53	
7b	>25	>25	>25	>25
7c	>25	>25	>25	>25
7d	19.49	>25	20.63	>25
	14.21–26.73		18.24–23.34	
7e	>25	>25	>25	>25
7f	>25	>25	>25	>25
Dox	0.02	0.48	0.04	0.24
	0.01–0.02	0.34–0.66	0.03–0.05	0.17–0.36

Data are present as IC_{50} (μg/mL) values and 95 % confidence interval obtain by nonlinear regression. Date represent two experiments performed in triplicate. Doxorubicin (Dox) was used as positive control

the lowest inhibitory activity against the three carcinoma cell lines tested, especially against the MDA-MB-435 cell line. In addition, compounds **5**, **7a**, and **7d** exhibited potent inhibitory activity against the MDA-MB-435 and HCT-8 cell lines, with greater than 85 % of inhibition in the HCT-8 cell line. The drugs concentrations that inhibited cell growth by 50 % compared with control cells (IC50) were under 25 μg/mL for the compounds **5**, **7a**, and **7d**. Thus, the compounds that had the best antitumor activity presented the smallest values at the IC_{50} assays.

The biological activity of acridines is mainly attributed to the planarity of these aromatic structures, which allows them to intercalate within the double-stranded DNA structure, thus interfering with the cellular machinery (Belmont *et al.*, 2007). In this way, connecting two planar intercalating moieties to obtain a bisacridine derivative generally increases the DNA binding affinity and the drug's residence times in the DNA-bound form (Antonini *et al.*, 2003). As expected, compound **5**, which contains two acridine nucleus, showed a promising result.

In general, electronegative substituents at *para* position on the phenyl ring contributed to the biological activity. First the bromo group (**7d**) having a electron withdrawing inductive effect and second the methoxy group (**7a**) having electron donating effect

It is noteworthy that the position and the nature of the substituent on the heterocyclic core are the determinants for the biological properties observed. Therefore, it could be concluded that the cell survival of the tumor cell lines tested with the thiazacridine derivatives decreases in the presence of a methoxy group (**7a**), or a bromo group (**7d**) both at *para* position of the phenyl ring. However, the increase in the steric bulk on the phenyl ring due to the association of the bromo group (in *meta*) and methoxy group (in *para*), as in compound **7f**, did not contributed to the activity.

Molecular modeling

In order to investigate the structures and properties of the thiazacridines, all optimized geometries were obtained using the AM1 semi-empirical method (Dewar *et al.*, 1985), available in the BioMedCAChe software, using the internal default settings for convergence criteria. In addition, some electronic properties, such as electron affinity, ionization potential and molecular dipole moment were

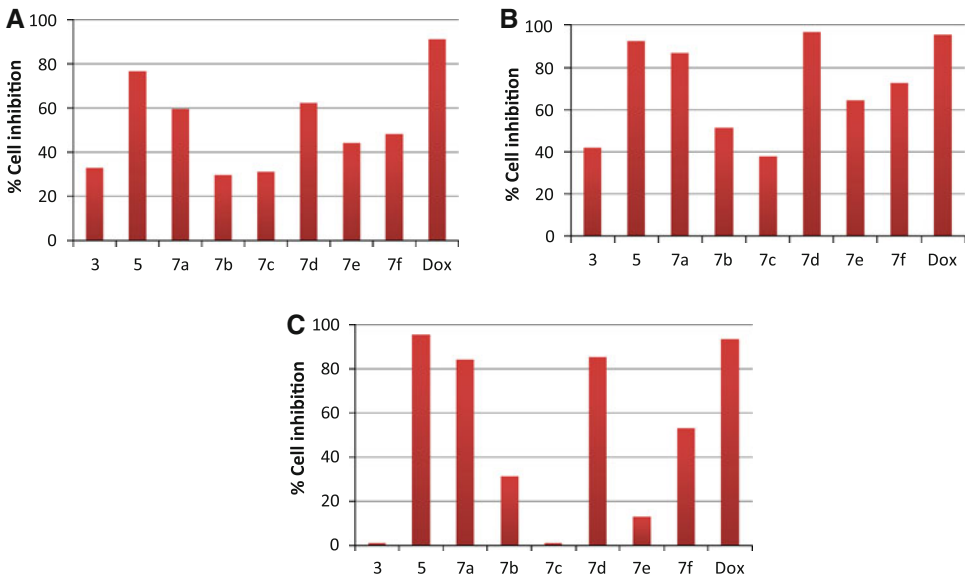


Fig. 2 MTT assay for three tumor cell lines: **a** SF-295, **b** HCT-8 and **c** MDA-MB-435. Date represent two experiments performed in triplicate. Doxorubicin (dox) was used as the positive control. All compounds were tested at a dose 25 μg/mL (Color figure online)

Table 3 Molecular properties and pharmacokinetic parameters, important for the good bioavailability of thiazacridine derivatives and doxorubicin (dox): number of hydrogen-bond acceptor and donor groups (nON and nOHNH, respectively), molecular weight (MW), calculated octanol/water partition coefficient (miLogP), number of rule's violations (Nviolat), number of rotatable bonds (Nrotb), topological polar surface area (TPSA), molecular volume (Vol), the scores to judge the compound's overall potential to qualify for a drug (druglikeness and drug-score), dipole moment, electron affinity, and ionization potential energies

Compounds	Lipinski's rule of five					Nrotb[a]	TPSA[a]	Vol[a]	Druglikeness[b]	Drug-score[b]	Dipole moment (debye)[c]	Electron affinity (eV)[c]	Ionization potential (eV)[c]
	nON[a]	nOHNH[a]	MW[a]	miLogP[a]	Nviolat[a]								
3	4	0	308.36	3.07	0	2	50.27	259.51	2.15	0.42	2.48	1.21	8.56
5	5	0	497.58	6.67[d]	1	3	64.86	425.36	4.38	0.14	2.66	1.34	8.52
7a	5	0	426.50	4.96	0	4	61.20	367.08	4.56	0.24	5.30	1.18	8.45
7b	4	0	410.50	5.35[d]	1	3	51.97	358.10	2.97	0.22	4.72	1.22	8.46
7c	4	0	430.92	5.58[d]	1	3	51.97	355.07	5.17	0.2	2.95	1.42	8.52
7d	4	0	475.37	5.71[d]	1	3	51.97	359.42	2.46	0.17	2.72	1.47	8.53
7e	6	0	474.56	3.78	0	4	86.11	389.53	4.54	0.24	1.98	1.84	8.63
7f	5	0	505.39[d]	5.7[d]	2	4	61.20	384.97	3.52	0.17	3.89	1.32	8.50
dox	12[d]	7[d]	543.52[d]	0.57	3	5	206.08	459.18	8.12	0.52	5.49	1.54	9.06

[a] http://www.molinspiration.com/cgi-bin/properties

[b] http://www.organic-chemistry.org/prog/peo

[c] BioMedCAChe software

[d] Parameters that violate the Lipinski's rule of five

calculated. Then, the molecules were submitted to the classical analysis of Lipinski *et al.* (1997) using the Molinspiration online tool. Lipinski's rule of five verifies molecular features related to the optimum bioavailability of a drug. The number of rotatable bonds also seems to be an important descriptor for this purpose (Wenlock *et al.*, 2003). Other properties were also calculated, such as the topological polar surface area (TPSA), which is a very useful parameter for the prediction of drug transport properties (Ertl *et al.*, 2000), and molecular volume. In addition, we evaluated the scores to judge the compound's overall potential to qualify as a drug (druglikeness and drug-score) using the Osiris Property Explorer online system (http://www.organic-chemistry.org/prog/peo). All of these data are summarized in Table 3.

The TPSA is calculated at Molinspiration on-line, based on the methodology published by Ertl *et al.* (2000) as a sum of fragment contributions. Only oxygen- and nitrogen-centered polar fragments are considered. While Bytheway *et al.* (2008) applied TPSA analysis for drug design, Hou and Xu (2003) have used TPSA data for ADME evaluation. The computation of molecule volume in molinspiration website is based on group contributions and has been obtained by fitting the sum of fragment contributions to real 3D volume for a training set of about twelve thousand, mostly drug-like molecules.

From the Osiris Property Explorer online system, the druglikeness approach is based on a list of about 5,300 distinct substructure fragments with associated scores. The fragment list was created with traded drugs and commercially available chemicals (Fluka), yielding a complete list of all available fragments. The distribution of the druglikeness values calculated for the 15000 Fluka chemicals remain in the range from −20 to 4 with the maximum occurrence around the value −1 for druglikeness score. The 3,300 traded drugs stay between −13 and 10 with the maximum around the value 2. This distribution shows that 80 % of the traded drugs have a positive druglikeness score, while the big majority of Fluka chemicals revealed negative values of druglikeness. Therefore, it is promising see that all the molecules calculated here have positive values of druglikeness ranging from 2.15 to 5.17 values (Table 2). The drug-score combines individual properties like druglikeness, cLogP, logS, molecular weight, and toxicity risks, in one value than may be used to judge the compound's overall potential to qualify for a drug. The drug-score is calculated by multiplying contributions of these individual properties, which are described as parametrized spline curves.

Thus, while the druglikeness values are based upon the occurrence frequency of each molecule's fragment in commercial drugs, the drug-score evaluates the compound's potential to qualify as a drug and is related to topological descriptors, fingerprints of druglikeness values, structural keys, and other properties such as cLogP, logs, molecular weight, and toxicity behavior. Several groups (Bernardino *et al.*, 2008; Campos *et al.*, 2009; Santos *et al.*, 2009) are using this scores (druglikeness and drug-score) to evaluate the potential of the studied molecules to become drugs.

According to Lipinski's rule, the violation of more than one of the specified criteria may decrease bioavailability. Our results demonstrated that all of the thiazacridine derivatives, except 3-acridin-9-ylmethyl-5-(3-bromo-4-methoxy-benzylidene)-thiazolidine-2,4-dione, compound **7f**, obey Lipinski's rule. It is important to stress that only the miLogP values, for five molecules (**5**, **7b-d**, and **7f**), slightly exceeds the value 5 preconized by Lipinski. In addition, only for molecule **7f** the molecular weight slightly passes over the limit (500). Table 2 shows that the reference drug, doxorubicin, seems to be very unlike the thiazacridine molecules, which is expected because of its peculiar molecular structure.

After a detailed analysis of the structure of thiazacridine molecules, we can see in Fig. 3 possible points of interactions of compound **5**. This derivative present high activity, at least against the HCT-8 and MDA-MB-435 cell lines (see Table 1). The benefits of these compounds, regardless of the pharmacological target, are the presence of two aromatic rings (π systems), significant planarity (intercalating ability) and the presence of three hydrogen-bond acceptors, two of which are stronger (oxygen atoms) than the other (sulfur atom).

Acridine and its derivatives are the most extensively studied DNA intercalating agents that bind reversibly but non-covalently to DNA (Hou and Xu, 2003). We evaluated the molecular properties and pharmacokinetic parameters of thiazacridine derivatives, which are considered potential drugs for anticancer treatment. Some compounds showed slightly better results when compared to the reference drug (doxorubicin), especially compounds 3-acridin-9-ylmethyl-5-(4-bromo-benzylidene)-thiazolidine-2,4-dione, compound **7d**, and 3-acridin-9-ylmethyl-5-acridin-9-ylmethylene-thiazolidine-2,4-dione, compound **5**, which were very active against the HCT-8 and MDA-MB-435 cell lines.

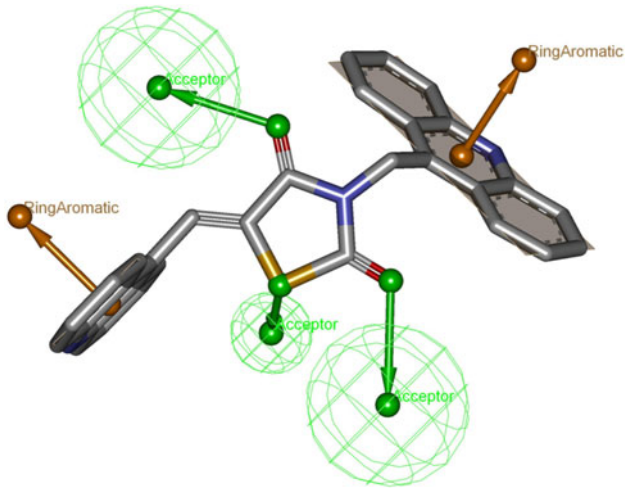


Fig. 3 Possible molecular points of interactions of thiazacridine derivatives using 3-acridin-9-ylmethyl-5-acridin-9-ylmethylene-thiazolidine-2,4-dione, compound **5**, as example. "Acceptor" corresponds to hydrogen-bond acceptor points and "Ring Aromatic" corresponds to aromatic rings. Carbon, oxygen, nitrogen and sulfur atoms are represented with *gray*, *red*, *blue*, and *yellow colors*, respectively. This figure was generated with DS visualizer software (Bytheway *et al.*, 2008) (Color figure online)

Materials and methods

All the synthesized compounds were analyzed with multiple analytical procedures. Melting points were determined in a capillary tube using a Quimis apparatus. Infrared spectra (IR) were recorded on a Bruker IFS66 spectrometer. ^{1}H NMR spectra were recorded on a Varian Plus 300 MHz spectrometer using DMSO-d_6 as a solvent and tetramethylsilane as an internal standard. The chemical shifts are expressed in ppm, and the following abbreviations are used: s is the singlet, d the doublet, t the triplet and m the multiplet. Mass spectra were recording using liquid chromatography/MS (LC/MS) with a HCT Ultra from Bruker Daltonics and were performed by electrospray ionization in positive or negative mode. The base peak of the MS spectrum is set to 100 (in percentage), and the height of the others peaks are measured relative to the base peak.

Experimental

*Synthesis of 3-acridin-9-ylmethyl-thiazolidine-2,4-diones (**3**)*

Thiazolidine-2,4-dione, compound **1**, (1.0 eq) and sodium hydroxide, previously solubilized in ethanol, were stirred for 10 min at room temperature (25 °C). 9-Bromomethyl-acridine, compound **2**, (1.0 eq) was added, and the mixture was stirred at 60 °C for 7 h. After completion of the reaction, the mixture was filtered and washed with water. The product obtained was a yellow solid. Formula: $C_{17}H_{12}N_2O_2S$. Melting point (m.p.): 196–197 °C. Yield: 51 %. IR (KBr, cm^{-1}): 2889 (–CH_2–), 1750 (C=O), 1694 (C=O), 750 (C–H). H^{1}NMR (300 MHz, DMSO-d_6) δ 8.42 (d, 2H, $J = 8.7$ Hz, Acr–H, 4 pos.), 8.17 (d, 2H, $J = 8.4$ Hz, Acr–H, 1 pos.), 7.83–7.88 (m, 2H, Acr–H, 3 pos.), 7.65–7.70 (m, 2H, Acr–H, 2 pos.), 5.75 (s, 2H, N–CH_2), 4.23 (s, 2H, S–CH_2). MS *m/z* (%): $(M+H)^+$ 309.1 (100), calculated 308; +MS2 309.1 (56), 235 (100), 192 (98).

*General preparation of 3-acridin-9-ylmethyl-5-arylidene-thiazolidine-2,4-dione (**5** and **7a–f**)*

3-Acridin-9-ylmethyl-thiazolidine-2,4-dione, compound **3**, (1.0 eq) and either 3-acridin-9-yl-2-cyano-acrylic acid

ethyl ester, compound **4**, (1.0 eq) or one of the phenyl-substituted 2-cyano-3-phenyl-acrylic acid ethyl esters, compounds **6a–f**, were refluxed in ethanol in the presence of piperidine and heated at 50 °C for 4 h. After this period, the mixture was filtered and washed with water and ethanol (Pitta *et al.*, 2004, 2007).

*3-Acridin-9-ylmethyl-5-acridin-9-ylmethylene-thiazolidine-2,4-dione (**5**)*

Yellow solid. $C_{31}H_{19}N_3O_2S$. m.p.: 243–245 °C. Yield: 11 %. IR (KBr, cm^{-1}): 1759 (C=O), 1694 (C=O), 760 (C–H). H^1NMR (300 MHz, DMSO-d_6) δ 8.75 (s, 1H, =CH), 8.54 (d, 2H, $J = 9.0$ Hz, 3-Acr–H, 4 pos.), 8.21 (d, 2H, $J = 8.7$ Hz, 5-Acr–H, 4 pos.), 8.13 (d, 2H, $J = 8.1$ Hz, 3-Acr–H, 1 pos.), 8.04 (d, 2H, $J = 8.4$ Hz, 5-Acr–H, 1 pos.), 7.87–7.92 (m, 2H, 5-Acr–H, 3 pos.), 7.81–7.84 (m, 2H, 3-Acr–H, 3 pos.), 7.72–7.77 (m, 2H, 5-Acr–H, 2 pos.), 7.60–7.68 (m, 2H, 3-Acr–H, 2 pos.), 5.96 (s, 2H, $–CH_2$). MS *m/z* (%): $(M+H)^+$ 498.2 (100), calculated 497; +MS2 498.2 (28), 305 (99), 236 (43), 193 (100).

*3-Acridin-9-ylmethyl-5-(4-methoxy-benzylidene)-thiazolidine-2,4-dione (**7a**)*

Yellow solid. $C_{25}H_{18}N_2O_3S$. m.p.: 225–226 °C. Yield: 52 %. IR (KBr, cm^{-1}): 1744 (C=O), 1679 (C=O), 1593 (C=C), 756 (C–H). H^1NMR (300 MHz, DMSO-d_6) δ 8.49 (d, 2H, $J = 9.0$ Hz, Acr–H, 4 pos.), 8.16 (d, 2H, $J = 8.4$ Hz, Acr–H, 1 pos.), 7.90 (s, 1H, =CH), 7.81–7.89 (m, 2H, Acr–H, 3 pos.), 7.60–7.71 (m, 2H, Acr–H, 2 pos.), 7.55 (d, 2H, $J = 8.7$ Hz, Ar–H, 2,6 pos.), 7.08 (d, 2H, $J = 8.7$ Hz, Ar–H, 3,5 pos.), 5.93 (s, 2H, N–CH_2), 3.81 (s, 3H, O–CH_3).). MS *m/z* (%): $(M+H)^+$ 427.2 (100), calculated 426; +MS2 427.2 (54), 235 (100), 193 (70).

*3-Acridin-9-ylmethyl-5-(4-methyl-benzylidene)-thiazolidine-2,4-dione (**7b**)*

Yellow solid. $C_{25}H_{18}N_2O_2S$. m.p.: 194–195 °C. Yield: 20 %. IR (KBr, cm^{-1}): 1729 (C=O), 1674 (C=O), 1604 (C=C), 760 (C–H). H^1NMR (300 MHz, DMSO-d_6) δ 8.46 (d, 2H, $J = 8.7$ Hz, Acr–H, 4 pos.), 8.19 (d, 2H, $J = 8.7$ Hz, Acr–H, 1 pos.), 7.90 (s, 1H, =CH), 7.81–7.86 (m, 2H, Acr–H, 3 pos.), 7.60–7.71 (m, 2H, Acr–H, 2 pos.), 7.47 (d, 2H, $J = 8.1$ Hz, Ar–H, 2,6 pos.), 7.33 (d, 2H, $J = 8.1$ Hz, Ar–H, 3,5 pos.), 5.92 (s, 2H, N–CH_2) 2.34 (s, 3H, $–CH_3$). MS *m/z* (%): $(M+H)^+$ 411.2 (100), calculated 410; +MS2 411.2 (48), 235 (100), 193 (39).

*3-Acridin-9-ylmethyl-5-(4-chloro-benzylidene)-thiazolidine-2,4-dione (**7c**)*

Yellow solid. $C_{24}H_{15}ClN_2O_2S$. m.p.: 229–231 °C. Yield: 47 %. IR (KBr, cm^{-1}): 1739 (C=O), 1689 (C=O), 1608 (C=C), 750 (C–H). H^1NMR (300 MHz, DMSO-d_6) δ 8.45 (d, 2H, $J = 8.7$ Hz, Acr–H, 4 pos.), 8.19 (d, 2H, J = 8.7 Hz, Acr–H, 1 pos.), 7.93 (s, 1H, =CH), 7.83–7.88 (m, 2H, Acr–H, 3 pos.), 7.66–7.71 (m, 2H, Acr–H, 2 pos.), 7.62–7.56 (m, 4H, Ar–H), 5.93 (s, 2H, N–CH_2). MS *m/z* (%): $(M+H)^+$ 431.1 (100), calculated 430; +MS2 431.1 (77), 235 (100), 193 (50).

*3-Acridin-9-ylmethyl-5-(4-bromo-benzylidene)-thiazolidine-2,4-dione (**7d**)*

Yellow solid. $C_{24}H_{15}BrN_2O_2S$. m.p.: 222–223 °C. Yield: 24 %. IR (KBr, cm^{-1}): 1744 (C=O), 1689 (C=O), 1608 (C=C), 750(C–H). H^1NMR (300 MHz, DMSO-d_6) δ 8.45 (d, 2H, $J = 9.0$ Hz, Acr–H, 4 pos.), 8.19 (d, 2H, $J = 8.7$ Hz, Acr–H, 1 pos.), 7.90 (s, 1H, =CH), 7.84–7.89 (m, 2H, Acr–H, 3 pos.), 7.70–7.73 (d, 2H, $J = 8.7$ Hz, Ar–H, 3,5 pos.), 7.66–7.71 (m, 2H, Acr–H, 2 pos.), 7.51 (d, 2H, $J = 8.4$ Hz, Ar–H, 2,6 pos.), 5.92 (s, 2H, N–CH_2). MS *m/z* (%): $(M+H)^+$ 475.1 (92), $(M+2+H)^+$ 477.1 (100), calculated 474; +MS2 475.1 (100), 235 (98), 193 (50).

*3-Acridin-9-ylmethyl-5-(4-methanesulfonyl-benzylidene)-thiazolidine-2,4-dione (**7e**)*

Yellow solid. $C_{25}H_{18}N_2O_4S_2$. m.p.: 227-228 °C. Yield: 28 %. IR (KBr, cm^{-1}): 3440 (O=S=O), 1744 (C=O), 1689 (C=O), 1608 (C=C), 1142 (O=S=O). H^1NMR (300 MHz, DMSO-d_6) δ 8,46 (d, 2H, $J = 8,4$ Hz, Acr–H, 4 pos.), 8,19 (d, 2H, $J = 8,1$ Hz, Acr–H, 1 pos.), 8,03 (d, 2H, $J = 7.2$ Hz, Ar–H, 3.5 pos.), 8,02 (s, 1H, = CH), 7,85 – 7,90 (m, 2H, Acr–H, 3 pos.), 7,83 (d, 2H, $J = 8,4$ Hz, Ar–H, 2,6 pos.), 7,67–7,73 (m, 2H, Acr–H, 2 pos.), 5,94 (s, 2H, N–CH_2), 3,25 (s, 3H, S–CH_3). MS *m/z* (%): $(M+H)^+$ 475.1 (100), 383 (99), calculated 474; +MS2 475.1 (65), 396 (100), 235 (90), 193(40), 192 (55).

*3-Acridin-9-ylmethyl-5-(3-bromo-4-methoxy-benzylidene)-thiazolidine-2,4-dione (**7f**)*

Yellow solid. $C_{25}H_{17}BrN_2O_3S$. m.p.: 230–232 °C. Yield: 62 %. IR (KBr, cm^{-1}): 1730 (C=O), 1684 (C=O), 1589 (C=C), 1267 (C–O), 756 (C–H). H^1NMR (300 MHz, DMSO-d_6) δ 8,46 (d, 2H, $J = 8,4$ Hz, Acr–H, 4 pos.), 8,19 (d, 2H, $J = 8,1$ Hz, Acr–H, 1 pos.), 7,89 (s, 1H, =CH), 7,86–7,87 (m, 2H, Acr–H, 3 pos.), 7,85 (d, 1H, $J = 3$ Hz, Ar–H, 2 pos.), 7,65–7,72 (m, 2H, Acr–H, 2 pos.), 7,58 (dd,

1H, $J = 8,7$ and 1,8 Hz, Ar–H, 6 pos.), 7,26 (d, 1H, $J = 9,0$ Hz, Ar–H, 5 pos.), 5,92 (s, 2H, N–CH_2), 3,99 (s, 3H, O–CH_3). MS *m/z* (%): $(M+H)^+$ 505.1 (99.5), $(M+2+H)^+$ 507.1 (100), calculated 504; +MS2 505.1 (14), 490 (71), 235 (100), 192 (71), 193 (72).

Conclusion

The eight newly synthesized 3-acridin-9-ylmethyl-thiazolidine-2,4-dione and 3-acridin-9-ylmethyl-5-arylidene-thiazolidine-2,4-dione analogues were evaluated for their anticancer activity against the human cancer cell lines consisting of central nervous system (SF-295), colon carcinoma (HCT-8) and the melanoma (MDA-MB-435). We have found that the acridine dimer 3-acridin-9-ylmethyl-5-acridin-9-ylmethylene-thiazolidine-2,4-dione demonstrates potent DNA binding affinity and a significant anticancer activity against all the three cancer cell lines tested. The great majority of the molecules presented in this paper seems to obey the Lipinski's rule of five regarding the bioavailability features. In addition, the compound 3-acridin-9-ylmethyl-5-(4-methoxy-benzylidene)-thiazolidine-2,4-dione having electron donating group methoxy as substituent on phenyl ring and the compound 3-acridin-9-ylmethyl-5-(4-bromo-benzylidene)-thiazolidine-2,4-dione having a electron withdrawing inductive effect of the bromo group on the phenyl ring also exhibited strong inhibition in the same cell lines.

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