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Molecular modeling of tricyclic compounds with anilino substituents and their intercalation complexes with DNA sequences☆

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ABSTRACT

Although 9-anilinoacridines are among the best studied antitumoral intercalators, there are few studies about the effect of isosteric substitution of a benzene moiety for a heterocycle ring in the acridine framework. According to these studies, this approach may lead to effective cytotoxic agents, but good cytotoxic activity depends on structural requirements in the aniline ring which differ from those in 9-anilinoacridines. The present paper deals with molecular modeling studies of some 9-anilino substituted tricyclic compounds and their intercalation complexes (in various DNA sequences) resulting from docking the compounds into various DNA sequences. As expected, the isosteric substitution in 9-anilinoacridines influences the LUMO energy values and orbital distribution, the dipole moment, electrostatic charges and the conformation of the anilino ring. Other important differences are observed during the docking studies, for example, changes in the spatial arrangement of the tricyclic nucleus and the anilino ring at the intercalation site. Semiempirical calculations of the intercalation complexes show that the isosteric replacement of a benzene ring in the acridine nucleus affects not only DNA affinity but also base pair selectivity. These findings explain, at least partially, the different structural requirements observed in several 9-anilino substituted tricyclic compounds for cytotoxic activity. Thus, the data presented here may guide the rational design of new agents with different DNA binding properties and/or a cytotoxic profile by isosteric substitution of known intercalators.

1. Introduction

Molecules are capable of interacting with duplex nucleic acids in various ways, one of the most significant of which is the intercalation of planar or approximately planar aromatic ring systems between base pairs without breaking up the hydrogen bonds between the bases. Although simple intercalators do not show great sequence selectivity, it has been reported that there is experimental evidence of intercalating agents showing sequence selectivity [1]. Many intercalators possess antitumor activity and seem to act by stabilizing the DNA-intercalator-topoisomerase II ternary complex [2,3]. Among these, 9-anilinoacridines have been extensively studied and some compounds such as amsacrine (*m*-AMSA) **1a** [4] and asulacrine **1b** [5] (Fig. 1) have entered clinical trials or are used in the treatment of leukemia and lymphoma. Studies on the mechanism action of *m*-AMSA by SERS (surface-enhanced Raman scattering) spectroscopy have shown that this compound interacts via its side chain with the Topo II alone, whereas its ortho-isomer, which is relatively inactive as antitumor compound, has not shown this interaction [6]. In the same study, it was proposed that the planar acridine moiety of *m*-AMSA intercalates within DNA through a π–π interaction between the intercalator and DNA base pairs, the same interaction was observed for the ortho-isomer [7]. We recognize that there is not a straightforward correlation between DNA intercalation potency and Topo II inhibition. Furthermore, some Topo II poisons are void of intercalating activity [8].

On the other hand, the analysis of some bioactive natural products with tricyclic templates in their structures led to the modification of acridine nucleus resulting in some interesting polycyclic templates [9–12], but few efforts have been undertaken on the isosteric substitution of the acridine nucleus. This consideration led to the synthesis of compounds such as 9-anilinothiazolo[5,4-*b*]quinoline **2** [13,14] and 4-anilinofuro[2,3-*b*]quinoline **3** [15] derivatives (Fig. 1), which have shown cytotoxic activity. Interestingly, structural requirements in the anilino ring for the improvement of cytotoxic activity differ from those of 9-anilinoacridines [14,15].

The aim of the present study is to investigate how the DNA intercalation properties of 9-anilinoacridine derivatives are affected by the isosteric substitution of a benzene moiety for a heterocyclic ring in the acridine framework. The present paper shows some theoretical studies on several tricyclic compounds such as

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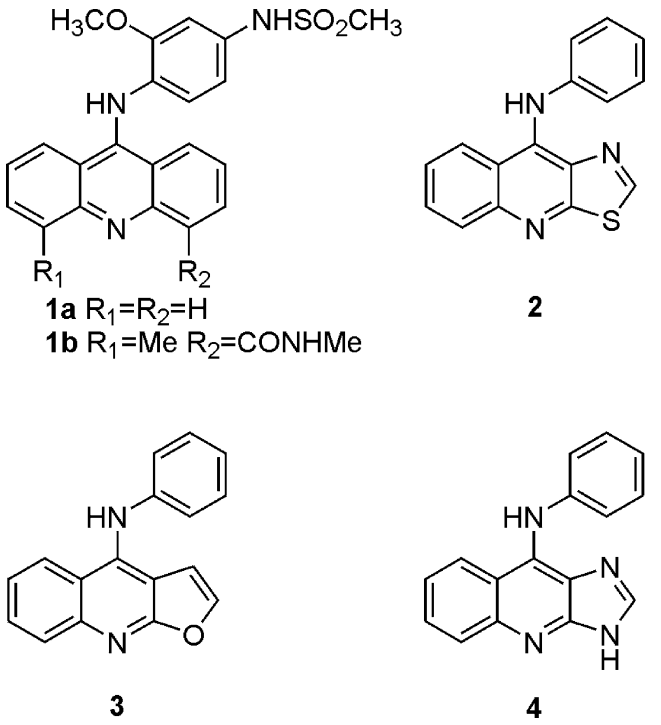

Fig. 1. Chemical structures of some anilino substitued tricycles.

9-anilinoacridines, 9-anilinothiazolo[5,4-*b*]quinolines and 9-anilinoimidazo[4,5-*b*]quinolines **4**. These studies consist of the molecular modeling of the isolated molecules by *ab initio* calculations, docking calculations of their intercalation complexes with several DNA sequences employing AMBER99 as force field, as well as, the analysis of the helical parameters of the intercalation complexes.

2. Computational procedure

The compounds analyzed are shown in Fig. 2. Calculations on isolated molecules were performed with Spartan'04 software. All molecules were built by assembling standard fragments, the resulting geometries being optimized by molecular mechanics. The conformational analysis of the compounds by Systematic Search protocol around dihedrals τa and τb (Fig. 2) was performed using the MMFF94 force field. The most frequent conformer for each compound was selected and the geometry optimization and calculation of their electronic properties were carried out with *ab initio* calculations (HF 6-31G*). For compounds **7a–i**, tautomer 3*H* was selected over tautomer 1*H* based on a lower heat of formation (5 kcal/mol).

In order to obtain more information on the effects of the isosteric substitution of the acridine template, it was decided to model DNA intercalation complexes of several aniline-substituted compounds. This was carried out with AMBER99 force field using the Hyperchem 7.0 software by docking the intercalators into the sequence 5′-CG*XY*CG-3′, where XY is every standard double base pair (AA, AC, AG, AT, CC, CG, GC, TA, TC TG). Relative dielectric constant 4r was used to simulate a solvent effect, van der Waals and electrostatic contribution being set to 0.5 each and a cut-off of 14 Å being used. Due to the heterotopic faces of some compounds, each ligand was docked in either a "face up" or "face down" orientation prior to initiating the conformational analysis of the anilino ring, and no difference was observed between these orientations.

Initially, the hexanucleotide was built by the nucleic acid database of Hyperchem; sodium counterions were added and the geometry was optimized to 0.1 kcal/Å mol with AMBER99 force field. Then the tricyclic compound (**5a–e**, **6a–e**, **7a–e, 8, 9, 10**, geometry optimized with AMBER99, charges calculated by AM1, single point) was manually stacked between the bases and the intercalation complex geometry was minimized by the steepest-descent protocol to 0.1 kcal/Å mol. The intercalator and the bases were allowed to move during energy minimization. Since previously reported data indicated that the anilino ring of 9-anilinoacridines was placed at the minor groove [16], it was decided to evaluate whether this behavior was true for compounds **6a** and **7a**. In all the sequences, lower energy values were obtained when the anilino ring was positioned within the minor groove.

A conformational analysis was then performed around dihedrals τa and τb to observe the preferential conformation of the anilino ring within the intercalation complex. The resulting geometry was minimized again to 0.1 kcal/Å mol. Energy of formation (E_{AMBER}) was calculated by the subtraction of the energy of the complex from the sum of the energies of compounds and the DNA fragment [$E_{AMBER} = E_{complex} - (E_{compound} + E_{DNA})$]. Although this binding energy does not accurately reflect the real binding energy, it still can be helpful in the identification of the most likely complexes. A similar approach has been used in other studies [17]. Though all the tricyclic compounds were also docked in their cationic forms, the final geometries obtained were not affected by this condition.

After finding the optimum AMBER geometry, AM1 semiempirical calculations were carried out to evaluate the electronic effects not considered in molecular mechanics. Calculations of electronic

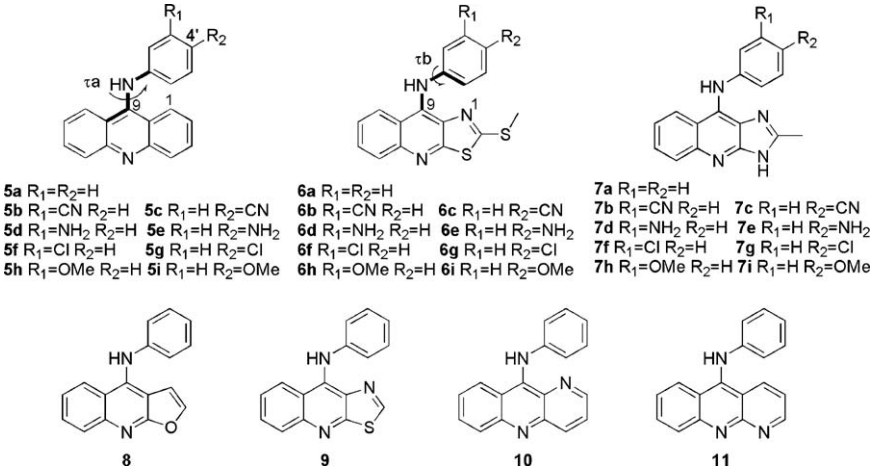

Fig. 2. Compounds analyzed by *ab initio* calculations, atom labeling and descriptions of dihedrals τa and τb.

properties of purines an pyrimidines in DNA and RNA using the AM1 semiempiric method have shown good correlation with experimental data [18]. In each intercalation complex, only the tricyclic compound, as a cationic entity, the adjacent two base pairs and their carbohydrate-phosphate backbone were selected for the AM1 calculations, the complex energy being calculated with no further geometry optimization. The heat of formation difference (ΔHf) was calculated by subtracting the quantity of the complex from the sum of the quantities of the compound and the corresponding pair of bases [$\Delta Hf = Hf_{complex} - (Hf_{compound} + Hf_{bases})$] [19].

3. Results and discussion

3.1. Molecular modeling of isolated molecules

Table 1 summarizes some calculated properties of the isolated molecules and the dihedral angle values obtained from their conformational analysis. Interesting differences were observed for dihedral angle values. In the case of acridine derivatives, τa was around −80° while in compound series **6** and **7**, it was around −60°/−70°, except in 4′-substituted derivatives (–NH_2 and –OCH_3 substituents), where the values were around −54°/−68°, in close agreement with experimental data for 9-anilinoacridines [20,21]. The τb value for acridines substituted in position 3′ of the anilino ring was around 3–9°, thus indicating that the anilino ring was nearly orthogonal to tricyclic nucleus, while in thiazolo and imidazoquinolines it laid between 10° and 18°. These observations are in agreement with NOESY experiments performed for **6b** and **6f**, which suggest that the anilino ring in these compounds is not orthogonal to tricyclic nucleus but slightly oblique [14]. In addition, a good correlation between the Hammet parameter of the substituent present in the anilino ring and τa was observed as indicated in Fig. 3, suggesting that the conformation of the anilino ring is affected by electronic factors.

Table 1
Some parameters of the compounds shown in Fig. 2 calculated with HF 6-31G*.

Comp	τa (°)	τb (°)	E HOMO (eV)	E LUMO (eV)	Bandgap (eV)	Dipole (Debye)
5a	−80.48	3.97	−7.4781	1.5047	8.9828	2.2066
5b	−84.39	7.19	−7.8013	1.2163	9.0177	3.3251
5c	−86.21	9.23	−7.8686	1.1610	9.0296	5.2428
5d	−82.90	6.47	−7.4512	1.5460	8.9973	3.1156
5e	−68.12	23.07	−7.1831	1.7102	8.8934	3.3401
5f	−83.29	6.52	−7.6629	1.3495	9.0123	0.6773
5g	−82.04	5.04	−7.6368	1.3554	8.9922	2.2833
5h	−84.24	9.08	−7.5014	1.5136	9.0150	2.7304
5i	−68.60	19.31	−7.2549	1.6637	8.9186	3.9872
6a	−59.54	18.72	−7.7398	2.0506	9.7903	3.7510
6b	−63.38	12.67	−8.0843	1.7063	9.7906	2.1396
6c	−68.38	1.20	−8.1635	1.5951	9.7586	4.0305
6d	−61.84	13.31	−7.7032	2.0693	9.7724	4.5764
6e	−54.29	33.25	−7.5350	2.2152	9.7501	4.8971
6f	−62.65	13.37	−7.9358	1.8663	9.8021	1.4274
6g	−61.34	15.88	−7.9013	1.8833	9.7846	2.2842
6h	−61.66	12.52	−7.7372	2.0456	9.7828	4.5177
6i	−54.68	30.95	−7.6177	2.1751	9.7928	5.2612
7a	−60.89	14.41	−7.4356	2.4239	9.8595	2.4416
7b	−63.95	9.95	−7.7912	2.0543	9.8454	6.5274
7c	−69.26	1.63	−7.8828	1.9570	9.8397	7.4377
7d	−62.40	10.12	−7.3918	2.4582	9.8499	3.5850
7e	−53.97	32.52	−7.2299	2.6145	9.8444	1.2184
7f	−63.46	10.13	−7.6390	2.2368	9.8758	3.9599
7g	−62.27	12.49	−7.6043	2.2530	9.8574	4.0910
7h	−62.83	10.62	−7.4601	2.3999	9.8600	3.0899
7i	−54.97	29.78	−7.3049	2.5707	9.8755	2.2630
8	−69.73	6.74	−7.7084	2.1345	9.8429	3.8045
9	−56.46	21.50	−7.7578	2.0155	9.7733	2.3456
10	−52.64	23.10	−7.4121	1.4985	8.9106	1.3549
11	−106.6	1.77	−7.7609	1.3178	9.0787	4.7082

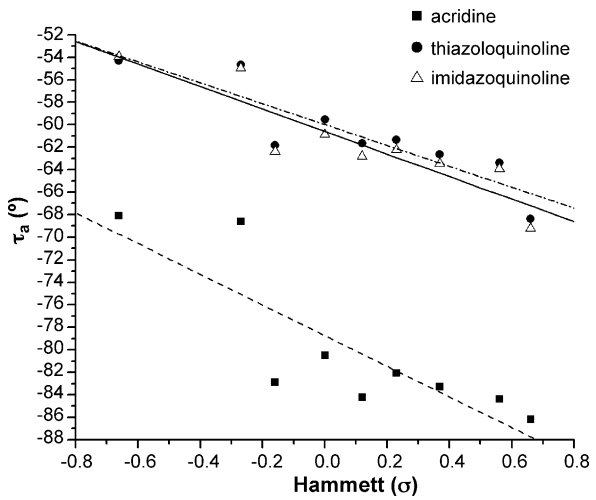

Fig. 3. Correlation between dihedral τa of compounds series and electronic Hammet parameter, acridine $\tau a = -13.63\sigma - 78.74$, $R^2 = 0.84$; thiazoloquinoline $\tau a = -9.30\sigma - 59.98$, $R^2 = 0.90$; imidazoquinoline $\tau a = -10.10\sigma - 60.61$, $R^2 = 0.90$.

To evaluate the above proposal, the spatial arrangement of compounds **8–11** was analyzed (Table 1). The results indicated that the dihedrals τa and τb for compounds **9** and **10** were similar to those in series **6** and **7**, whereas in compounds **8** and **11**, lacking a nitrogen atom at position 1, these dihedral angle values were similar to those of **5a**. Moreover, a rough correlation between the electrostatic charge at position 1 of the trycyclic nucleus (C1 for compounds **5a**, **8** and **11**) and dihedral angle τa was observed, while in **8** τa the value was around −70°; in **11** it was over −100° and the electrostatic charge value was more negative in **8** than in **11** or **5a** (Table 2), confirming the strong influence of electronic factors on the anilino ring conformation.

Since nitrogen atoms are frequently highly relevant to the interaction of small ligands with biomolecules, electrostatic charges of these atoms were calculated, several of which are listed in Table 2. The absolute value of the electrostatic charge of the nitrogen atom in the central ring (N4) is lower in compounds **6a** and **9** by effect of the vicinal sulfur atom. Because several studies have established the importance of a functional group in position 3′ or 4′ for interactions with topoisomerase II, their charges were also determined. In this case, these charges were not affected by changes in the tricyclic nucleus (data not shown).

The forces maintaining the stability of the DNA–intercalator complex include van der Waals, hydrogen bonding, hydrophobic, charge transfer and electrostatic complementarity [22–24]. The interaction between the LUMO orbital of the intercalator and the HOMO orbital of the adjacent bases also has been suggested as an

Table 2
Electrostatic nitrogen atomic charges for selected compounds.

Comp	N1[a]	N3[b]	N4[c]	N1′
5a	−0.133	–	−0.706	−0.756
6a	−0.363	–	−0.464	−0.641
7a	−0.691	−0.606	−0.653	−0.662
8	−0.372	–	−0.703	−0.757
9	−0.562	–	−0.463	−0.600
10	−0.598	–	−0.661	−0.559
11	+0.294	−0.772	−0.757	−0.768

[a] (C1) for compounds **5a–i**, **8** and **11**.
[b] (N4) for compound **11**.
[c] (N5) for compounds **10** and **11**.

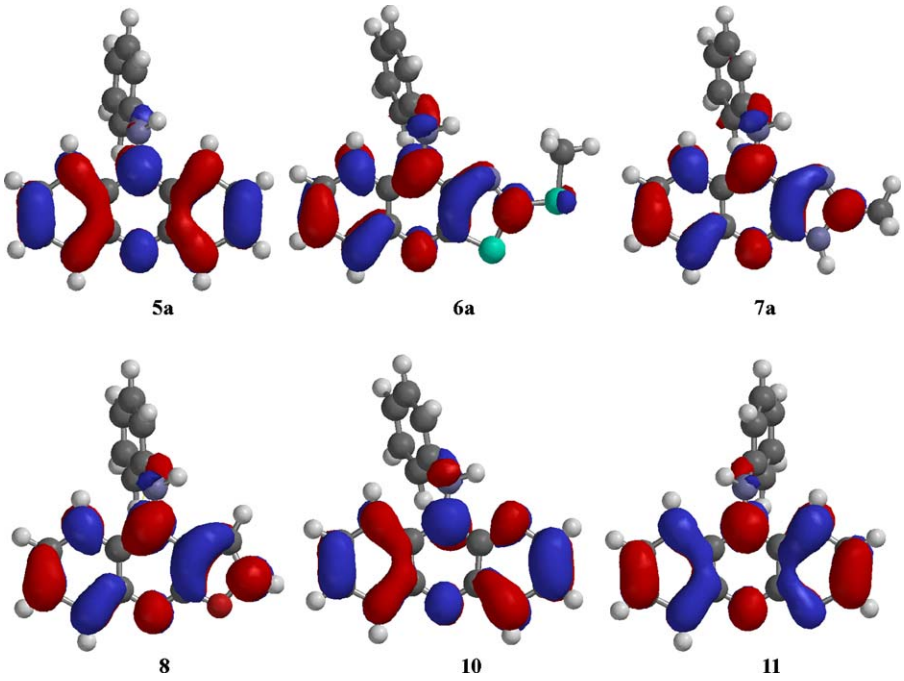

Fig. 4. LUMO distribution for some tricyclic compounds.

important stabilizing factor [25,26]. LUMO and HOMO energy values are related to the substituents present in the anilino ring, as expected, with lower values in those compounds with electron-withdrawing groups. LUMO energy values are lower in acridines and benzonaphthyridines (Table 1), suggesting these should be better intercalators than the other compounds. This is true, at least, for thiazoloquinolines which exhibited lower DNA binding affinity than did acridines, as shown in a previous study [14]. However, intercalation properties do not always correlate with cytotoxic activity; for instance, *o*-AMSA possesses a high affinity for DNA but lower cytotoxic activity than its isomer amsacrine (*m*-AMSA) [27]. LUMO orbitals for some compounds are shown in Fig. 4. Distribution maps for compound **8** and **9** (**9** not shown) are similar to **6a**, while compounds **10** and **11** resemble **5a**. LUMO orbital of the studied compounds is located on the tricyclic template and its distribution is symmetric in series **5**, **10** and **11** but not in the other compounds. Our data do not allow discrimination as to whether this asymmetry affects base selectivity, but it is a possibility to be considered.

The dipole moment might play an important role in cytotoxic activity of intercalators, such as in the ellipticine derivatives [28], or it may affect the intercalation process itself [29]; thus, in the present study the dipole moment vector and magnitude were determined. In the analyzed compounds with non-substituent or electron-releasing groups in the aniline ring, the dipole vector is oriented towards the tricyclic template, whereas when an electron-withdrawing group is attached, the vector is directed towards the aniline ring. Interestingly, compounds with a high dipole value have shown poor cytotoxic activity (**5b**, **6d** and **6i**) [14,30]. Although there is no agreement between dipole magnitude and cytotoxic activity, the available data suggests that compounds with a dipole value lower than 4.00 might have good cytotoxic activity [14,30]. In a similar fashion, ellipticine-like derivatives with a dipole moment below 2.6 have shown a better cytotoxic activity [28].

Medhi [31] has proposed a central role for the molecular electrostatic potential (MEP) in the binding strength of the chemically modified chromophore. Fig. 5 shows isovalue volumes for some of the compounds under study. Whereas MEP was concentrated around nitrogen atoms in all compounds, an additional lobule was found in **5a**, **10** and **7a** around the benzene ring, which was absent in **6a**. Importantly, a lower MEP surrounds the anilino ring in compounds **6a** and **8** in comparison with **5a**. These changes correlate with a different hypothetical mode of binding to the interaction site for the aniline ring in the compounds of series **5** when compared with those of series **6** and **8**. MEP could play a significant role in understanding these differences.

These results show how the isosteric substitution of the benzene in acridine template affects geometric and electronic properties of the resulting tricyclic compound. Changes in LUMO energy values and the orbital distribution of the tricyclic compounds and spatial arrangement of the anilino ring could exert some influence on binding and base selectivity, thus partially explaining the differences observed in cytotoxic activity and the DNA binding between these compounds [14,15].

3.2. Intercalation complexes of 9-anilino substituted tricyclic compounds with various DNA sequences

The complexes with lower calculated energy for compounds **5a** and **6a** with 5′-CG*C*G*C*G-3′ hexanucleotide are shown in Fig. 6. It was observed, that isosteric substitution of the tricyclic template did not affect the position of the chromophore in the intercalation site, but it had influence on the position of the aniline ring, the same behavior was observed during the *ab initio* calculations. Calculated binding energy values for each complex are shown in Table 3. From this data, the order of binding is **6a–e** ≈ **9** ≈ **5a–e** < **8** ≈ **7a–e**. In general, compounds **6a–e** and **9** exhibited *in silico* higher selectivity for sequence 5′-CG*C*G*C*G-3′ than did the other analyzed compounds (see Supplementary Table S1); thus, isosteric replacement seems to have an important impact on the geometry of the intercalation complex, which is reflected in base selectivity.

The preferred sequence in these calculations was 5′-CG*C*G*C*G-3′. Other favored sequences were those with adenine in direction 5′ (for compounds **5a–e** and **10**), those with cytosine in 3′ (for **6a–e** and **9**) and with thymine in 5′ (for **7a–e**). Hydrogen bonds between DNA and the intercalator were observed in several complexes,

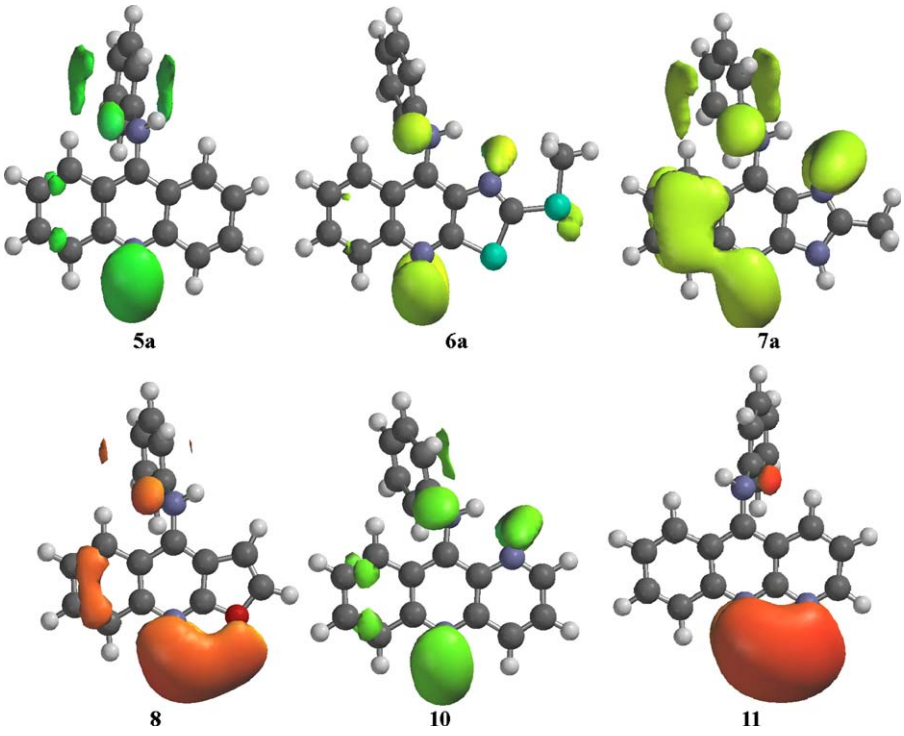

Fig. 5. Isovalue volumes of electrostatic potential (−20 kcal/mol) for **5a**, **6a**, **7a**, **8**, **10** and **11**.

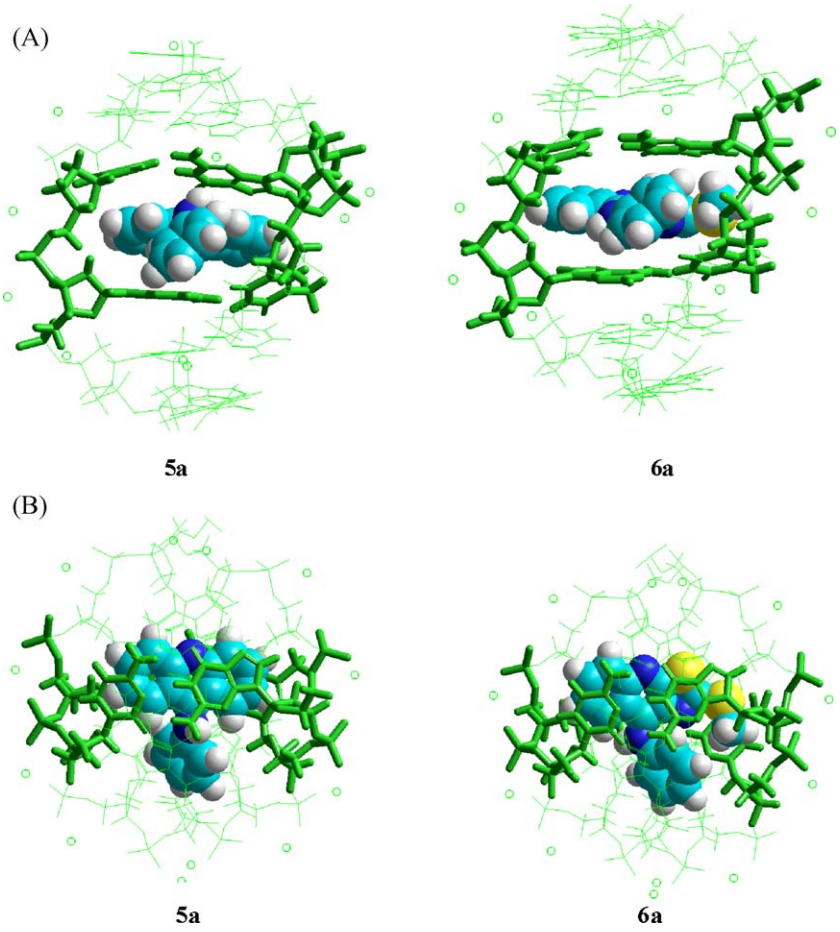

Fig. 6. 5a and **6a** intercalation complexes with 5′-CG*A*CCG-3′ DNA sequence viewed from minor groove (A) and from direction 5′ (B).

Table 3
Energy of complex formation (E_{AMBER}) for DNA-intercalator complexes analyzed (kcal/mol).

	AA	AC	AG	AT	CC	CG	GC	TA	TC	TG	AVG
5a	−58.64	−58.73	−53.75	−62.79	−58.28	−65.80	−57.91	−58.36	−56.71	−56.05	−58.70
5b	−60.22	−58.76	−66.33	−58.70	−54.07	−64.46	−54.35	−57.29	−56.50	−54.21	−58.49
5c	−62.68	−53.34	−62.06	−58.41	−52.84	−65.68	−58.35	−55.94	−63.33	−52.67	−58.53
5d	−61.53	−54.69	−56.00	−59.22	−59.59	−64.58	−60.02	−60.02	−56.15	−55.15	−58.70
5e	−60.92	−52.57	−60.46	−58.52	−55.21	−65.26	−54.98	−64.74	−61.28	−54.42	−58.84
6a	−44.69	−55.36	−49.76	−57.01	−58.28	−66.52	−63.68	−59.18	−64.12	−58.02	−57.66
6b	−46.19	−52.18	−46.11	−52.46	−60.11	−67.89	−61.73	−46.82	−58.38	−47.45	−53.93
6c	−46.25	−54.41	−51.63	−58.58	−60.59	−67.72	−45.77	−56.16	−55.93	−47.72	−54.48
6d	−45.47	−54.19	−47.19	−54.32	−60.16	−67.07	−51.43	−42.88	−66.16	−52.87	−54.18
6e	−49.75	−56.93	−58.19	−49.95	−59.27	−68.01	−48.53	−54.08	−60.96	−60.67	−56.63
7a	−42.85	−64.56	−59.42	−65.23	−59.77	−69.52	−63.88	−65.34	−61.72	−57.63	−60.99
7b	−45.10	−62.47	−53.51	−62.12	−62.31	−71.90	−62.14	−61.13	−59.98	−58.77	−59.94
7c	−45.00	−60.39	−56.90	−61.72	−61.82	−71.30	−60.03	−61.34	−63.18	−57.07	−59.87
7d	−44.69	−61.44	−54.45	−60.80	−60.95	−71.25	−62.00	−65.07	−59.08	−58.78	−59.85
7e	−44.44	−55.97	−58.41	−63.09	−62.93	−71.25	−66.28	−63.74	−63.38	−56.39	−60.59
8	−59.84	−61.46	−58.10	−63.34	−55.56	−63.76	−66.29	−58.68	−61.33	−57.58	−60.59
9	−49.35	−60.13	−55.04	−60.95	−56.13	−66.98	−62.45	−59.90	−57.74	−55.64	−58.43
10	−63.00	−56.69	−53.08	−60.87	−62.12	−62.84	−55.76	−58.25	−56.54	−56.96	−58.61

some of which were between amino groups in position 3′ or 4′ or the amino group (N1′), the link between aniline, the tricyclic template and carbonyl groups of the bases.

When the conformation of the anilino ring was analyzed, it was oriented, in some cases preferentially to direction 5′, while in others, to direction 3′, and without preference in the rest, (Fig. 7). In acridines **5a–e** the preferred orientation was to 5′, while in thiazoloquinolines **6a–e**, it was to 3′. For compounds **7a–e**, **8**, **9**, the orientation is alike in thiazoloquinolines and acridines. The values of τa were roughly orthogonal in acridines but were oblique in the rest of the compounds, (see Supplementary Table S2) in agreement with *ab initio* calculations.

The geometric variations in the DNA caused by the intercalating agents affected the helical parameters [32], which were calculated by 3DNA software [33]. Only the complementary base pair parameters were used for the analysis [34], a subscript 1 being assigned to the helical parameters of the base pair with 5′ to 3′ direction whereas a subscript 2 was assigned to the base pair 3′ to 5′ direction (Table 4). In general, the largest observed effects were on the two base pairs next to the tricyclic nucleus, as expected. Important changes were found in the buckle (κ), propeller (ω), opening (σ) and rise (D_z) parameters (step parameter), with only minor effects on Sx, Sy and Sz values.

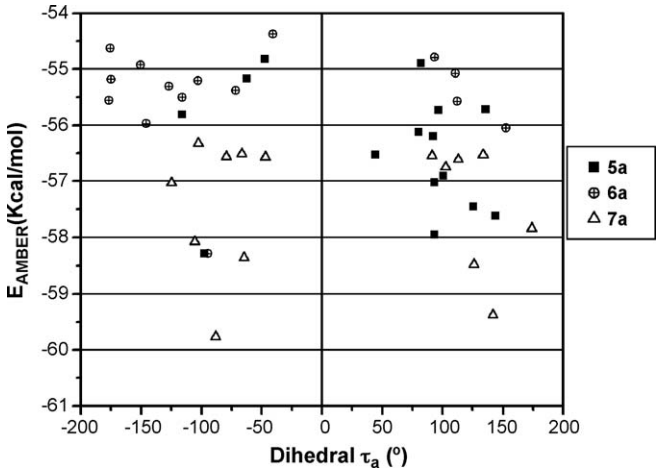

Fig. 7. Values of dihedral τa against E_{AMBER} for complexes of **5a** (direction 5′ preferred), **6a** (direction 3′ preferred) and **7a** (none direction preferred) with 5′-CGCCCG-3′.

The average angular values in Table 4 are arranged by the heterocyclic nucleus, as well as the AMBER and the Δ*Hf* average calculated energies. It is evident that the replacement of a benzene moiety by a thiazole or imidazole ring in the acridine skeleton affects mainly the buckle and propeller angular values, which is attributed to the different arrangement of the acridine skeleton within the DNA sequence. The position and the electronic nature of the substituent slightly diminish the values of the same parameters (seeSupplementary Table S3). This could be related to the negative steric effect which has been observed in QSAR studies on DNA affinity and the cytotoxicity of acridine derivatives [30]. On the other hand, there was a rough correlation between the κ and ω parameters and the Δ*Hf* energy values because higher values of κ and lower values of ω showed lower energy values of Δ*Hf*. In order to determine whether there was a correlation between the helical parameters and the DNA-intercalating agent complex energy values, calculated by AM1, several models for each sequence and type of compound were generated by means of the BuildQSAR program [35]. Though a global equation accounting for the calculated Δ*Hf* values against all sequences was not found, the κ and ω parameters showed a relevant role in almost all models

Table 4
Average values of helical parameters for all DNA sequences arranged by heterocycle ring.

	Heterocyclic nucleus		
	ACR	TZQ	IMQ
AMBER	−58.65	−56.15	−61.14
Δ*Hf*	6.96	13.34	10.21
Sx_1	−0.45	−0.13	−0.42
Sy_1	0.23	0.27	0.17
Sz_1	0.94	0.46	0.71
κ_1	24.51	16.48	16.10
ω_1	−15.98	−21.39	−20.85
σ_1	4.69	−2.09	2.63
Sx_2	0.42	0.87	0.50
Sy_2	0.33	0.50	0.37
Sz_2	0.77	0.80	0.70
κ_2	−23.07	−15.48	−18.49
ω_2	−17.82	−23.09	−21.57
σ_2	5.61	3.24	4.37

ACR: acridine; TZQ: thiazoloquinoline; IMQ: imidazoquinoline.

Table 5
Average value of rise (D_z) parameter for all DNA sequences.

sequence	ACR	TZQ	IMQ
AA	8.27	7.45	7.29
AC	8.53	7.78	8.37
AG	8.41	8.22	8.49
AT	8.84	8.17	8.68
CC	8.29	7.12	7.10
CG	8.17	7.11	7.20
GC	8.57	8.15	8.07
TA	7.76	7.52	7.46
TC	7.99	7.68	7.68
TG	7.93	8.34	7.53
AVG	8.28	7.75	7.79

evaluated. It is possible the structural requirements within the intercalation site might be sequence-specific.

The acridine compounds had a greater effect on the rise (D_z, opening between two adjacent pair bases) parameter than the other compounds (Table 5). This was consistent with the intercalation mode of the acridine derivatives found in the docking study. All compounds studied resulted in greater changes for the sequences 5′-CG*An*CG-3′, where *n* was G, C, T. Although the weaker AT pair at position 5′ rendered these sequences prone to distortion, AT pairs at 3′ being less sensitive, other factors had to participate.

The electronic effect of the substituents at positions 3′ and 4′ of the anilino ring was evaluated from semiempirical calculations of the intercalation complex core. The results for ΔHf are shown in Table 6. As expected, substituents with higher calculated DNA binding have electron releasing substituents in series **5** for almost all of the sequences. However, no clear trend was observed for series **6** or **7**. In general, all compounds showed a calculated preference for the sequence 5′-CG*AT*CG-3′, as it has been reported in calculations carried out with amsacrine intercalation complexes [36]. The order of ΔHf was **10** > **9** ≈ **8** > **5a** > **7a** > **6a** in agreement with experimental data, because the compounds of series **6** were weaker intercalators than some 9-anilinoacridines [14].

The comparison of ΔHf values for thiazoloquinolines **6a** and **9** complexes indicated that compound **9** not only could have better DNA binding properties than **6a** but also different base sequence selectivity because their sequence-preferences were quite different. This behavior was also observed in AMBER calculations implying that lateral chains, even small ones such as methylthio, could have an important effect on base selectivity. Further studies on this topic are necessary to evaluate this proposal.

The spatial disposition of the aniline substituent and of the tricyclic template is a factor relevant to the DNA binding and, in particular, to the complex geometry. In turn, the complex geometry could be the cause of the different cytotoxic profiles observed in previous studies. Another example of the importance of geometrical properties in cytotoxicity was observed for acenaphtho[1,2-*b*]pyrrole derivatives, where the binding geometry and not only the binding affinity contributed to the antitumor capacity of these compounds [37]. Thus, even subtle changes in the electronic features (such as LUMO energy and orbital disposition, dipole moment) and geometrical features (dihedral angles of the lateral chains respect to the template) of the tricyclic compounds should be considered in the design of intercalators as potential antitumorals because both properties are surely involved in the factors that result in different structural requirements for cytotoxic activity and DNA binding.

Our data offer possible grounds for the rational design of new agents with different intercalation properties and/or a cytotoxic activity profile by the isosteric replacement in known polycyclic nuclei, but clearly experimental data will be needed to test the validity of such designs.

4. Conclusion

The isosteric replacement of benzene moiety in 9-anilinoacridines for a heterocyclic ring appears to have an important influence on both the geometry and electronic properties of the resulting compounds and their DNA binding properties. The most important differences are (i) the position of the anilino ring, which is nearly orthogonal in acridines but oblique for the rest of analyzed compounds, (ii) the LUMO distribution density which is symmetric in **5a–i** but not in the other studied compounds and (iii) the effect on the electrostatic charge of N4 and MEP isovalues, which are lower in thiazolo[5,4-*b*]quinoline derivatives. Additional important differences were observed in the simulation of DNA-intercalator complexes, particularly those relating to the position of the aniline ring in the intercalation site and the sequence selectivity which was different depending on the nature of the tricyclic compound and the susbtituents attached to the anilino ring. These are factors that could have a strong influence on cytotoxicity, affecting the structural requirements for biological

Table 6
Calculated heat of complex formation (ΔHf, kcal/mol , AM1 calculations.) for the intercalation complexes analyzed.

	AA	AC	AG	AT	CC	CG	GC	TA	TC	TG	AVG
5a	9.02	18.17	8.59	−5.25	6.16	18.63	3.27	0.97	2.88	11.97	7.44
5b	4.65	14.63	6.65	−4.39	4.76	23.87	−5.69	5.60	−2.46	14.06	6.17
5c	1.49	5.12	6.09	2.26	7.68	29.74	−0.56	6.78	5.35	11.03	7.50
5d	3.14	0.50	8.71	−0.08	16.16	18.36	13.62	9.09	1.93	18.88	9.03
5e	0.84	0.41	12.49	1.19	−3.75	16.73	1.37	1.79	2.76	12.86	4.67
6a	11.26	15.93	29.63	2.23	8.21	18.35	15.81	15.24	−0.87	19.59	13.61
6b	5.36	4.39	39.39	−0.11	8.19	21.03	8.39	8.58	13.43	27.44	13.61
6c	11.33	8.63	26.05	−5.64	11.46	15.34	4.37	10.80	0.44	28.84	11.22
6d	12.68	27.11	32.05	7.01	1.63	15.69	10.96	13.98	1.21	19.91	14.29
6e	12.59	15.55	8.45	12.73	13.17	15.35	7.34	11.86	15.41	26.64	13.96
7a	0.61	11.00	12.45	−0.98	5.58	11.07	15.81	2.85	4.96	12.70	7.65
7b	7.45	5.74	20.88	7.52	9.78	19.01	7.42	9.00	9.86	16.05	11.30
7c	9.29	17.53	14.63	5.37	6.82	25.45	20.57	15.56	0.32	10.43	12.65
7d	5.89	5.40	10.89	0.05	4.05	14.21	8.80	1.70	12.12	13.82	7.72
7e	17.47	6.51	26.87	−1.28	3.63	18.79	18.86	3.33	7.83	14.41	11.72
8	−0.39	7.91	8.14	−5.40	8.12	10.01	6.75	10.60	0.52	12.37	5.89
9	1.98	7.54	5.00	3.23	8.71	14.61	5.77	6.18	−2.20	6.69	5.77
10	12.48	−0.49	1.02	−3.63	−7.29	12.11	1.27	7.45	−4.27	5.57	2.42

activity and should be considered in the design of new intercalators with potential antitumoral activity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmgm.2009.02.001.

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