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Synthesis and cytotoxic activity of new acridine-thiazolidine derivatives

Francisco W.A. Barros [a], Teresinha Gonçalves Silva [b], Marina Galdino da Rocha Pitta [b], Daniel P. Bezerra [c], Letícia V. Costa-Lotufo [a], Manoel Odorico de Moraes [a], Cláudia Pessoa [a], Maria Aline F.B. de Moura [d], Fabiane C. de Abreu [d], Maria do Carmo Alves de Lima [b], Suely Lins Galdino [b], Ivan da Rocha Pitta [b,*], Marilia O.F. Goulart [d,*]

[a] *Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil*
[b] *Laboratório de Planejamento e Síntese de Fármacos – LPSF, Grupo de Pesquisa em Inovação Terapêutica – GPIT, Universidade Federal de Pernambuco, Avenida Moraes Rego 1235, Cidade Universitária, CEP 50670-901 Recife, Pernambuco, Brazil*
[c] *Departamento de Fisiologia, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brazil*
[d] *Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, Maceió, Alagoas, Brazil*

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ABSTRACT

Although their exact role in controlling tumour growth and apoptosis in humans remains undefined, acridine and thiazolidine compounds have been shown to act as tumour suppressors in most cancers. Based on this finding, a series of novel hybrid 5-acridin-9-ylmethylene-3-benzyl-thiazolidine-2,4-diones were synthesised via N-alkylation and Michael reaction. The cell viability was analysed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and DNA interaction assays were performed using electrochemical techniques.

1. Introduction

Acridine derivatives have been used for commercial purposes for more than a century. They are capable of interacting with nuclear DNA in a sequence-specific manner and with biological targets, such as topoisomerase I and II (topo I and II) and telomerase. These compounds play an important role in a variety of diseases, and they have been used clinically in past decades as antiviral,[1,2] antiprion,[3] antiprotozoal,[4] anti-inflammatory, antineoplasic[5] and as analgesic compounds.[6]

The biological activity of acridines has been attributed to the planarity of these aromatic structures, which can intercalate within double-stranded DNA, thus interfering with cellular functions.[7] Indeed, the mode of action of acridines has been the focus of continuous and exciting research.[8] In cancer chemotherapy, the biological targets of acridines include DNA topoisomerases I and/or II, telomerase/telomeres and protein-kinases.[9–15]

Amsacrine is the best-known acridine, and it exhibits potent cytotoxic activity and has been found to be clinically useful. It was also one of the first DNA-intercalating agents to be considered as a topoisomerase II inhibitor. Amsacrine is active in the treatment of acute leukaemias and lymphomas but is ineffective in solid tumours. Widespread clinical use of this compound has been limited by problems, such as side effects, drug resistance and poor bioavailability, which have encouraged the further structural modification of this compound.[16]

In contrast, thiazolidine compounds have emerged as antineoplastic agents with a broad spectrum of antitumour activity against many human cancer cells.[17–23] These molecules are agonists of peroxisome proliferator-activated receptor γ (PPARγ), which is expressed in many human tumours, including lung, breast, colon, prostate and bladder,[24] and they modulate the proliferation and apoptosis of many cancer cell types. Arylidene-thiazolidine-2,4-diones were also synthesised and screened as anti-inflammatory compounds, showing considerable biological efficacy, when compared to rosiglitazone, agonist of PPARγ and used as a reference drug.[25] As cytotoxic compounds, they were shown to be moderately active in a range higher than 30 μM.[26] Troglitazone and derivatives were assayed as anticancer against PC-3 and androgen dependent LNCaP cells. The respective IC_{50} values for troglitazone and its Δ2-dehydro derivative were 30 ± 2 and 20 ± 2 μM in PC-3 cells and 22 ± 3 and 14 ± 1 μM in LNCaP cells.[27]

Considering these facts, our strategy was to couple acridine and thiazolidine nucleus to obtain a new class of compounds, the thiazacridine derivatives. By assaying their biological activities using diverse techniques based on various mechanisms of action, we found

* Corresponding authors.
E-mail address: mofg@qui.ufal.br (M.O.F. Goulart).

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these derivatives to be a new class of drugs that are effective in cancer therapy.

It is clearly of fundamental importance to explore the factors that determine the affinity and selectivity of DNA-binding compounds to ascertain the nature and potency of such molecules, particularly with respect to their potential to cause DNA damage. In this context, the need for stable, low cost, and readily adaptable analytical tools for the detection of DNA damage has been the driving force in the development of DNA-biosensor technology.[28,29] An electrochemical DNA-biosensor is a receptor-transducer that employs double-stranded DNA (dsDNA) immobilised onto the surface of an electrochemical transducer as a molecular recognition element through which specific DNA-binding processes may be assessed.[28,30] The interaction of an analyte (i.e., a drug, pro-drug or in situ-generated intermediate) with dsDNA may lead to the rupture of hydrogen bonds and the consequential opening of the double helix, resulting in increased accessibility to the constituent bases. The extent of DNA damage may be determined by monitoring the oxidation of the exposed bases by voltammetric methods. The electrochemical characteristics of such dsDNA-biosensors have been evaluated, and it is clear that this approach can provide a greater understanding of the mechanism of interaction between drugs and DNA and can offer new insights in rational drug design.[30,31]

Herein, we describe the synthesis of the following acridine-thiazolidine derivatives: 5-(acridin-9-ylmethylene)-3-(4-methyl-benzyl)-thiazolidine-2,4-dione (**9**), 5-(acridin-9-ylmethylene)-3-(4-bromo-benzyl)-thiazolidine-2,4-dione (**10**), 5-(acridin-9-ylmethylene)-3-(4-chloro-benzyl)-thiazolidine-2,4-dione (**11**), and 5-(acridin-9-ylmethylene)-3-(4-fluoro-benzyl)-thiazolidine-2,4-dione (**12**). Moreover, the cytotoxic properties of these derivatives and of some of their precursors were examined in different histotype cancer cell lines, and they were also tested for DNA interaction, using electrochemical methods and DNA biosensors.

2. Results and discussion

2.1. Chemistry

The thiazacridine derivatives **9–12** were synthesised by the nucleophilic addition of substituted 3-benzyl-thiazolidine-diones **5–8** on 3-acridin-9-yl-2-cyano-acrylic acid ethyl ester **3** (Scheme 1). Indeed, the direct condensation of 9-acridinaldehyde **2**, with the substituted thiazolidines **5–8**, did not lead to the expected acridinylidene-thiazolidines. 9-Methyl-acridine **1** was prepared from diphenylamine with zinc dichloride in acetic acid according to Tsuge et al. (1963).[32] Subsequently, the oxidation of **1** with pyridinium chlorochromate, according to Mosher and Natale (1995),[33] gave the 9-acridinaldehyde **2**. The synthetic pathways are illustrated in Scheme 1.

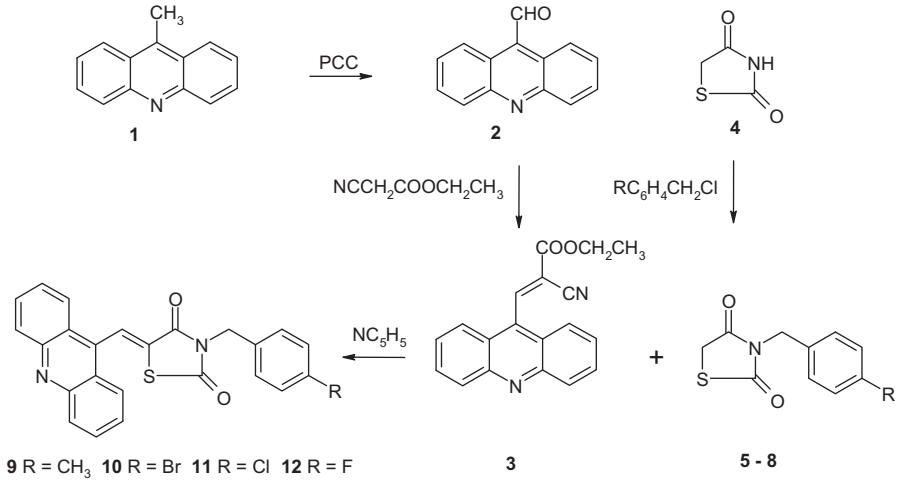
The acridinylidene-thiazolidine **9** was isolated as a single isomer. X-ray crystallographic studies and ^{13}C NMR have demonstrated a preferred *Z* configuration for 5-benzylidene-thiazolidinones and 5-benzylidene-imidazolinones.[34,35] The $^{3}J_{CH}$-coupling constant value between the ethylene hydrogen and the carbon atom located at the 4-position of the heterocyclic ring is of special interest in this structural elucidation.[36] In contrast, compounds **10** and **12** were isolated as isomeric mixtures. The *Z* isomer was the major stereoisomer formed, and the isomers were readily identified by ^{1}H NMR, as the ethylene hydrogen is more deshielded in the *Z* isomer than it is in the *E* isomer. The *Z* isomer was isolated for the derivative **11**.

2.2. Biological assays

2.2.1. Cytotoxicity assay

The cytotoxicity of the acridine-thiazolidine derivatives and of some of the precursors: **3** (3-acridin-9-yl-2-cyano-acrylic acid ethyl ester), **5** (3-(4-methyl-benzyl)-thiazolidine-2,4-dione) and **6** (3-(4-bromo-benzyl)-thiazolidine-2,4-dione) was evaluated against tumour cell lines of different histotypes using a previously described MTT assay.[37] Amsacrine was used as a positive control. Table 1 summarises the IC_{50} data for cytotoxic activity. The results indicated that the precursor thiazolidine-2,4-diones **5** and **6** were inactive (IC_{50} >25 μg/mL) (not shown in Table 1) and that the 3-acridin-9-yl derivative was significantly active against HL-60 (4.07 μM). The thiazacridines exhibit relatively high cytotoxicity against colon carcinoma and glioblastoma tumour cell lines, possessing IC_{50} values in the range of 7.4–46.4, 7.2–35.5, 5.8–29.0, and 5.6–58.0 μM for (**9**, **10**, **11**) and (**12**), respectively. However, amsacrine was most active showing IC_{50} values ranging from 0.08 to 3.3 μM.

The cytotoxicity of the quimeric thiazacridines was also evaluated against normal cells (PBMC and V79). The results presented in Table 1 show that the cytotoxic effects of these acridine-thiazolidine derivatives were less pronounced in normal cells, with a selectivity index (SI) for glioblastoma (SF-295) of 3.7, 3.1, 3.1 and 5.9 for **9–12**, respectively. In addition, these compounds were not found to be active in leukaemia, breast carcinoma or normal lymphoblast cells.



Scheme 1. Synthetic route involved in the preparation of acridine-thiazolidine derivatives.

Table 1
Cytotoxic activity of acridine-thiazolidine derivatives on cancer and normal cells, in μg/mL (μM)

Cell line[b]	Histotype	Acridine derivatives[a]					
		9	**10**	**11**	**12**	**3**	Amsacrine[c]
Tumour cell							
HL-60	Promyelocytic leukaemia	>25 (60.9)	>25 (52.6)	>25 (58.0)	>25 (60.3)	1.23 (4.07) 0.98–1.55	0.03 (0.08) 0.01–0.09
K-562	Myeloblastoid leukaemia	>25 (60.9)	>25 (52.6)	>25 (58.0)	>25 (60.3)	Nd	Nd
CEM	Lymphoblastic leukaemia	>25 (60.9)	>25 (52.6)	>25 (58.0)	>25 (60.3)	Nd	Nd
HCT-8	Colon carcinoma	3.1 (7.4) 2.4–3.8	5.3 (11.2) 4.5–6.3	3.6 (8.3) 3.0–4.3	2.3 (5.6) 1.8–3.0	Nd	0.1 (0.3) 0.03–0.3
HCT-15	Colon carcinoma	11.9 (29.2) 7.5–19.0	9.4 (19.8) 4.9–18.0	9.3 (21.5) 6.6–13.1	24.0 (58.0) 13.4–43.2	Nd	0.1 (0.3) 0.1–1.7
SW-620	Colon carcinoma	11.0 (26.8) 6.7–18.1	16.9 (35.5) 11.2–23.3	8.7 (20.1) 5.8–13.1	12.9 (31.3) 7.8–19.4	Nd	0.1 (0.3) 0.1–0.2
COLO-205	Colon carcinoma	19.1 (46.4) 8.6–42.3	>25 (52.6)	2.7 (6.3) 1.8–4.1	9.5 (23.0) 5.5–16.4	Nd	0.4 (1.1) 0.4–2.0
MDA-MB-231	Breast carcinoma	>25 (60.9)	Nd	>25 (58.0)	Nd	Nd	Nd
HS-578-T	Breast carcinoma	>25 (60.9)	Nd	>25 (58.0)	Nd	Nd	Nd
MX-1	Breast carcinoma	>25 (60.9)	Nd	>25 (58.0)	Nd	Nd	Nd
PC-3	Prostate carcinoma	7.3 (17.7) 5.5–9.5	4.1 (8.6) 2.4–6.8	5.6 (13.0) 3.7–8.5	11.2 (27.1) 6.1–20.8	Nd	1.3 (3.3) 0.5–3.2
DU-145	Prostate carcinoma	>25 (60.9)	Nd	4.1 (9.5) 3.5–4.8	Nd	Nd	0.10 (0.3) 0.06–0.18
SF-295	Glioblastoma	3.2 (7.8) 2.6–4.0	3.4 (7.2) 2.8–4.1	2.5 (5.8) 1.8–2.8	2.3 (5.6) 1.8–2.9	16.52 (54.68) 11.60–23.52	0.18 (0.5) 0.08–0.43
OVCAR-8	Ovarian carcinoma	8.1 (19.7) 5.6–11.7	17.7 (37.2) 13.4–23.4	2.5 (5.7) 1.8–3.5	3.9 (9.3) 2.6–5.8	7.04 (23.30) 5.73–8.66	0.5 (1.4) 0.3–0.9
UACC-62	Melanoma	8.6 (21.1) 5.3–14.11	24.1 (50.7) 17.8–32.6	12.5 (29.0) 8.4–18.7	3.9 (9.5) 2.7–5.6	Nd	Nd
MDA-MB-435	Melanoma	6.4 (15.6) 4.9–8.2	12.3 (25.9) 10.2–14.9	9.6 (22.2) 7.7–11.8	5.8 (14.1) 4.9–7.0	Nd	Nd
Normal cells							
PBMC[d]	Peripheral lymphoblast	>25 (60.9)	>25 (52.6)	>25 (58.0)	>25 (60.3)	Nd	8.5 (21.7) 6.8–9.9
V79	Lung fibroblasts[e]	11.7 (28.5) 7.8–18.5	10.7 (22.6) 6.4–18.1	7.7 (17.9) 5.7–10.5	13.5 (32.7) 9.1–20.1	Nd	2.3 (5.8) 1.7–3.6

[a] Data are presented as IC_{50} values in μg/mL (μM) and as the 95% confidence interval obtained by nonlinear regression for all of the cell lines from two independent experiments, performed in duplicate, after 72 h of incubation.
[b] Cell survival was evaluated by the MTT assay, as reported in the Section 4.
[c] Amsacrine was used as a positive control.
[d] Cell survival was evaluated by the Alamar blue assay, as reported in the Section 4.
[e] Chinese hamster cell line. Nd: not determined.

The selectivity index of amsacrine to glioblastoma was of 11.6 showing a greater selectivity for tumour cells compared with thiazacridines. The SI was calculated with the following formula: SI = IC_{50}(V79)/IC_{50}(SF-295).

Although the thiazacridines were shown to be less active and less selective than amsacrine, the selectivity for solid tumour cell lines is interesting and deserves attention as a target for study. In fact, some compounds, such as bisannulated acridines, have been shown to have solid tumour-selective cytotoxicity.[38] Some aminoderivatives of azapyranoxanthenone have shown greater cytotoxic potential against colon tumour cells than against leukaemia cells.[39] However, the basis for this solid tumour selectivity remains unclear.

None of the compounds described here were able to cause haemolysis in mouse erythrocytes, even at the highest concentration (200 μg/mL) (data not shown). The absence of lytic effects suggests that the cytotoxicity of these compounds is not related to membrane disruption and is likely related to more specific cellular pathways. The target could be DNA, as has been observed for acridine compounds.[40,41]

2.2.2. DNA interaction assay

Results from the dsDNA biosensor show positive interactions for all of the acridines, as represented by the appearance of guanosine (E_p = 0.8 V) and adenosine (E_p = 1.0 V) peaks (Figs. 1A–4A). This clearly demonstrates that damage from the compounds caused a distortion of the double helix and exposure of the bases to oxidation. In the experiments using ssDNA in solution, a decrease of the oxidation waves of the nucleobases, guanosine and adenosine, with eventual anodic potential shifts (mainly for **11**), were observed, confirming the interaction with DNA (Figs. 1B–4B).

3. Conclusion

The acridine-thiazolidines derivatives herein investigated showed promising cytotoxic activity. The synthesis of the quimeric compounds is worthy once there was an increase of cytotoxic activity compared to precursors. Although less active and selective than the positive control amsacrine, all of them exhibited relatively high cytotoxicity, predominantly on colon carcinoma and glioblastoma tumour cell lines, whereas no activity on leukaemia, breast carcinoma, or normal lymphoblast cells was observed. Taking into account the selectivity for cells of solid tumours, as well as, the positive interaction with the DNA shown in the electrochemical tests, we propose that the modified acridine-thiazolidines could be promising key structures in anticancer drug development.

4. Experimental part

4.1. Chemistry

The melting points were measured in capillary tubes on a Buchi (or Quimis) apparatus. Thin layer chromatography was performed on silica gel plates from Merck (60_{F254}). The infrared spectra of 1% KBr pellets were recorded on a Bruker IFS66 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AC 300 P spectrophotometer using DMSO-d_6 as the solvent, with tetramethylsilane as an internal standard. Electronic impact mass spectra were measured at 70 eV on a Finnigan GCQ Mat Quadrupole Ion-Trap. The MS data fully agreed with the proposed structures.

The chemical data on 5-acridin-9-ylmethylene-3-(4-methyl-benzyl)-thiazolidine-2,4-dione **9**, 5-acridin-9-ylmethylene-3-(4-bromo-benzyl)-thiazolidine-2,4-dione **10**[42] and 5-(acridin-9-ylmethylene)-3-(4-fluoro-benzyl)-1,3-thiazolidine-2,4-dione **12**[43] are reported elsewhere.[42,43] Thiazolidine-2,4-dione was N-(3)-alkylated in the presence of potassium hydroxide, which enabled the thiazolidine potassium salt to react with substituted benzyl halide in a hot alcohol medium.

4.1.1. (5*Z*)-5-(Acridin-9-ylmethylene)-3-(4-chloro-benzyl)-1,3-thiazolidine-2,4-dione 11

4-Chloro-benzylthiazolidine (0.9 mmol) and 3-acridin-9-yl-2-cyano-acrylic acid ethyl ester (0.9 mmol) were dissolved in absolute ethanol (8 mL). The solution was refluxed for 4 h in the presence of a small amount of piperidine as a catalyst. The precipitate obtained was filtered and washed with water. $C_{24}H_{15}ClN_2O_2S$. Yield 51%. Mp 204–206 °C. TLC R_f 0.73, (*n*-hexane/ethyl acetate 6:4). IR cm^{-1} (KBr) *ν* 1748, 1695, 1624, 1379, 1334, 1149, and 760. ^{1}H NMR (*δ* ppm, DMSO-d_6) 4.88 (s, CH_2); 7.46 (s, 4H benzyl), 7.69 (dt, 2H *2,7*-acridin, *J* = 7.8 and 1.2 Hz), 7.92 (dt, 2H *3,6*-acridin, *J* = 7.8 and 1.2 Hz), 8.15 (d, 2H *1,8*-acridin, *J* = 8.4 Hz), 8.24 (d, 2H *4,5*-acridin, *J* = 8.4 Hz), 8.79 (s, 1H, ethylene). ^{13}C NMR (DMSO-d_6, DEPT): *δ* 44(CH2), 122.3(2C), 125.6 (2CH), 126.9 (2CH), 128.5 (2CH), 129 (C), 129.7 (C), 129.9 (2CH), 130.7 (2CH), 131.9 (C), 132.5 (C), 134.2 (C), 138.0 (2C), 148 (2CH), 164 (CO), 166.8 (CO). Ms EI 70 eV, *m*/*z* (%) 430 (M^+ 2.5), 305(20.3), 235(100), 234(75.1), 231(80.1), 203(16.8), 190(16.9), and 125(28.8).

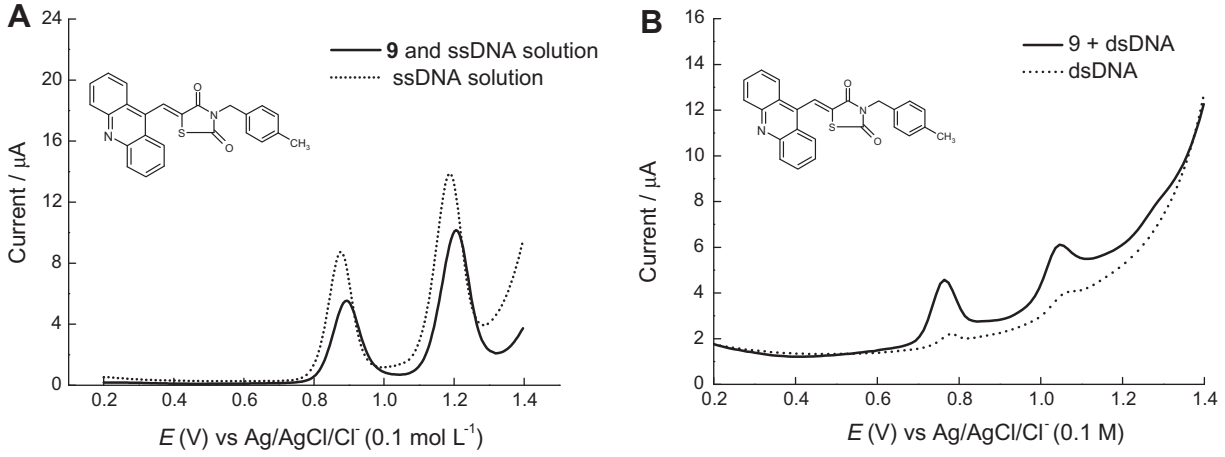


Figure 1. (A) Differential pulse voltammogram (DPV) at pH 4.5 for the oxidation of **9** attached to the surface of carbon paste electrode (CPE) in the presence of a ssDNA solution (1.0×10^{-4} M). (B) Differential pulse voltammogram, at pH 4.5, of the dsDNA biosensor in the absence and in the presence of **9** in solution (1.0×10^{-4} M). Equilibration time = 5 s, ΔE_s = 2 mV, ΔE_{dpv} = 50 mV, v = 5 mV s^{-1}.

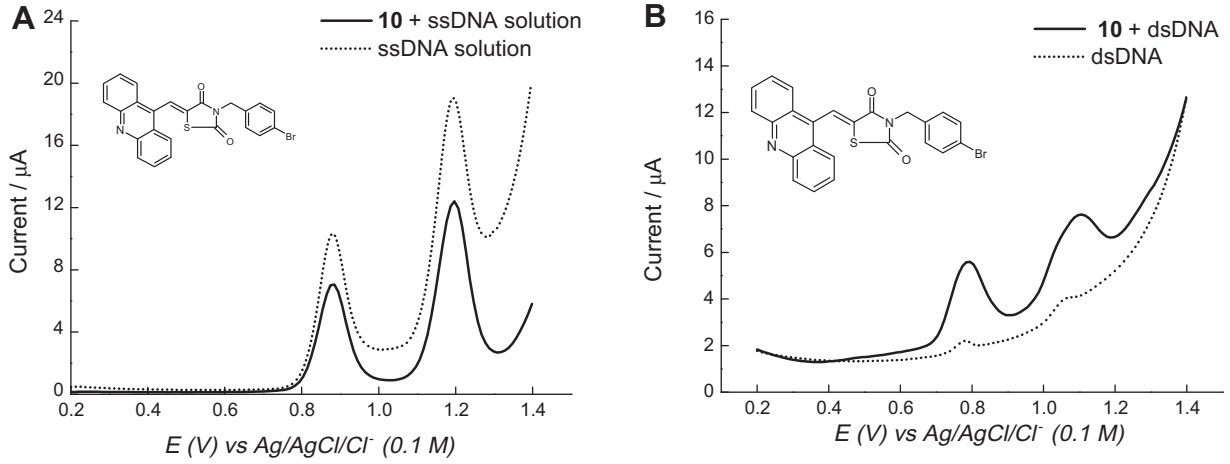


Figure 2. (A) Differential pulse voltammogram at pH 4.5 for the oxidation of **10** attached to the surface of CPE in the presence of a ssDNA solution (1.0×10^{-4} M). (B) Differential pulse voltammogram at pH 4.5 of the dsDNA biosensor in the absence and in the presence of **10** in solution (1.0×10^{-4} M). Equilibration time = 5 s, ΔE_s = 2 mV, ΔE_{dpv} = 50 mV, v = 5 mV s^{-1}.

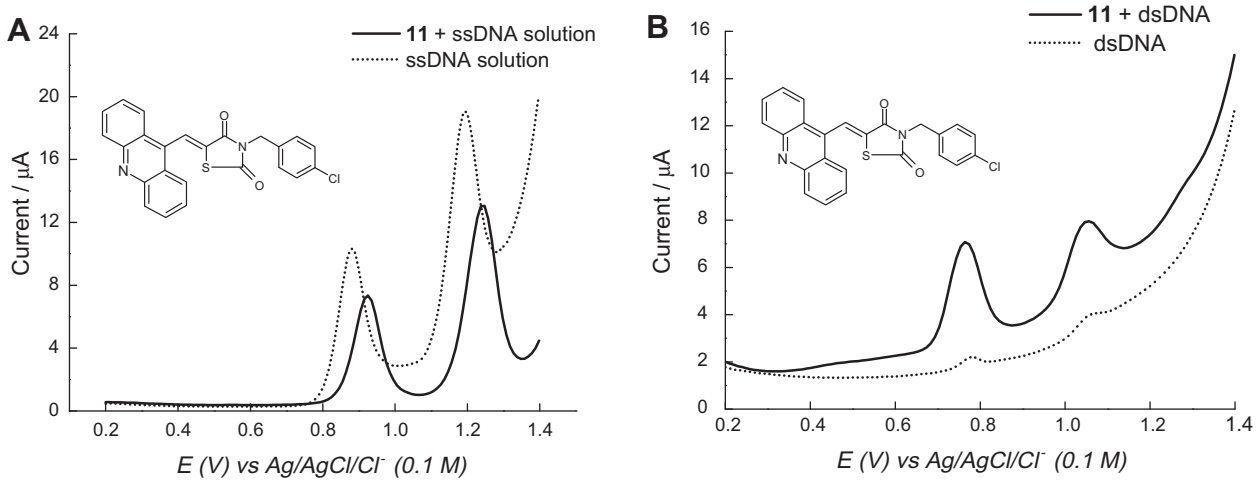


Figure 3. (A) Differential pulse voltammograms at pH 4.5 for the oxidation of **11** attached to the surface of CPE in the presence of a ssDNA solution (1.0×10^{-4} M). (B) Differential pulse voltammogram at pH 4.5 of the dsDNA biosensor in the absence and in the presence of **11** solution (1.0×10^{-4} M). Equilibration time = 5 s, ΔE_s = 2 mV, ΔE_{dpv} = 50 mV, v = 5 mV s^{-1}.

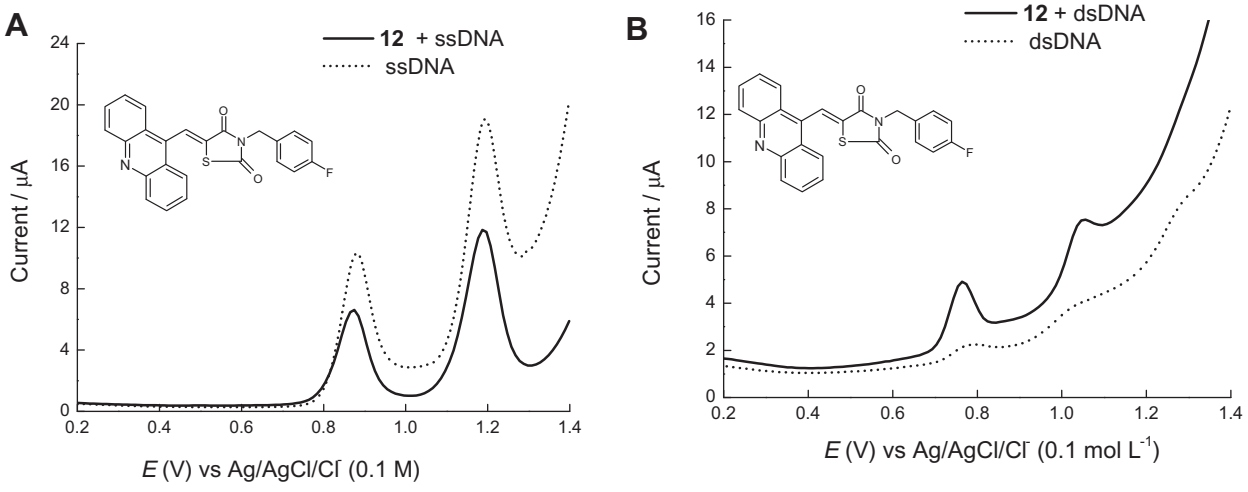


Figure 4. (A) Differential pulse voltammogram at pH 4.5 for the oxidation of **12** attached to the surface of CPE in the presence of a ssDNA solution (1.0×10^{-4} M). (B) Differential pulse voltammogram at pH 4.5 of the dsDNA biosensor in the absence and in the presence of **12** in solution (1.0×10^{-4} M). Equilibration time = 5 s, ΔE_s = 2 mV, ΔE_{dpv} = 50 mV, v = 5 mV s^{-1}

4.2. Biological

4.2.1. MTT assay

The cytotoxic effects of the synthesised compounds were evaluated against the following human cancer cell lines, all obtained from the National Cancer Institute, Bethesda, MD, USA: HL-60 (promyelocytic leukaemia), K-562 (myeloblastoid leukaemia), CEM (lymphoblastic leukaemia), HCT-8 (colon carcinoma), HCT-15 (colon carcinoma), SW-620 (colon carcinoma), COLO-205 (colon carcinoma), MDA-MB-231 (breast carcinoma), HS-578-T (breast carcinoma), MX-1 (breast carcinoma), PC-3 (prostate carcinoma), DU-145 (prostate carcinoma), SF-295 (glioblastoma), OVCAR-8 (ovarian carcinoma), UACC-62 (melanoma), and MDA-MB-435 (melanoma). Chinese hamster lung fibroblasts (V79 – normal cells), kindly provided by Dr. J.A.P. Henriques (Federal University of Rio Grande do Sul, Porto Alegre, Brazil), were also used. The cell lines were maintained in RPMI-1640 medium (cancer cells) or MEM with Earle's salts (V79 cells) supplemented with 10% foetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C with 5% CO_2.

Cell growth was quantified by the ability of living cells to reduce a yellow dye, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), to a purple formazan product.[37] For all of the experiments, the cells were seeded in 96-well plates (0.7×10^5 cells/well for adherent cells and 0.3×10^6 cells/mL for suspended cells). After 24 h, the compounds (0.048–25 μg/mL), dissolved in DMSO, were added to each well (using an HTS (high-throughput screening) Biomek 3000, Beckman Coulter, Inc. Fullerton, California, USA) and incubated for 72 h. Amsacrine (Sigma Aldrich Co., St. Louis, MO, USA) was used as a positive control. At the end of the incubation, the plates were centrifuged, and the medium was replaced by fresh medium (150 μL) containing 0.5 mg/mL MTT. After 3 h, the formazan product was dissolved in 150 μL DMSO, and the absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter, Inc., Fullerton, California, USA). The substance effect was quantified as the percentage of the control absorbance at 595 nm.

4.2.2. Alamar blue assay

The cytotoxic effects of the synthesised compounds were evaluated against PBMC (peripheral blood mononuclear cells) from healthy donors using the Alamar blue assay.[44] Heparinised blood (from healthy, non-smoker donors who had not taken any drug for at least 15 days prior to sampling) was collected, and the PBMC were isolated by a standard method of density-gradient centrifugation using Ficoll-Hypaque. PBMC were cultivated in RPMI-1640 medium supplemented with 20% foetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C with 5% CO_2. Phytohaemagglutinin (2%) was added at the beginning of the culture period.

Briefly, the PBMC were plated in 96-well plates (3×10^5 cells/mL in 100 mL of medium). After 24 h, the compounds (0.048–25 μg/mL), dissolved in DMSO, were added to each well (using an HTS Biomek 3000, Beckman Coulter, Inc., Fullerton, California, USA) and incubated for 72 h. Amsacrine (Sigma Aldrich Co., St. Louis, MO, USA) was used as a positive control. At 24 h before the end of the incubation, 10 mL of a stock solution (0.312 mg/mL) of Alamar blue (resazurin, Sigma Aldrich Co., St. Louis, MO, USA) was added to each well. The absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter, Inc., Fullerton, California, USA), and the effect of the drug was quantified as the percentage of the control absorbance at 570 and 595 nm.

4.2.3. Haemolysis assay

The test was performed in 96-well plates using a 2% mouse erythrocyte suspension in 0.85% NaCl containing 10 mM $CaCl_2$ following the method described by Costa-Lotufo et al. (2002).[45] The diluted compounds were tested at concentrations ranging from 1.5 to 200 mg/mL. Triton X-100 (1%) was used as a positive control. After incubation at room temperature for 30 min followed by centrifugation, the supernatant was removed, and the liberated haemoglobin was measured spectrophotometrically at 540 nm.

4.3. DNA interaction assay

4.3.1. Electrochemical approaches

The following experiments were conducted to evaluate the interaction of acridine-thiazolidine derivatives with DNA by electrochemical methods. Electrochemical experiments including differential pulse voltammetry (DPV) were performed using an Autolab (Echo-Chemie, Utrecht, Netherlands) PGSTAT 20 or PGSTAT-30. The working electrodes were a BAS (Bioanalytical Systems, West. Lafayette, IN, USA) GC electrode of 3-mm diameter or a carbon paste electrode and a carbon paste electrode modified with acridine-thiazolidine derivatives. The counter electrode was a platinum coil, and the reference electrode was Ag|AgCl, Cl^- (0.1 M); all electrodes were contained in a single-compartment electrochemical cell with a 10 mL capacity. The optimised differential pulse voltammetry parameters were as follows: pulse amplitude (ΔE_{sw}) of 50 mV, pulse width of 70 ms and a scan rate of 5 mV s^{-1} (using a step potential [ΔE_s] of 2 mV). The glassy carbon electrode was polished with alumina on a polishing felt (BAS polishing kit). After mechanical cleaning, the electrochemical pretreatment of the glassy carbon electrode involved a sequence of five cyclic potential scans from 0 to +1.4 V in acetate buffer at pH 4.5. All of the experiments were carried out at room temperature (25 ± 1 °C).

4.3.1.1. Preparation of the dsDNA-GC biosensor and its interaction with acridine-thiazolidine derivatives. The electrochemical procedure for the investigation of the acridine-thiazolidine derivatives and dsDNA interaction involved three steps: preparation of the electrode surface, immobilisation of the dsDNA gel and voltammetric transduction. The GC electrode was first polished with alumina, using a Metrohm felt-polishing pad, until the surface displayed a mirror-like appearance. The electrode was then electrochemically pretreated with a sequence of five cyclic potential scans from 0 to +1.4 V versus Ag|AgCl, Cl^- (0.1 M) in acetate buffer,[28] washed thoroughly with distilled/deionised water, dried and placed in an upright position in the cell.

To immobilise the dsDNA (calf thymus, type 1), the surface of the electrode was coated with 10 μL of calf thymus DNA solution (containing 12.0 mg of dsDNA in 1.0 mL of acetate buffer). The quantity (0.36 mg) of dsDNA employed was estimated to be sufficient to cover the entire surface of the GC electrode.[28] The dsDNA was allowed to dry at room temperature under a stream of nitrogen. Immediately after drying, 20 μL of an ethanolic solution of the acridine-thiazolidine derivatives (10^{-4} M) was added with sequential drying. The prepared biosensor was then put into the appropriate cell, covered with 5 mL of aqueous acetate buffer and analysed at $E = 0$ to 1.4 V.[28] For each series of experiments, an identical dsDNA-GC electrode was prepared as a reference blank as a control; this electrode was not treated with substrate but received the same pre- and post-treatments as the test electrode.

The procedure produced a thick-layer dsDNA-modified electrode. Because uniform coverage of the electrode surface had been achieved, any new peaks observed in the presence of the additive were due solely to the interaction of the analyte with the DNA film not from the diffusion process in solution.[29]

4.3.1.2. Preparation of the carbon paste electrode modified with acridine-thiazolidine derivatives. Analyses of the interaction between test substances and ssDNA are generally

performed in solution. Due to the insolubility of acridines in aqueous solution, it was necessary to adapt a methodology to analyse them. This one consisted of incorporating acridine-thiazolidine derivatives in carbon paste.[46] Activated graphite (45 mg) was mixed with 1 mL of an ethanol solution of the acridine-thiazolidine samples (10^{-4} mol L^{-1}) and stirred for approximately 20 min until the solvent was completely evaporated. To ensure complete evaporation, the mixture was subjected to a nitrogen atmosphere. Next, 20 μL of commercial mineral oil was added and mixed strongly to achieve a uniform texture. In this case, a blank (control) was also produced by the addition of ethanol only (no substance) to graphite and a subsequent mixture with mineral oil.

4.3.1.3. Preparation of ssDNA and its interaction with acridine-thiazolidine derivatives. Single-stranded DNA (ssDNA) was prepared by dissolving 3.0 mg of dsDNA in 1.0 mL of hydrochloric acid (1 M) and heating for 1 h until complete dissolution. This treatment was followed by neutralising the solution with 1.0 mL of sodium hydroxide (1 M) and 9 mL of acetate buffer was then added.[28,29]

Freshly prepared ssDNA solution was added to the cell, and single-scan DPV experiments were conducted in the range of 0 to +1.4 V versus Ag|AgCl, Cl^- (0.1 M). Two peaks corresponding to the oxidation of the guanosine and adenosine bases appeared at potentials of +0.815 and +1.131 V, respectively. To ensure reproducibility, this assay format was repeated at least three times, and the oxidation current and potential of the bases were very similar (rsd of 5%). After this process, the carbon paste electrode modified with acridine-thiazolidine derivatives was inserted into the ssDNA solution, and the DPV experiment was performed. An unmodified carbon paste electrode was also employed in the DPV experiments involving the ssDNA solution and was used for comparison.

4.3.1.4. Statistical analysis. The IC_{50} values and their 95% confidence intervals (CI 95%) were obtained by nonlinear regression using the GRAPHPAD program (Intuitive Software for Science, San Diego, CA, USA).

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Supplementary data

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