J. Chem. Sci. Vol. 128, No. 2, February 2016, pp. 269–277.
DOI 10.1007/s12039-015-1023-7

Unexpected regiospecific formation and DNA binding of new 3-(acridin-9-yl)methyl-2-iminothiazolidin-4-ones

JÁN IMRICH[a]*, DANICA SABOLOVÁ[b], MÁRIA VILKOVÁ[a] and JÚLIA KUDLÁČOVÁ[b]
[a]Department of Organic Chemistry [b]Department of Biochemistry, Institute of Chemistry, Faculty of Science, P J Šafárik University, Moyzesova 11, 041 01 Košice, Slovakia
e-mail: jan.imrich@upjs.sk

MS received 18 June 2015; revised 26 October 2015; accepted 7 December 2015

Abstract. New 3-(acridin-9-yl)methyl-2-substituted imino-1,3-thiazolidin-4-ones were regiospecifically synthesized from unstable (acridin-9-yl)methyl thioureas and methyl bromoacetate (MBA) or bromoacetyl bromide (BAB). Unexpected formation of only one thiazolidinone regioisomer with both the reagents was due to a new mechanism involving a transient spiro 9,10-dihydroacridine intermediate. These results are in contrast with the reactions of acridin-9-yl thioureas with MBA/BAB that afforded two different thiazolidinone regioisomers with these reagents. UV-vis titrations, CD spectra, and fluorescence quenching have shown that new products intercalated into calf thymus (CT) DNA, and displaced ethidium bromide (EB) from a CT DNA–EB complex. Intrinsic binding constants, K_b, and Stern-Volmer constants, K_{SV}, were found in the range 0.79×10^5 – 2.85×10^5 M^{-1} and 17950 – 3360 M^{-1}, respectively. The strongest binding affinity was found for an electron-donated 2-(4-methoxyphenylimino)thiazolidinone. Additional evidence for DNA intercalation was obtained from thermal denaturation studies. Gel electrophoresis has proven that thiazolidinone products nicked the supercoiled plasmid DNA in 5.0 μM concentration.

Keywords. CT DNA; acridine; thiazolidinone; intercalation; DNA binding constants; Stern-Volmer constants.

1. Introduction

In the recent years, an increasing attention has been devoted to small aromatic molecules able to interact with nucleic acids by covalent[1] or non-covalent binding resulting from electrostatic interaction, intercalation between the DNA base pairs, or binding to the minor/major DNA groove.[2] Such drugs can modify DNA structure and influence transcription, replication, and repair processes[3] in the cancer cells as well as endothelium, extracellular matrix, and immune system of the host cells.[4,5] As many of them are toxic toward healthy cells as well,[6] novel compounds with better antitumor activity and lower toxicity are highly desired. Among these, new fluorescent acridine derivatives with intercalating properties have been broadly examined as antitumor drugs,[7–10] some even in clinical trials.[11] Tethering of acridines to five- or six-membered *S*,*N*-heterocycles with established biological activity may represent a way to decrease the toxicity and enhance the activity of the target products. Within this family, most studied were 2-imino-1,3-thiazolidin-4-ones conveniently obtainable from haloacyl derivatives and thioureas.[12–14] A drawback of this method was a limited control of regioselectivity, particularly when starting compounds were 1,3-diaryl or 1,3-dialkyl thioureas.[15] In our previous studies of 1-alkyl-3-anthracenyl/acridinyl thioureas[16–20] we have shown that regioselectivity could be successfully controlled by using two bifunctional electrophilic reagents, methyl bromoacetate (MBA) and bromoacetyl bromide (BAB) that afforded, sometimes even regiospecifically, two different 2-imino-1,3-thiazolidin-4-one regiomers with anthracene/acridine in the position 3 and 2 of the heterocycle, respectively, depending on the reagent used. Improved regioselectivity resulted from a different reaction mechanism with each of these reagents. The better one has been observed for 1-alkyl-3-anthracenyl/acridinyl thioureas than for the 1-aryl ones.

As continuation, we wanted to prepare now two novel regioisomeric 2-imino-1,3-thiazolidin-4-ones possessing a more flexible (acridin-9-yl)methyl pharmacophoric moiety instead of the acridin-9-yl one from 1-(acridin-9-yl)methyl-3-substituted thioureas and MBA/BAB. We expected that extended size, motional freedom, and lipophilicity of new acridinylmethyl-thiazolidinone products could improve their bioavailability and accommodation within the target biomolecules. Contrary to our expectations, however, we obtained only a one acridinylmethyl-thiazolidinone regioisomer with both the reagents, so

*For correspondence

we concentrated on a mechanism of thiazolidinone formation in this case. To shed light on a biological function of the introduced acridin-9-ylmethyl moiety, we also examined interactions of the products with nucleic acids by UV-Vis, fluorescence and CD spectroscopy, and electrophoresis.

2. Experimental

2.1 *Preparation of 3-(acridin-9-yl)methyl-2-substituted imino-1,3-thiazolidin-4-ones* ***5a-d***

To an aqueous solution of 1-(acridin-9-yl)methanamine dihydrochloride (**1**) (101 mg, 0.36 mmol),[21] an aqueous solution of sodium carbonate (38 mg, 0.36 mmol) was added. A free amine **2** was extracted with dry benzene (3 × 5 mL), organic layers were combined, dried over magnesium sulfate, filtered, and evaporated to 5 mL. To this well-stirred solution, a corresponding isothiocyanate (0.36 mmol) in 1–2 mL of dry benzene was added at once at r. t. and stirred for 30 min, then methyl bromoacetate (A) (55 mg, 0.034 mL, 0.36 mmol) or bromoacetyl bromide (B) (73 mg, 0.031 mL, 0.36 mmol) was added and the reaction mixture was stirred overnight at room temperature. Afterwards, triethylamine (36 mg, 0,050 mL, 0.36 mmol) was added and the reaction mixture was stirred for another 3 h. A triethylammonium salt that precipitated was filtered off and washed with benzene. Combined organic phases were concentrated *in vacuo* and flash-chromatographed over silica gel (10 g, eluent: cyclohexane/ethyl acetate, 4:1). Appropriate fractions were combined and the solvent was removed *in vacuo*. A solid crude product was purified by crystallization from the mixture ethyl acetate/*n*-heptane.

2.1a *3-(Acridin-9-yl)methyl-2-(4-methoxyphenyl)imino-1,3-thiazolidin-4-one (**5a**)*: Yield 33 mg (22%, bright yellow solid, reagent A); 35 mg (24%, bright yellow solid, reagent B). M.p.: 172−173°C. ^{1}H NMR (600 MHz, DMSO-d$_6$)δ 8.63 (dd, J = 9.0, 1.8 Hz, 2H, H-1′,8′), 8.16 (dd, J = 9.0, 1.8 Hz, 2H, H-4′,5′), 7.84 (ddd, J = 9.0, 6.6, 1.8 Hz, 2H, H-3′,6′), 7.66 (ddd, J = 9.0, 6.6, 1.8 Hz, 2H, H-2′,7′), 6.89 (d, J = 9.0 Hz, 2H, H-3″,5″), 6.76 (d, J = 9.0 Hz, 2H, H-2″,6″), 5.96 (s, 2H, NCH$_2$Acr), 4.02 (s, 2H, H-5), 3.72 (s, 3H, OCH$_3$) ppm. ^{13}C NMR (150 MHz, DMSO-d$_6$)δ 172.0 (C=O), 156.2 (C-4″), 153.9 (C=N), 148.0 (C-4′a,10′a), 140.1 (C-1″), 139.2 (C-9′), 129.9 (C-4′,5′), 129.7 (C-3′,6′), 125.9 (C-2′,7′), 125.3 (C-1′,8′ and C-8′a,9′a), 121.8 (C-2″,6″), 114.5 (C-3″,5″), 55.2 (OCH$_3$), 39.4 (NCH$_2$Acr, C-6), 32.0 (CH$_2$-5). ^{15}N NMR (61 MHz, DMSO-d$_6$) δ -72.3 (N-10′), -123.9 (C=N), -220.0 (N-3). Anal. Calcd. for $C_{24}H_{19}N_3O_2S$: C, 69.71; H, 4.63; N, 10.16. Found: C, 69.55; H, 4.71; N, 10.06.

2.1b *3-(Acridin-9-yl)methyl-2-allylimino-1,3-thiazolidin-4-one (**5b**)*: Yield 42 mg (34%, yellow solid, reagent A); 32 mg (26%, yellow solid, reagent B). M.p. 175−178°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.54 (dd, J = 8.4, 1.2 Hz, 2H, H-1′,8′), 8.23 (dd, J = 8.4, 1.2 Hz, 2H, H-4′,5′), 7.76 (ddd, J = 8.4, 6.8, 1.2 Hz, 2H, H-3′,6′), 7.56 (ddd, J = 8.4, 6.8, 1.2 Hz, 2H, H-2′,7′), 5.88 (s, 2H, NCH$_2$Acr), 5.76 (ddt, J = 16.8, 10.4, 5.6 Hz, 1H, H-2″), 4.98 (ddt, J = 10.4, 3.6, 1.6 Hz, 1H, H$_{cis}$-3″), 4.90 (ddt, J = 16.8, 3.6, 1.6 Hz, 1H, H$_{trans}$-3″), 3.84 (dt, J = 5.6, 1.6 Hz, 2H, H-1″), 3.77 (s, 2H, H-5). ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.5 (C=O), 151.8 (C=N), 148.7 (C-4′a,10′a), 138.2 (C-9′), 134.5 (C-2″), 130.3 (C-4′,5′), 129.6 (C-3′,6′), 125.9 (C-2′,7′; C-8′a,9′a), 125.1 (C-1′,8′), 115.5 (C-3″), 54.1 (C-1″), 39.4 (C-6), 32.3 (C-5). Anal. Calcd. for $C_{20}H_{17}N_3OS$: C, 69.14; H, 4.93; N, 12.09. Found: C, 69.02; H, 4.90; N, 11.95.

2.1c *3-(Acridin-9-yl)methyl-2-phenylimino-1,3-thiazolidin-4-one (**5c**)*: Yield 37 mg (27%, beige solid, reagent A); 37 mg (27%, beige solid, reagent B). M.p. 201−203°C. ^{1}H NMR (600 MHz, DMSO-d$_6$) δ 8.64 (dd, J = 8.4, 1.8 Hz, 2H, H-1′,8′), 8.17 (dd, J = 8.4, 1.8 Hz, 2H, H-4′,5′), 7.84 (ddd, J = 8.4, 6.6, 1.8 Hz, 2H, H-3′,6′), 7.67 (ddd, J = 8.4, 6.6, 1.8 Hz, 2H, H-2′,7′), 7.34 (t, J = 7.2 Hz, 2H, H-3″,5″), 7.11 (t, J = 7.2 Hz, 1H, H-4″), 6.83 (d, J = 7.2 Hz, 2H, H-2″,6″), 5.98 (s, 2H, H-6), 4.02 (s, 2H, H-5). ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 172.1 (C=O), 154.7 (C=N), 148.0 (C-4′a,10′a), 147.3 (C-1″), 139.0 (C-9′), 129.9 (C-3′,6′), 129.8 (C-4′,5′), 129.3 (C-3″,5″), 125.9 (C-2′,7′), 125.3 (C-1′,8′; C-8′a,9′a), 124.4 (C-4″), 120.7 (C-2″,6″), 39.4 (C-6), 32.0 (C-5). ^{15}N NMR (61 MHz, DMSO-d$_6$) δ -72.1 (N-10′), -122.9 (C=N), -219.8 (N-3). Anal. Calcd. for $C_{23}H_{17}N_3OS$: C, 72.04; H, 4.47; N, 10.96. Found: C, 71.90; H, 4.29; N, 10.90.

2.1d *3-(Acridin-9-yl)methyl-2-methylimino-1,3-thiazolidin-4-one (**5d**)*: Yield 43 mg (37%, beige solid, reagent A), 49 mg (42%, beige solid, reagent B). M.p. 210−211°C. ^{1}H NMR (600 MHz, DMSO-d$_6$) δ 8.51 (dd, J = 8.4, 1.2 Hz, 2H, H-1′,8′), 8.15 (dd, J = 8.4, 1.2 Hz, 2H, H-4′,5′), 7.83 (ddd, J = 8.4, 6.6, 1.2 Hz, 2H, H-3′,6′), 7.63 (ddd, J = 8.4, 6.6, 1.2 Hz, 2H, H-2′,7′), 5.82 (s, 2H, H-6), 3.98 (s, 2H, H-5), 3.03 (s, 3H, CH$_3$). ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 171.9 (C=O), 153.6 (C=N), 148.0 (C-4′a,10′a), 139.3 (C-9′),

129.8 (C-3′,6′), 129.7 (C-4′,5′), 125.8 (C-2′,7′), 125.3 (C-1′,8′; C-8′a,9′a), 39.0 (C-6), 37.8 (CH_3), 31.7 (C-5). ^{15}N NMR (61 MHz, DMSO-d_6) δ -222.4 (N-3), N-10′ and C=N cross-peaks not found because of their low intensity. Anal. Calcd. for $C_{18}H_{15}N_3OS$: C, 67.27; H, 4.70; N, 13.07. Found: C, 67.14; H, 4.62; N, 13.17.

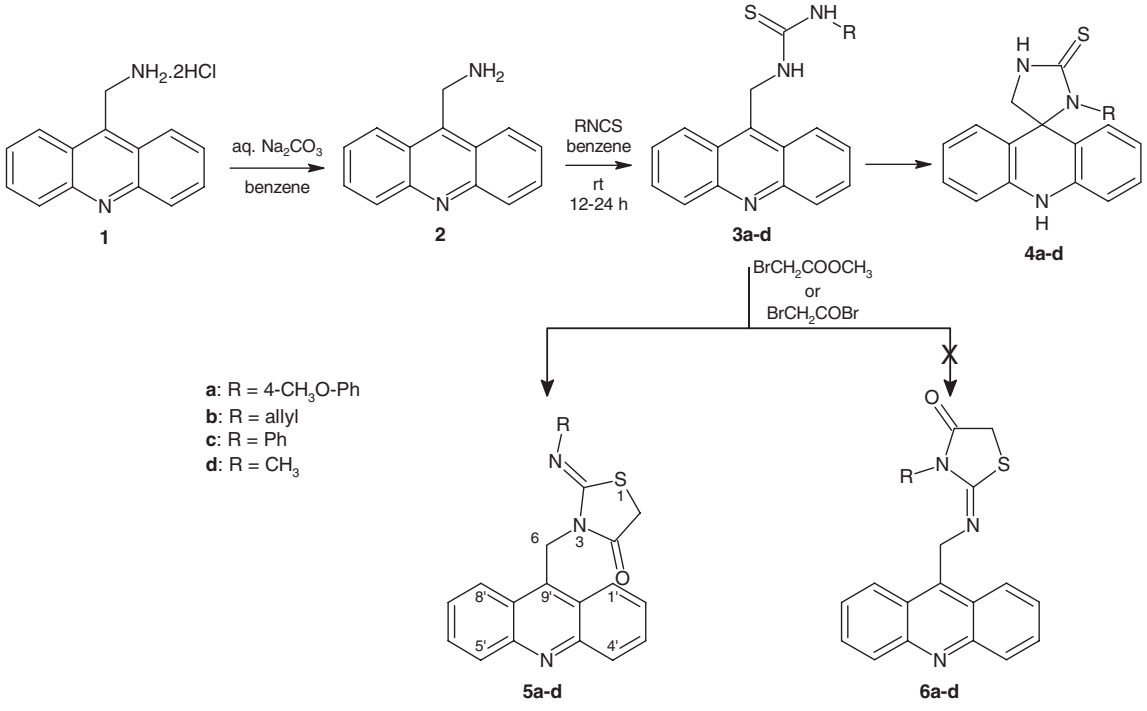
3. Results and Discussion

3.1 *Synthesis and characterization of 3-(acridin-9-yl) methyl-2-R-imino-1,3-thiazolidin-4-ones* ***5a–d***

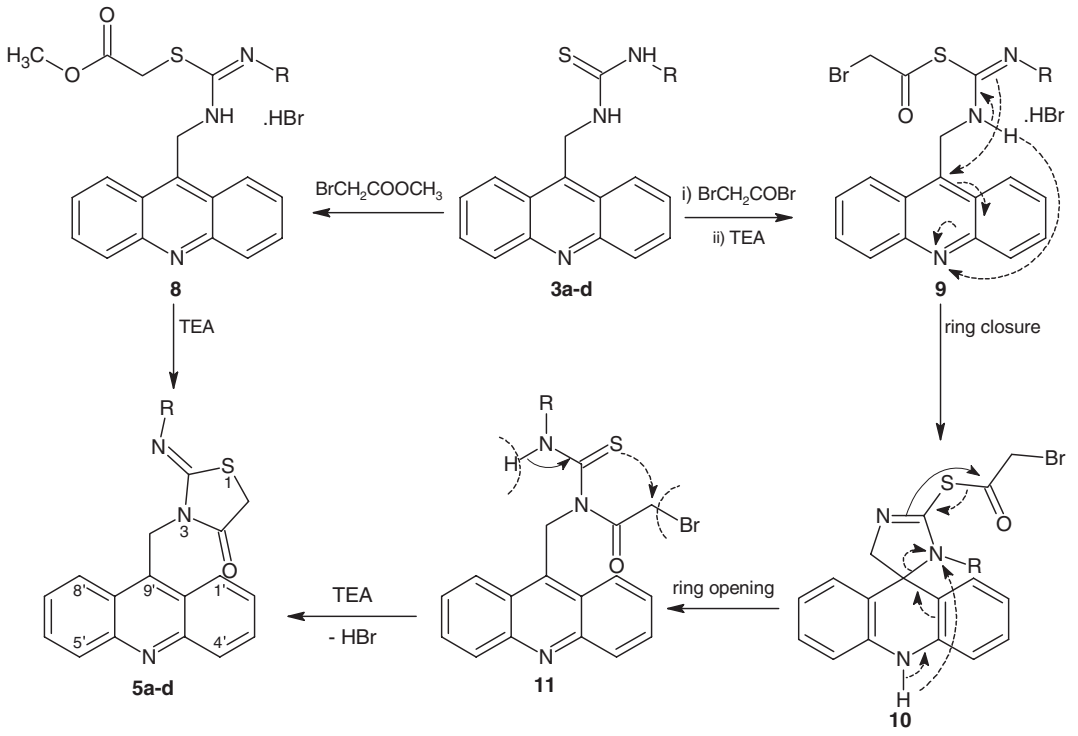
To prepare target thiazolidinones, starting 1-(acridin-9-yl)methanamine (**2**) obtained from its dihydrochloride **1**[21] and aqueous sodium carbonate reacted first with isothiocyanates R-NCS (R = 4-CH_3O-phenyl (a), allyl (b), phenyl (c), and methyl (d)) at room temperature to give reactive intermediate 1-(acridin-9-yl)methyl-3-substituted thioureas **3a**−**d** (scheme 1). As described in our recent paper,[22] these spontaneously and readily cyclized to spiro[dihydroacridine-9′[10′*H*],5-imidazolidine]-2-thiones **4a**−**d** upon standing in chloroform. In this study, however, we were interested in regioselective transformation of these thioureas to novel regioisomeric 3-(acridin-9-yl)methyl-2-R-imino-1,3-thiazolidin-4-ones **5a**−**d** and 2-(acridin-9-yl)methylimino-3-R-1,3-thiazolidin-4-ones **6a**−**d**, analogous to products of 1-alkyl-3-(acridin-9-yl)thioureas, by using the MBA or BAB reagent. Novel products **5** and **6** were hoped to possess a better biological activity than the previous ones.[16–18]

To suppress undesirable spirocyclization of **3a**−**d** to **4a-d**, intermediate thioureas **3a**−**d** were set into reactions with MBA or BAB 30 min after mixing of the amine **2** with the isothiocyanates R-NCS, when sufficient amount of thioureas had already been formed (proven by NMR in ref.[22], scheme 1). Contrary to our expectation, only one type of thiazolidinones, **5a**−**d**, was obtained with both the reagents and no regiomeric thiazolidinones **6a**−**d** have been isolated or even observed in NMR spectra of the crude reaction mixtures. The reaction rates of **3a**−**d** with MBA/BAB were significantly different - BAB reacted much faster as indicated by an immediate color change of the reaction mixture upon addition of BAB.

In our previous studies[16–18] we succeeded to isolate intermediate isothiourea hydrobromides analogous to **8** (with MBA) or **9** (with BAB) (scheme 2). In this work, however, due to the complexity of the reaction mixtures, the intermediate isothioureas **8** and **9** (scheme 2) could not be isolated but readily cyclized to target 3-(acridin-9-yl)methyl-2-(R-imino)-1,3-thiazolidin-4-ones **5a**−**d** in 22–42% yields after chromatography and crystallization, based on the starting amine **1**. The products of both the reagents MBA and BAB had identical melting points and NMR spectra. Lower yields of thiazolidinones **5a**−**d** were due to unavoidable parallel spirocyclization of thioureas **3a**−**d** to the spiro products



Scheme 1. Intended transformation of the thioureas **3a**−**d** with MBA or BAB to regioisomeric thiazolidinones **5a**−**d** and **6a**−**d**.



Scheme 2. Mechanism of the reactions of thioureas **3a**–**d** with MBA and BAB leading to the same products **5a**–**d** (R = 4-CH_3O-C_6H_4 (a), allyl (b), phenyl (c), methyl (d)).

4a–**d**. ^{1}H NMR was of prime importance for structural assignment confirming the regioisomer **5** that was deduced from a three-bond HMBC correlation between the NCH_2 proton signal and the thiazolidinone C=O and C=N peaks. For the regioisomer **6**, by contrast, such a correlation between NCH_2 proton and C=O carbon would not be possible due to a too long five-bond distance between these atoms (Figure 1). ^{13}C NMR spectra also corroborated the structure **5** and corresponded with model acridinyl thiazolidinones.[16–18] ^{15}N NMR data were in accord with expected values from the literature.[22–25] Acridine N-10 at ca. -72 ppm as well as thiazolidinone 2-C=N at ca. -122 ppm are both typical, downfield shifted, sp^2 nitrogens, whereas thiazolidine 3-N nitrogen of the sp^3 type is distinctly shifted upfield to -220 – -222 ppm.

An apparent enigma of a sole product being produced by two reagents, MBA and BAB, which previously yielded regioisomeric products, though, is easily rationalized and, furthermore, is entirely consistent with the reaction mechanisms previously proposed for these reagents and for the substrates under study.[16–18] In these previous works, in the case of MBA and anthracen-9-yl/acridin-9-yl thioureas, the reaction first occurred by alkylation of the sulfur atom to give corresponding isothioureas analogous to **8**, possessing antracen-9-yl/acridin-9-yl substituent instead of the (acridin-9-yl) methyl substituent shown in scheme 2. Displacement

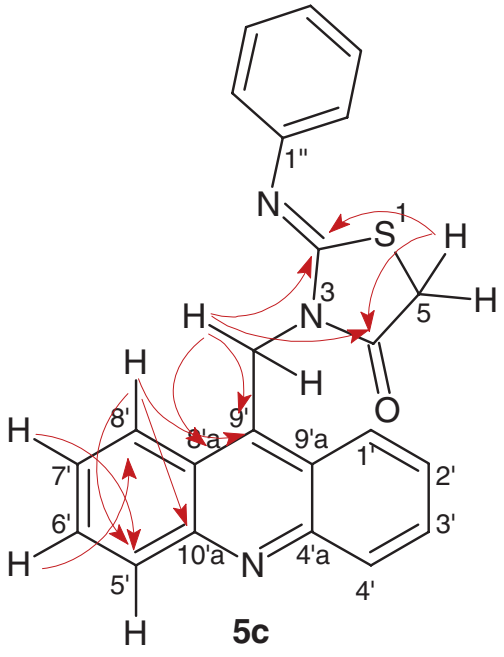


Figure 1. HMBC (→)transfers in 2-phenylimino thiazolidinone **5c**.

of a methoxide ion by amine nitrogen followed then to form the 2-R-imino-3-(anthracen-9-yl/acridin-9-yl)-1,3-thiazolidin-4-ones analogous to **5a**–**d**. Reaction with BAB followed a strikingly different path: acylation of the sulfur atom occurred first, followed by

Table 1. UV-Vis absorption characteristics of thiazolidinones **5a**–**d** and Stern-Volmer constants characterizing the binding of **5a**–**d** to CT DNA.

Compound	R	λ_{max} [nm] Free ligand	λ_{max} [nm] Bound ligand	$\Delta\lambda$ [nm]	K_b[M^{-1}]	Hypochromism [%]	K_{SV} [M^{-1}]
5a	4-CH_3O-C_6H_4	371	374	3	1.20×10^5	15.0	17.95×10^3
5b	allyl	345	346	1	0.79×10^5	21.2	3.36×10^3
5c	Ph	370	374	4	Nd[a]	47.5	3.75×10^3
5d	Me	360	361	1	2.85×10^5	65.0	8.37×10^3

[a] Not determined.

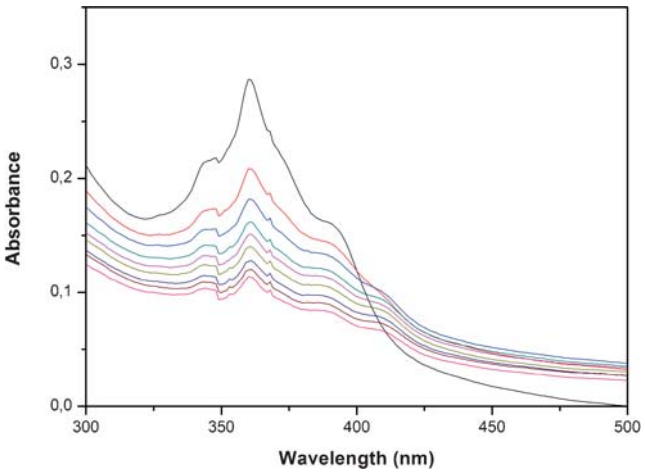


Figure 2. Spectrophotometric titration of 2-methylimino thiazolidinone **5d** (6×10^{-6}M) in the 0.01 M Tris buffer (pH 7.3, 24°C). The absorption descended with increasing concentration of CT DNA from top to bottom (0–16 μM bp, in 2 μM intervals).

acyl migration to imine nitrogen to produce reversed 2-(acridin-9-yl)imino-3-R-thiazolidin-4-ones analogous to **6a**−**d** (again with antracen-9-yl/acridin-9-yl instead of the shown (acridin-9-yl)methyl substituent).[16–18] In the system under study here (scheme 2), as the acyl transfer is slow, the *S*-acylated isothiourea **9**, which results from the reaction with BAB, has time to adopt − if it hasn't already done so − the spirocyclic form **10**, a strongly-favored structure given the above results.[22] The adoption of the spirocyclic form results in CH_2N= being the imine-type nitrogen and as such it is favored to receive the acyl group when it migrates. Following acyl transfer to CH_2N= nitrogen, however, the system must ring open again to an intermediate **11** for further reaction to occur. The resultant thiazolidinone **5** then formed from **11** is the same as that resulting from the reaction with MBA. It is worth noting that spirocyclization is inconsequential in the reaction with MBA as further reaction after *S*-alkylation can only occur for the open-chain form. Furthermore, whether acylation of the sulfur in the reaction with BAB does indeed occur prior to spirocyclisation or afterwards is also immaterial.

3.2 *UV-Vis titration studies*

Spectral titration of thiazolidinones **5a**−**d** was followed by monitoring UV-Vis absorbance changes in the range of 300–500 nm. Upon addition of CT DNA, absorption bands showed a significant hypochromism and a slight red shift (Table 1). The results are similar to those reported for our analogous intercalators, methyl 2-[2-(acridin-9-yl)imino-3-R-4-oxo-1,3-thiazolidin-5-ylidene]acetates (R = *sec*-Bu, *tert*-Bu, 4-Br-C_6H_4) prepared from thioureas and dimethyl acetylenedicarboxylate.[26] The representative UV-vis spectrum of 2-methylimino derivative **5d** is shown in Figure 2 and remaining spectra in Supporting Information (Figures S11–S13). The hypochromism observed is caused by DNA binding of the investigated drugs involving a partial unwinding of the DNA double helix and consequent conformational changes.[27] An absence of the isosbestic point in the titration UV-Vis spectra of **5a-d** is similar to our previous study of acridin-9-ylalkenoic derivatives interaction with thiols[28] and indicates that non-classical interaction is active. The intrinsic binding constants K_b, which we found in the range 0.79×10^5 – 2.85×10^5 M^{-1} (Table 1), correspond well with the data on CT DNA interaction with analogous acridin-9-yl thiosemicarbazones[29] ($K_b = 1.74\times10^4$– 1.0×10^6 M^{-1}) and 9-benzylamino-2-methoxyacridine ($1.42\times10^5M^{-1}$).[30] This indicates a similar mode of interaction based on a common structural element, i.e., the acridine skeleton. As to diversity of the 9-substituents within these three series (2-iminothiazolidinones-this study, -CH=N-NH-C(=S)-NH-R/Ar[29] and benzylamine/amide[30]), a preferential intercalation of our ligands between the DNA base pairs overwhelms the other possible interaction modes, namely major or minor groove binding of the 9-substituent on acridine.

3.3 *Competitive binding of thiazolidinones **5a–d** to the complex CT DNA-EB*

The binding of **5a**–**d** to CT DNA was studied by competitive binding fluorometric experiment with the complex CT DNA – ethidium bromide (EB) (Figure 3 – **5a**,

Figures S14–S16 – **5b-d**). The fluorophore EB has a negligible fluorescence when not bound to DNA which becomes stronger when EB is intercalated between the DNA base pairs. Many quenching studies have been conducted to evaluate substituent and solvent effects on a Stern-Volmer quenching using competitive binding compounds. Fluorescence emission spectra of the EB-DNA complex excited at 510 nm (Figure 3) showed a distinct fluorescence with a characteristic strong emission band at 610 nm pertaining to intercalated EB molecules, which were sufficiently protected by neighbour DNA base pairs from being quenched by external polar solvent molecules such as H_2O.[31] Upon titration of the EB-DNA complex with increasing concentration of **5a**, the EB emission band gradually decreased owing to a competitive binding of non-fluorescent **5a**. Calculated Stern-Volmer constants, K_{SV}, of the tested compounds are given in Table 1. As can be seen, the compound **5a** with the highest K_{SV} value was most strongly bound to CT DNA. Removal of the methoxy group from 2-phenylimino substituent in the derivative **5c** decreased its quenching ability almost five times indicating that electron-donor on the phenyl ring of **5a** contributes to stronger binding to CT DNA. On the other hand, the methyl derivative **5d** proved to be 2.5-fold better quencher and thus a better binder to CT DNA than the allyl derivative **5b**, maybe owing to its smaller volume and tighter accommodation within the CT DNA. A similar fluorescence quenching has also been observed for a new similar quencher acridine glucuronate, with a little greater $K_{SV} = 1.54\times10^4$ M^{-1} owing to a dual binding interaction mode.[32]

3.4 *Thermal denaturation studies*

The binding of a small molecule to DNA stabilizes the helix against the thermal denaturation. A usual mark of this stabilization for a double- to single-stranded form morphing of DNA is a rise of the transition temperature, T_m. The experiment is accomplished by comparing T_m of DNA in the solution without and with the intercalator, whilst monitoring some property dependent on the DNA helical structure, e.g., UV absorbance. Owing to a difference between extinction coefficients of DNA bases in the double-stranded *vs.* single-stranded form at 260 nm, the absorbance increases sharply at T_m as DNA strands separate.[33] We monitored the DNA helix denaturation as a function of temperature by recording absorbance at 260 nm. As shown in Figure 4, the DNA melting experiment revealed T_m of the calf thymus DNA at 68.0°C which increased to 76.0°C in the presence of **5a**. Also T_m values for other derivatives (**5b** – 73.0°C, **5c** - 70.0°C, **5d** - 74.0°C) definitely confirmed a heightened helix stability resulting from intercalation of investigated drugs into CT DNA and their stabilization by $\pi - \pi$ stacking interactions.[19] Interesting was a

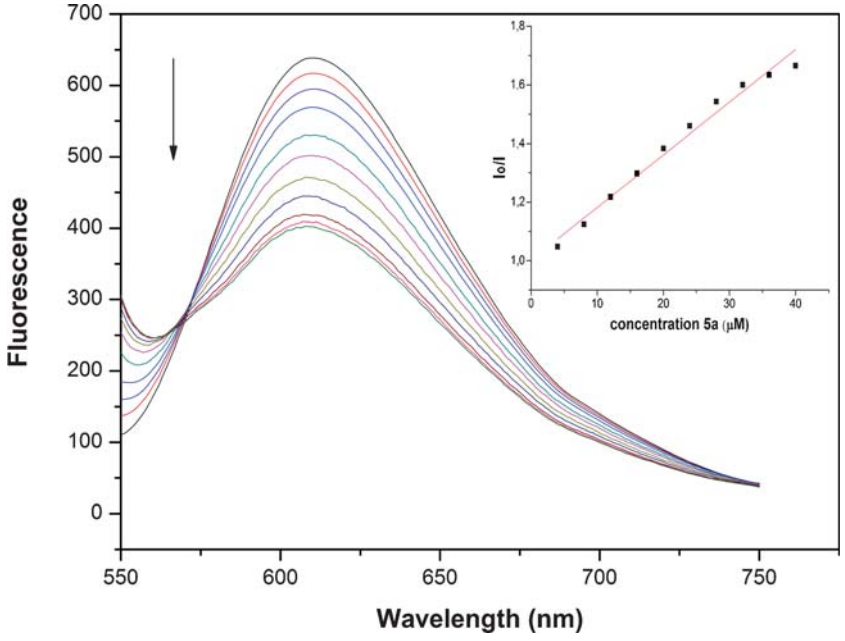


Figure 3. Fluorescence emission spectra of EB bound to DNA in the absence and presence of **5a**; (0–40 μM, in 4 μM intervals), $\lambda_{ex} = 510$ nm, DNA (17 μM), EB (4 μM) in 10 mM Tris (pH 7.3). Inset: Stern-Volmer plot for quenching of EB by **5a**. The fluorescence decreased with increasing concentration of **5a** from top to bottom.

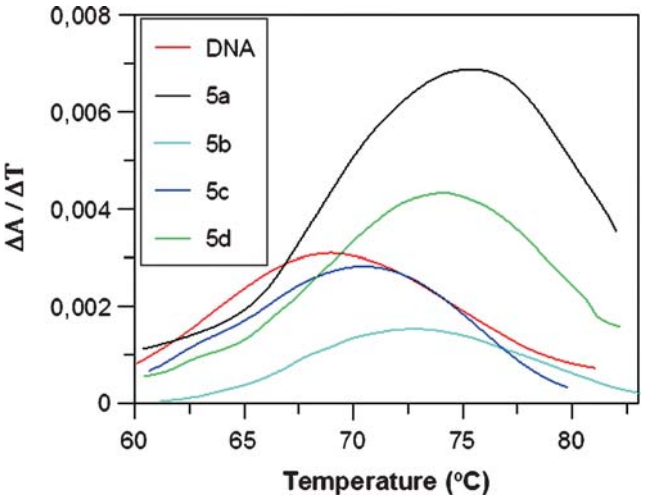


Figure 4. First derivative of helix denaturation curves for CT DNA alone (red line), and in the presence of compounds **5a**, **5b**, **5c**, and **5d**, measured at 260 nm in the BPE buffer, pH 7.3.

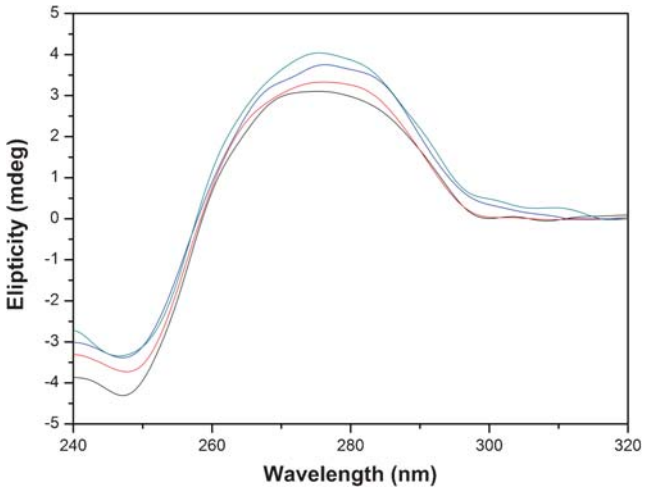


Figure 5. Circular dichroism spectra of CT DNA (17 μM bp) in the absence (black line) and presence (red) of the derivative **5a**: 5 μM (green), 10 μM (red), 15 μM (blue), in 0.01 M Tris buffer (pH 7.4, 24°C).

similar trend of growth of the T_m values and Stern-Volmer constants K_{SV} for the DNA–**5a-d** complexes in the order phenyl → allyl → methyl → *p*-methoxyphenyl indicating a strengthened association upon increased electron donation within the ligand by its substituents on 2-imino nitrogen.

3.5 *CD measurements*

Circular dichroism spectroscopy (CD) is an optical technique measuring a difference in the absorption of the left and right circularly polarized light. The B conformation of CT DNA shows two conservative CD bands, a positive one at 275 nm caused by the base stacking and a negative one at 245 nm corresponding to the right-handed helicity. The CD absorption spectrum of DNA is very sensitive to conformational changes. When small molecules bind to CT DNA by intercalation or covalent binding, conformational modifications of DNA and a significant CD spectral perturbation occur.[34] Here, intensity of the positive band increased and showed a small red shift upon increasing the concentration of **5a**−**d** (5 μM, 10 μM, 15 μM) in CT DNA solution similarly to our recent work on interaction of acridin-9-ylalkenoic derivatives.[28] The observation corresponds to a stabilization of DNA by intercalation.[35] Intensity of the negative band decreased (became more positive) with a slight red shift of about 2.0 nm (**5a**) upon addition of **5a**−**d** (Figure 5). Positive circular dichroic band in the vicinity of 275 nm reflects the helix winding angle such that the higher the intensity, the smaller the winding angle. Similar changes in the CD spectra have been reported and explained diversely. Some authors assume that changes in the spectra reflect a change of the B-like DNA structure toward one possessing contributions from the A-like one.[36,37] We suppose that increase of the CD signal at 275 nm along with the growing acridine complexation is a significant indication of intercalation of our products between the DNA base pairs in accord with other authors.[38,39] Furthermore, a small red shift of the CD band at 245 nm suggests that interactions between aromatic rings of the complex and the base pairs of DNA may be also present.[40]

3.6 *The cleavage of pUC19 DNA*

When the circular plasmid DNA is subjected to electrophoresis, a relatively fast migration is observed for the intact supercoiled form (Form I). If scission occurs just in the one strand (nicking), the supercoils relax

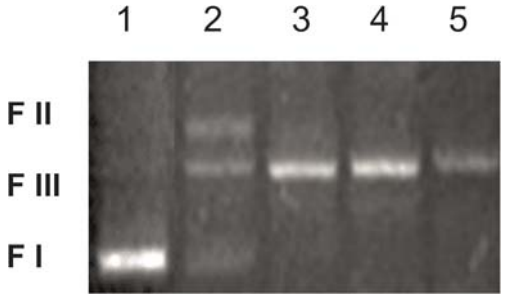


Figure 6. Cleavage of the supercoiled pUC19 DNA (0.5 μg/μL) with the derivative **5d** in the Tris-HCl, 10 mM NaCl buffer at pH 7.0, 37°C. Lane 1: DNA alone; lane 2–5: DNA + **5d** (5, 15, 30, 40 μM). The Forms I, II, III are supercoiled DNA, nicked DNA, and linear open circular DNA, respectively. The electrophoresis was carried out for ~2 h at 78 V.

to generate a slower-moving open circular form (Form II).[41] If both the strands are cleaved, a linear form (Form III) that migrates between the Form I and the Form II should occur.[42] As reported, many organic molecules interacting with DNA can cleave the DNA strands and change the Form I to Form II.[43,44] Our results suggest that all the drugs **5a**−**d** cause nicking of the supercoiled plasmid DNA already at 5.0 μM concentration indicating that the new compounds behave as a chemical nuclease mediating an interconversion of the supercoiled DNA, Form I mainly to the linear open circular DNA, Form III (Figures 6, S17).

4. Conclusion

Four new 3-(acridin-9-yl)methyl-thiazolidin-4-one ligands **5a-d** have been prepared and characterized by NMR, UV-Vis, fluorescence, and CD spectroscopy. Expected regioselective formation of two different 2-imino-1,3-thiazolidin-4-ones with MBA and BAB, 3-(acridin-9-yl)methyl-substituted and 2-(acridin-9-yl)methyl-substituted, respectively, has not been achieved, instead, only the former regioisomer had been obtained. The mechanism of its formation involving a spirocyclic intermediate has been proposed. Titration of the acridinylmethyl-thiazolidinone products with CT DNA afforded intrinsic binding constants K_b in the range 0.79×10^5 – 2.85×10^5 M^{-1} as well as reduction of the peak intensities and bathochromic shifts confirming the binding of the compounds to DNA. A preferential intercalation of **5a-d** ligands between DNA base pairs overwhelms other interaction modes, namely major or minor groove binding of the 9-substituent on acridine. Increase of the T_m values in thermal denaturation experiments as well as CD spectra also indicated that investigated drugs intercalated into CT DNA. Competitive fluorescence binding studies have shown that the target thiazolidinones pushed out ethidium bromide from its complex with DNA. The strongest binding affinity found for 4-methoxyphenyl derivative **5a** emphasized an importance of the electron-dopped aromate for the binding. Electrophoretic studies demonstrated that investigated compounds induced a DNA cleavage activity. In general, the new (acridin-9-yl)methyl thiazolidinones seem to be promising candidates for their further biological examination. The compounds prepared showed physicochemical properties and biological activity comparable with these of our recent 3,6-bis(1,3-thiazolidin-4-one)acridine products.[19]

Supplementary Information (SI)

See Supporting Information for Chemicals, NMR measurements and CHN analysis, plots of ^{1}H and ^{13}C NMR spectra of the compounds **5a-d**, conditions of measurements of UV-Vis, fluorescence, and CD spectra, determination of the thermal denaturation melting points T_m, DNA-cleaving activity, and plots of the UV-Vis and fluorescence titration spectra of ligands with CT DNA. Supplementary Information is available at www.ias.ac.in/chemsci.

Acknowledgements

Financial support from the Slovak Grant Agency VEGA (1/0672/11 and 1/0001/13), Slovak Research and Development Agency (APVV-0280-11) and internal grants VVGS-PF-2013-114, VVGS-2013-127 are gratefully acknowledged. Doc. Dr. Karel D. Klika is thanked for valuable suggestions concerning the reaction mechanism.

References

1. Dervan P B 2001 *Bioorg. Med. Chem.* **9** 2215
2. Nelson S M, Ferguson L R and Denny W A 2007 *Mutat. Res.* **623** 24
3. Hampshire A J and Fox K R 2008 *Biochimie* **90** 988
4. Gibson D 2002 *Pharmacogenomics J* **2** 275
5. Espinosa E, Yamora P, Feliu J and Baron M G 2003 *Cancer Treat. Rev.* **29** 515
6. Slapak C A, Kufe D W 1998 In *Harrison's Principles of Internal Medicine* 14th ed. (New York: McGraw-Hill, Inc.) p. 523
7. Bacherikov V A, Chou T Ch, Dong H J, Chen Ch H, Lin Y W, Tsai T J and Su T L 2004 *Bioorg. Med. Chem. Lett.* **14** 4719
8. Demeunynck M, Charmantray F and Martelli A 2001 *Curr. Pharm. Des.* **7** 1703
9. Srivastava A and Nizamuddin A 2004 *Indian J. Heterocycl. Chem.* **13** 261
10. Kristian P, Bernát J, Imrich J, Danihel I, Suchár G, Chomča I, Hočová S, Bušová T, Guspanová J and Linden A 1996 *Molecules* **1** 181
11. Denny W A and Baguley B C 1994 In *Molecular Aspects of Anticancer Drug-DNA Interaction* (London: Macmillan) p. 270
12. Verma A and Saraf S K 2008 *Eur. J. Med. Chem.* **43** 897
13. Jain A K, Vaidya A, Ravichandran V, Kashaw S K and Agrawal R K 2012 *Bioorg. Med. Chem.* **20** 3378
14. Tripathi A C, Gupta S J, Fatima G N, Sonar P K, Verma A and Saraf S 2014 *Eur. J. Med. Chem.* **72** 52
15. Laurent St. D R, Gao Q, Wu D and Serrano-Wu M H 2004 *Tetrahedron Lett.* **45** 1907
16. Klika K D, Janovec L, Imrich J, Suchár G, Kristian P, Sillanpää R and Pihlaja K 2002 *Eur. J. Org. Chem.* 1248
17. Klika K D, Valtamo P, Janovec L, Suchár G, Kristian P, Imrich J, Kivelä H, Alföldi J and Pihlaja K 2004 *Rapid Commun. Mass Spectrom.* **18** 87

18. Géci I, Valtamo P, Imrich J, Kivelä H, Kristian P and Pihlaja K 2005 *J. Heterocyclic Chem.* **42** 907
19. Janovec L, Sabolová D, Kožurková M, Paulíková H, Kristian P, Ungvarský J, Moravčíková E, Bajdichová M, Podhradský D and Imrich J 2007 *Bioconjugate Chem.* **18** 93
20. Janovec L, Böhm S, Danihel I, Imrich J, Kristian P and Klika K D 2007 *Coll. Czech. Chem. Commun.* **72** 1435
21. Rzeszotarski W and Ledochowski Z 1963 *Ann. Soc. Chim. Polonorum* **37** 1631
22. Vilková M, Prokaiová M and Imrich J 2014 *Tetrahedron* **70** 944
23. Marek R and Lyčka A 2002 *Curr. Org. Chem.* **6** 35
24. Barbry D, Ricart G and Couturier D 1990 *J. Chem. Soc. Perkin Trans.* **2** 133
25. Chen B Ch, von Philipsborn W and Nagarajan K 1983 *Helv. Chim. Acta.* **66** 1537
26. Paulíková H, Vantová Z, Hunáková L, Čižeková L, Čarná M, Kožurková M, Sabolová D, Kristian P, Hamul'aková S and Imrich J 2012 *Bioorg. Med. Chem.* **20** 7139
27. Shahabadi N and Mohammadi S 2012 *Bioinorg. Chem. Appl.* ID571913 1
28. Salem O, Vilkova M, Plsikova J, Grolmusova A, Burikova M, Prokaiova M, Paulikova H, Imrich J and Kozurkova M 2015 *J. Chem. Sci.* **127** 931
29. de Almeida S M V, Lafayette E A, da Silva L P B G, Amorim C A C, de Oliveira T B, Gois Ruiz A L T, de Carvalho J E, de Moura R O, Beltrao E I C, de Lima M C A and de Carvalho Jr. L B 2015 *Int. J. Mol. Sci.* **16** 13023
30. Lang X, Li L, Chen Y, Sun Q, Wu Q, Liu F, Tan C, Liu H, Gao C and Jiang Y 2013 *Bioorg. Med. Chem.* **21** 4170
31. Kumar C V, Barton J K and Turro N J 1985 *J. Am. Chem. Soc.* **107** 5518
32. Zhao J, Li W, Ma R, Chen S, Ren S and Jiang T 2013 *Int. J. Mol. Sci.* **14** 16851
33. Vantová Z, Paulíková H, Sabolová D, Kožurková M, Sucháňová M, Janovec L, Kristian P and Imrich J 2009 *Int. J. Biol. Macromol.* **45** 174
34. Brabec V and Nováková O 2006 *Drug Resist. Updat.* **9** 111
35. Janovec L, Kožurková M, Sabolová D, Ungvarský J, Paulíková H, Plšíková J, Vantová Z and Imrich J 2011 *Bioorg. Med. Chem.* **19** 1790
36. Poklar N, Pilch D S, Lippard S J, Redding E A, Dunham S U and Breslauer K J 1996 *Proc. Natl. Acad. Sci. USA* **93** 7606
37. Li F H, Zhao G H, Wu H X, Lin H, Wu X X, Zhu S R and Lin H K 2006 *J. Inorg. Biochem.* **100** 36
38. Dalgleish D G, Fey G and Kersten W 1974 *Biopolymers* **13** 1757
39. Shahabadi N, Kashanian S and Fatahi A 2011 *Bioinorg. Chem. Appl.* ID 687571 1
40. Zhong W, Yu J S, Liang Y, Fan K and Lai L 2004 *Spectrochim. Acta A* **60** 2985
41. Li W, Ji Y Y, Wang J W and Zhu Y M 2012 *DNA Cell Biol.* **31** 1046
42. Reddy P R, Raju N and Reddy K V 2009 *Indian J. Chem. A* **48** 1638
43. Kumar R S and Arunachalam S 2006 *Polyhedron* **25** 3113
44. Ishikawa Y, Yamashita A and Uno T 2001 *Chem. Pharm. Bull.* **49** 287