

Physicochemical interaction of antitumor acridinone derivatives with DNA in view of QSAR studies

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Abstract The acridinone derivatives with antitumor activity and ability with respect to noncovalent DNA binding were investigated for their quantitative structure–activity relationships (QSAR). Multiple regression analysis was used to model relationships between molecular descriptors and antileukemia activity, or between molecular descriptors and DNA-duplexes stabilization. Studies were performed on molecular modeling using HyperChem and Dragon computer programs, and molecular geometry optimization using MM+ molecular mechanics and semi-empirical AM1 method. Two multiple regression equations were derived and characterized as good and with statistically significant correlations, $R = 0.9384$ and $R = 0.8388$, for quantitative structure–antitumor activity relationships and quantitative structure–ability to DNA-duplexes stabilization relationships, respectively. Moreover, hydrophobic and total molecular symmetry properties are important for antitumor activity of acridinone derivatives, and electronic and topological properties are important for physicochemical (noncovalent) DNA-duplexes stabilization of these compounds. The obtained equations can be used for prediction of acridinone derivatives' activity and their ability to noncovalent interaction with DNA which, as it was shown earlier, play important role in the antitumor mechanism of action of these compounds.

Keywords Quantitative structure–activity relationships (QSAR) · Multiple linear regression (MLR) analysis · Acridinones · Antitumor activity · Physicochemical interaction with DNA

Introduction

The acridinones represented by imidazo- and triazoloacridinones are a new group of potent antitumor compounds (Cholody *et al.*, 1990, 1992, 1996) from which one of the most active derivatives called as C-1305 has been selected for extended preclinical trials and the other one called C-1311 (for review see Mazerska *et al.*, 1998) is currently undergoing phase II clinical trials as drug SymadexTM (Burger *et al.*, 1996; Den Brok *et al.*, 2005a, b, c). Imidazoacridinones exhibit *in vivo* activity against various tumor cell lines as leukemia, melanoma, colon adenocarcinoma, and colon carcinoma. Triazoloacridinones exhibit *in vivo* activity against leukemia, murine carcinoma, lung carcinoma, breast carcinoma, and colon carcinoma (Cholody *et al.*, 1990, 1992, 1996; Kusnierczyk *et al.*, 1994; Burger *et al.*, 1996a, b; Lamb and Wheatley, 1996; Calabrese *et al.*, 1998, 1999; Alami *et al.*, 2007; De Marco *et al.*, 2007; Bram *et al.*, 2007). As was previously shown (Skladanowski *et al.*, 1999; Lemke *et al.*, 2004; Augustin *et al.*, 2004, 2006; Wesierska-Gadek *et al.*, 2004; Koba and Konopa, 2007; Koba *et al.*, 2009), cellular DNA is important target for the triazoloacridinone drugs, and hence interactions with DNA are naturally the crucial point in view of the biological activity of these compounds. In previous article (Skladanowski *et al.*, 1999; Lemke *et al.*, 2004), it was indicated that triazoloacridinones inhibit cleavable complexes of topoisomerase II with DNA. They inhibit also nucleic acid or protein synthesis induced by G₂ block of cell

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cycle followed by apoptosis (Augustin *et al.*, 2004, 2006; Wesierska-Gadek *et al.*, 2004), intercalating to DNA and binding in minor groove (Koba and Konopa, 2007; Koba *et al.*, 2009) and/or forming of interstrand DNA crosslinks (Koba and Konopa, 2007). In addition, it was shown that intercalation to DNA takes place preferentially in guanine triplet regions inducing changes in DNA structures (Lemke *et al.*, 2005). For imidazoacridinones, it was demonstrated that intercalation to DNA undergoes at physiological condition with parallel stabilization of double-stranded DNA and unwinding of supercoiled DNA (Burger *et al.*, 1999; Dziegielewski *et al.*, 2002). The intercalative binding mode of acridinone derivatives was also confirmed with the use of molecular-modeling studies (Mazerski and Muchniewicz, 2000). Similar to other DNA-binding agents, treatment of tumor cells with imidazoacridinones induces topoisomerase II-associated DNA strand breaks (Składanowski *et al.*, 1996), arrests cells in G₂ phase, and stimulates apoptosis (Zaffaroni *et al.*, 2001; Skwarska *et al.*, 2007) or mitotic catastrophe (Hyzy *et al.*, 2005; Skwarska *et al.*, 2007). However, after testing imidazoacridinone and triazoloacridinone derivatives, it has been concluded that although the intercalative binding to DNA seems to be necessary for their biological activity (the most active compounds have usually the highest binding affinity), it is not sufficient (some inactive analogs also bind strongly with DNA) (Dziegielewski *et al.*, 2002; Koba and Konopa, 2007). Moreover, acridinones undergo enzymatic oxidation, and this reaction is important for their biological activity as intercalation to DNA and covalent adducts formation (Dziegielewski and Konopa, 1996; Mazerska *et al.*, 1999, 2003). In this context, noncovalent interaction of acridinones may help position drug molecules on DNA for the covalent reaction.

In this article, physicochemical interactions of acridinones with DNA were evaluated in view of quantitative structure–activity relationships (QSAR). Multiple regression analysis was used to model relationships between molecular structural descriptors and biological (antitumor) activity of molecules, or between molecular structural descriptors and physicochemical interaction of acridinone derivatives with DNA. The biological data expressed as anti-leukemia P388 activity and parameter describing ability to physicochemical (noncovalent) interaction with DNA as value of DNA-duplexes stabilization were applied in this study.

Materials and methods

Antitumor and physicochemical DNA-binding activity data of acridinones

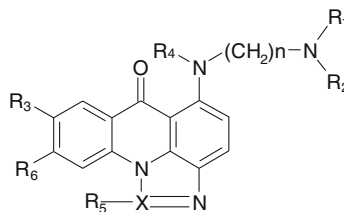
The acridinone derivatives examined in this study have been selected to collect analogue compounds differing in

chemical structures as well as anticancer activities (Table 1). The data of acridinones' antitumor activity against P388 leukemia in mice in vivo and expressed as the percentage of increase in survival time of the treated to that of the control mice with P388 leukemia at optimal dose (ILS) were taken from the literature (Table 1) (Cholody *et al.*, 1990, 1992; Koba and Konopa, 2007; Mazerska *et al.*, 1996). The data of physicochemical binding of acridinones to DNA (as values of DNA-duplexes stabilization), which were expressed as an increase in DNA melting temperature in centigrade degrees of ctDNA at drug to DNA base pairs 0.25 M ratio were taken from the literature (Table 1) (Koba and Konopa, 2007; Dziegielewski *et al.*, 2002).

Structural parameters

The structure of the tested compounds was studied by molecular modeling using HyperChem 7.5 Release software (Kaliszan *et al.*, 1995; Ivanciuc, 1996) and Dragon software (Todeschini *et al.*, 2000). The structures of the compounds were first pre-optimized with the Molecular Mechanics Force Field (MM+) procedure included in the Hyperchem 6.03 (Hypercube) <http://www.hyper.com>, and the resulting geometries were further refined by means of the Semi-Empirical Molecular Orbitals Method AM1 using the Polak-Ribiere algorithm and a gradient norm limit of 0.01 kcal Å⁻¹.

The following molecular descriptors taken from HyperChem software were considered among quantum and chemical indices: total energy (TE), binding energy (BE), isolated atomic energy (IAE), electron energy (EE), core–core energy (CCE), heat flow (HF), energy of the highest occupied molecular orbitals (E_HOMO), energy of the lowest unoccupied molecular orbitals (E_LUMO), and difference between HOMO and LUMO energies referred to as EG = *energy gap*; ionization energy (potential) (IE) and electron affinity (EA) were calculated as a difference between the HF of positive molecular ion and electrically neutral molecule, and electronegativity (EN) calculated as average arithmetic potential of ionization and EA. In addition, other parameters were used as the value of electron density of atom orbitals from the lowest to the highest (ED_MIN and ED_MAX, respectively), the highest positive electron charge on the atoms (MAX_POS), and the highest negative electron charge on the atoms (MAX_NEG), the difference between the highest positive and negative charge (DELTA_Q), distribution of dipolar moment along x, y, and z axes (X_DM, Y_DM, and Z_DM, respectively), total dipolar moment (TDM), mean polarizability of molecules (in atom units) MP (*Mean Polarizability*), energy equal to the length of the wave with the greatest long-wave transfer of electrons, for which the

Table 1 Chemical structures of acridinones studied

Compound	X	n	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	ILS ^a	ΔT _m ^b
C-1310	C	2	CH ₂ CH ₃	CH ₂ CH ₃	OH	H	CH ₃	H	185	15.3
C-1311	C	2	CH ₂ CH ₃	CH ₂ CH ₃	OH	H	H	H	93	13.7
C-1330	C	2	CH ₂ CH ₃	CH ₂ CH ₃	OCH ₃	H	H	H	96	11.5
C-1415	C	2	CH ₂ CH ₃	CH ₂ CH ₃	H	H	H	H	55	7.2
C-1419	C	2	CH ₂ CH ₃	CH ₂ CH ₃	H	H	H	OH	27	8.3
C-1558	C	2	CH ₂ CH ₃	CH ₂ CH ₃	C(CH ₃) ₃	H	H	H	0	2.4
C-1176	C	2	CH ₃	CH ₃	H	H	H	H	90	9.5
C-1263	C	2	CH ₃	CH ₃	OH	H	H	H	110	12.3
C-1212	C	3	CH ₃	CH ₃	H	H	H	H	25	11.5
C-1371	C	3	CH ₃	CH ₃	OH	H	H	H	120	3.5
C-1554	C	5	CH ₂ CH ₃	CH ₂ CH ₃	CH ₃	H	H	H	20	10.5
C-1266	C	5	CH ₃	CH ₃	H	H	H	H	10	9.9
C-1492	C	5	CH ₃	CH ₃	OH	H	H	H	85	13.1
C-1233	N	2	CH ₃	CH ₃	H	H	–	H	77	9.1
C-1303	N	2	CH ₃	CH ₃	OH	H	–	H	102	13.1
C-1533	N	2	CH ₃	CH ₃	OH	CH ₃	–	H	10	8.1
C-1567	N	2	CH ₃	CH ₃	C(CH ₃) ₃	H	–	H	0	6.8
C-1410	N	2	H	CH ₂ CH ₃	OH	H	–	H	78	7.1
C-1296	N	3	CH ₃	CH ₃	CH ₃	H	–	H	18	11.5
C-1305	N	3	CH ₃	CH ₃	OH	H	–	H	165	15.1

^a The percentage of increase in survival time of treated to control mice with P388 leukemia at optimal dose

^b The increase in DNA melting temperature (expressed in centigrade degrees) at drug to DNA base pairs 0.25 M ratio

value of oscillator force was different from zero (EL)—the value of wave figures were converted to eV—and the value of the most intensive electron transfer (EMAX—the maximum value of oscillator force calculated with the use of AM1 method—as well as oscillator maximum force used for the transfer (OS_EMAX). Moreover, additional parameters were calculated with the use of QSAR Properties Module of HyperChem. They include the following descriptors: surface area of the molecule available for solvent (SA), molecule volume (V), hydration energy (HE), the calculated distribution coefficient logarithm (logP), refraction (R), and polarizability (P).

On the other hand, using Dragon software, over 1,300 molecular descriptors were calculated and considered for QSAR analysis. They include molecular parameters from different group and class of descriptors as constitutional, topological, walk and path counts, connectivity indices, information indices, 2D autocorrelations, edge adjacency

indices, topological charge indices, eigenvalue-based indices, geometrical, 3D-Morse, WHIM, GETAWAY, functional group counts, atom-centred fragments, charge, molecular properties and other group of descriptors, and describing some properties of compound as geometry, symmetry, topology, electronic, steric or thermodynamic and other properties. The definitions of these descriptors are reviewed by Todeschini (Todeschini *et al.*, 2000).

Statistical analysis

The statistical QSAR analysis was performed with the use of stepwise regression analysis that used Statistica 8.0 software (StatSoft, Tulsa, OK, USA). The objective of stepwise regression is to construct a multivariate regression model (QSAR equation) for a certain property, y, based on several selected explanatory variables. In stepwise regression, the first selected explanatory variable has the highest

correlation with dependent variable, y . Then, explanatory (independent) variables are consecutively added to the model in a forward selection procedure. A new variable is added to the model if a significant change in residuals of the model can be observed. The significance is evaluated using a statistical test, usually F -test (the value of the F -test of significance, F). In addition, the multiple correlation coefficients (R), the standard error of estimate (S), and the significance levels of each term and of whole equation (p) are calculated for the derived QSAR equations. Whenever a new variable is included into a model, a backward elimination step follows in which an F -test detects the earlier selected variables, which can be removed from the model without any significant change on the level of the residuals. The variable selection procedure stops when no additional variable significantly improves the model. Stepwise regression is very much popular in QSAR studies, since the stepwise procedure is simple and based on the classical multiple linear regression (MLR) approach. Moreover, it is implemented in almost all the statistical software packages. One of the drawbacks of the method is the fact that no optimal variable selection is guaranteed, since the new variables are found based on the previously included variables into the model (Put *et al.*, 2006).

During model building, the model fit can be improved proportional to the model complexity. Therefore, the more the factors are included into the model, the better the model fits the training data. Usually, the model fit is evaluated by the root mean-squared error (RMSE), computed for the training data.

The determination of the optimal complexity of the model requires an estimation of its predictive ability, to prevent overfitting to the calibration data. After all, the main goal of QSAR models is to obtain a reasonable prediction of the retention for future samples. To evaluate the prediction by means of an internal validation procedures, cross validation can be used. The predictive ability of a model is characterized by the cross-validated root mean-squared error (RMSECV); test values were calculated with the Matlab software (MathWorks, Natick, MA, USA). The RMSECV as values, which quantify the predictive power of the QSAR model, were calculated by the leave-one-out method and leave-ten-out method.

Results and discussion

The chemical structures of the 20 compounds considered for this study and their antitumor and noncovalent DNA-binding activities are presented in Table 1. In this study, QSAR analysis using multiple regression method was performed

for biological activity data (ILS) or physicochemical interaction with DNA (ΔT_m), and non-empirical parameters (molecular descriptors). Owing to a large number of non-empirical parameters (over 1,300), treated as independent variables and according to QSAR strategy and multi-parameter regression rule in derived multi-parameter regression equation, the number of independent variables must be 5–6 times less than the number of cases considered in this study. In practice, for obtaining statistically significant equation, one independent variable (in our case structural descriptor) falls, generally out of five to maximum six cases considered, in dependent-variable activity (in our case, activity of acridinones). In the research done, the data set of 20 acridinone derivatives (dependent variables) was taken to QSAR analysis, and for this reason the derived QSAR equations were maximally limited to four statistically significant independent variables (structural descriptors).

Moreover, correlations were limited to the value of regression coefficient $R \geq 0.8$ and an additional criterion, considered as relevant to particular independent variables, was established at the significance level $p \leq 0.05$.

The calculated equations are presented in Table 2 and characterized by four statistically significant independent variables with a good value of regression coefficient $R \geq 0.8$ ($R = 0.9384$ and $R = 0.8388$ for quantitative structure–antitumor activity relationships and quantitative structure–ability to DNA-duplexes stabilization relationships, respectively). Moreover, all the regression coefficients are highly statistically significant ($p < 0.05$) as is the whole equation ($p < 7 \times 10^{-4}$ for quantitative structure–antitumor activity relationships and $p < 9 \times 10^{-7}$ for quantitative structure–ability to DNA-duplexes stabilization relationships, respectively). The values of the multiple correlation coefficient, R ; the standard error of the estimate, s ; and the value of the F -test of significance, F , are also statistically significant.

Statistically significant parameters used in QSAR analysis (values of molecular descriptors are presented in the Table 3) as logarithm of theoretically calculated n -octanol–water partition coefficient ($\log P$) from the class of hydrophobic descriptors, 3st component symmetry directional WHIM index weighted by atomic masses (G3m), 2st component symmetry directional WHIM index by atomic polarizabilities (G2p), and 3st component symmetry directional WHIM directional index/weighted by atomic polarizabilities (G3p) from class of WHIM (Weighted Holistic Invariant Molecular) descriptors had the influence upon antitumor activity of acridinones. The WHIM descriptors are molecular descriptors based on statistical indices calculated on the projections of the atoms along principal axes. They are built in such a way as to capture relevant molecular 3D information regarding molecular size shape, symmetry, and atom distribution with respect to invariant reference frames (Todeschini *et al.*, 2000).

Table 2 Multiple regression QSAR equation (dependent variable = $k_0 + k_1A + k_2B + k_3C + k_4D$)

Dependent variable	Coefficients and statistically significant molecular descriptors								Statistical parameters				
	k_0	k_1	A	k_2	B	k_3	C	k_4	D	R (R^2) ^a	S ^b	F ^c	p ^d
ΔT_m	97.44 ± 55.09	-6.59 ± 1.50	GATS7e	3.03 ± 0.88	μ_i	0.64 ± 0.30	H-047	-147.44 ± 83.58	Mp	0.8388 (0.7036)	2.15	8.90	7×10^{-4}
	$p = 1 \times 10^{-2}$	$p = 5 \times 10^{-4}$		$p = 4 \times 10^{-3}$		$p = 5 \times 10^{-3}$		$p = 1 \times 10^{-2}$					
ILS	88.80 ± 153.44	8914.33 ± 1225.69	G3m	-36.31 ± 6.02	logP	-4691.69 ± 1227.99	G2p	-4744.01 ± 1451.51	G3p	0.9384 (0.8806)	21.03	27.65	9×10^{-7}
	$p = 1 \times 10^{-2}$	$p = 3 \times 10^{-6}$		$p = 2 \times 10^{-5}$		$p = 2 \times 10^{-3}$		$p = 5 \times 10^{-3}$					

^a Multiple correlation coefficient (determination coefficient)

^b Standard error of estimate

^c Value of the F-test of significance

^d Significance level

Table 3 Values of molecular descriptors used in QSAR analysis

Compound	Molecular descriptors							
	GATS7e	μ_i	H-047	Mp	G3m	logP	G2p	G3p
C-1310	1.07	3.70	13	0.66	0.16	-1.98	0.15	0.15
C-1311	0.92	3.06	16	0.66	0.15	-2.19	0.15	0.15
C-1330	1.19	3.16	16	0.66	0.15	-2.15	0.15	0.15
C-1415	0.90	2.32	14	0.67	0.15	-1.16	0.15	0.15
C-1419	0.89	2.01	13	0.66	0.15	-2.19	0.15	0.16
C-1558	2.13	2.28	13	0.65	0.15	0.15	0.15	0.15
C-1176	0.94	2.50	16	0.68	0.16	-1.12	0.16	0.16
C-1263	0.90	3.34	15	0.67	0.16	-2.87	0.16	0.16
C-1212	1.01	2.61	16	0.67	0.16	-1.79	0.16	0.16
C-1371	0.94	2.11	15	0.67	0.15	-2.82	0.15	0.15
C-1554	0.83	2.66	13	0.66	0.15	-1.01	0.15	0.15
C-1266	0.86	2.60	13	0.66	0.15	-0.95	0.15	0.16
C-1492	0.86	3.10	15	0.66	0.15	-1.97	0.15	0.15
C-1233	0.99	2.99	16	0.68	0.17	-1.12	0.17	0.16
C-1303	0.87	2.48	15	0.67	0.16	-2.14	0.16	0.16
C-1533	0.91	1.11	15	0.67	0.16	-1.78	0.17	0.16
C-1567	2.15	3.53	15	0.66	0.15	0.2	0.15	0.15
C-1410	0.86	2.39	11	0.67	0.16	-2.16	0.16	0.16
C-1296	0.94	3.08	19	0.67	0.16	-1.06	0.17	0.16
C-1305	0.81	2.44	18	0.67	0.17	-2.09	0.16	0.16

In general, the obtained data indicate that hydrophobic and total molecular symmetry properties are important for antitumor activity of acridinones. These observations are in partial agreement with the data obtained by Mazerska (Mazerska *et al.*, 1996), for which antitumor activity of imidazoacridinones is dependent on lipophilicity. However, impact of lipophilicity on the biological activity of these compounds was observed only in the case of derivatives with 8-hydroxyl group, which undergo metabolic activation (Mazerska *et al.*, 1999, 2003). Moreover, non-hydroxyl or 9-hydroxyl derivatives also exhibited lipophilic properties, but its effect was not crucial when metabolic activation did not occur. Relocation of hydroxyl group from position 8 to 9 drastically decreases antitumor activity (C-1311 8-hydroxyl, C-1419 9-hydroxyl). In addition, hydrophobic properties of acridinones can play important role in transport and accumulation of these compounds in cells in view of fastening of metabolic activation (Składanowski *et al.*, 1996). On the other hand, diaminoalkyl side chain has also crucial influence on antitumor activity of acridinones. For compounds without 8-hydroxyl group, the increase in number of carbon atoms between nitrogen atoms from two to three or five (C-1415, C-1176, and C-1233 two; C-1212 and C-1296 three; and C-1266 five) generally decreases antitumor activity of imidazo- and triazoloacridinones. In case of derivatives

bearing 8-hydroxyl group, the increase in number of carbon atoms (C-1311, C-1263 two, C-1371 three, and C-1492 five) rather do not augment antitumor activity for imidazoacridinones, while good increase in antitumor activity is observed in case of triazoloacridinones (C-1303, C-1410 two, and C-1305 three carbon atoms). These observations indicate that length and conformation of diaminoalkyl side chain attached to flat aromatic acridinone ring have some influence on proper conformation of acridinone derivatives molecule and is in accordance with earlier modeling studies (Mazerski and Muchniewicz, 2000) The observations also proved our suggestion that total molecular symmetry properties in context of interaction with DNA as intercalation and DNA groove binding are crucial for antitumor activity of acridinones.

On the other hand, statistically significant parameters—values of molecular descriptors are presented in the Table 3—such as dipole moment (μ_i) from class of electronic descriptors, mean atomic polarizability scaled on carbon atom (Mp) from class of constitutional descriptors, Geary autocorrelation-lag 7 weighted by atomic Sanderson electronegativities (GATS7e) from class of 2D autocorrelations descriptors, and H attached to C1(sp3)/CO(sp2) (H-047) from class of atom-centered fragments descriptors had the influence upon physicochemical (noncovalent) DNA-duplexes stabilization of acridinone derivatives. The obtained data indicate that electronic and topological properties are important for noncovalent DNA-duplexes stabilization of these compounds. It is known that drug–DNA binding induces changes in DNA structure and topology and is closely connected with conformation of drug molecule and its electronic and topological properties. The presence of a hydroxyl group in position 8 of acridinone ring slightly increases the affinity for DNA compared to unsubstituted or alkyl-substituted derivatives, possibly because of additional hydrogen bonds with the DNA phosphate backbone. As it was mentioned earlier (Mazerski and Muchniewicz, 2000), the charged diaminoalkyl side chain of acridinone compounds can interact with DNA in the minor groove, in addition to intercalation. In addition, some other data (Koba and Konopa, 2007) indicated that intercalation is not involved in stabilization of secondary structure of DNA. However, for the biologically non-active compounds, C-1558 and C-1567, bearing a *t*-butyl group in position 8, the ΔT_m values were 2.4 and 6.8°C, respectively, indicating that stabilization of the DNA duplex by these compounds especially by C-1567 probably derived from electrostatic interactions of the side chain with DNA and not from its intercalation to DNA. This means that intercalation to DNA is necessary for the biological activity of acridinones via positioning the drug molecules within DNA before the covalent reaction and formation of interstrand DNA crosslink (Koba and Konopa,

2007). This also indicated that topological and electronic properties of acridinone derivatives are important for their physicochemical interactions with DNA. Moreover, the molecular modeling studies (Mazerski and Muchniewicz, 2000) evidenced that when acridinone C-1311 is intercalated between GC, the highly reactive position 8 on acridinone core is in close proximity to nucleophilic N7 position on guanine. It is plausible to postulate that drug molecule first intercalates into DNA and then, after in situ activation, binds covalently to the neighboring base. These observations are compatible with recent findings demonstrating that electrochemically activated C-1311 forms covalent adducts with deoxyguanine (Mazerska *et al.*, 2003). On the other hand, the structure of acridinones suggests that there are at least two possible sites for enzymatic oxidation/activation, which potentially could be involved in the covalent binding to DNA. One is the diaminoalkyl side chain at position 5 which is necessary for covalent binding of mitoxantrone to DNA (Składanowski and Konopa, 2000). The other one is the potential quinone–imine group formed by hydroxyl group in position 8

Table 4 Values of experimental and calculated data for DNA-duplexes stabilization and antitumor activity of acridinones

Compound	ΔT_m exp. ^a	ΔT_m calc.	Δ^b	ILS exp. ^c	ILS calc.	Δ
C-1310	15.3	12.6	2.7	185	172	13
C-1311	13.7	13.6	0.1	93	90	3
C-1330	11.5	12.1	0.6	96	89	7
C-1415	7.2	8.8	1.6	55	53	2
C-1419	8.3	8.7	0.4	27	43	16
C-1558	2.4	2.8	0.4	0	5	5
C-1176	9.5	8.9	0.6	90	46	44
C-1263	12.3	12.5	0.2	110	110	0
C-1212	11.5	10.2	1.3	25	70	45
C-1371	3.5	8.5	5.0	120	113	7
C-1554	10.5	11.1	0.6	20	47	27
C-1266	9.9	10.7	0.8	10	−2	8
C-1492	13.1	13.5	0.4	85	82	3
C-1233	9.1	10.0	0.9	77	88	11
C-1303	13.1	10.1	3.0	102	83	19
C-1533	8.1	5.7	2.4	10	23	13
C-1567	6.8	6.3	0.5	0	3	3
C-1410	7.1	7.3	0.2	78	84	6
C-1296	11.5	14.0	2.5	18	−3	15
C-1305	15.1	12.3	2.8	165	170	5
Mean value of Δ			1.4			13

^a The increase in DNA melting temperature (expressed in centigrade degrees) at drug to DNA base pairs 0.25 M ratio

^b Difference between experimental and calculated values

^c The percentage of increase in survival time of treated to control mice with P388 leukemia at optimal dose

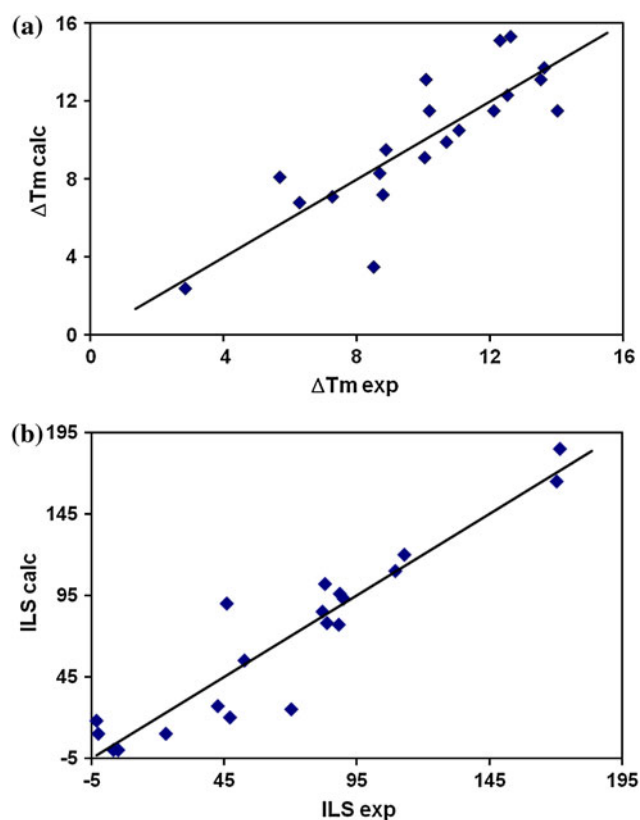


Fig. 1 Correlation between the experimental data and the calculated data from the derived multiple regression QSAR equation for **a** DNA-duplexes stabilization of acridinones expressed as ΔT_m (the increase in DNA melting temperature at drug to DNA base pairs 0.25 M ratio) and **b** antitumor activity of acridinones expressed as ILS (survival time of treated to control mice with P388 leukemia at optimal dose)

(8-OH) and heterocyclic nitrogen atom in acridinone nucleus (Mazerska *et al.*, 2003). Recently proposed mechanism of oxidation involves highly unstable carbocations generated in these two positions (Mazerska *et al.*, 2003). It is suggested that C-1311 carbocations react

rapidly with nucleophiles present in the environment, including DNA bases forming covalent adducts. These observations indicate that topological and electronic properties of acridinone derivatives are also important for their covalent interactions with DNA.

Moreover, the calculated values of ILS and ΔT_m obtained for other compounds (Table 4) and the plots of the experimental data versus the calculated data (Fig. 1a–b) for DNA-duplexes stabilization of acridinones expressed as ΔT_m (the increase in DNA melting temperature at drug to DNA base pairs 0.25 M ratio) and antitumor activity of acridinones expressed as ILS (survival time of treated to control mice with P388 leukemia at optimal dose) proved good correlation and predictive potency of proposed QSAR models. In addition, the RMSECV as value, which quantifies the predictive power of the proposed QSAR model, are calculated by the leave-one-out and the leave-ten-out methods and presented in the Table 5. The obtained values of RMSECV test (22.79 and 22.27 for quantitative structure–antitumor activity relationships as well as 2.39 and 2.41 for quantitative structure–ability to DNA-duplexes stabilization relationships) performed for all the four statistically significant independent variables proved the predictive power of the derived QSAR models.

Conclusions

Statistically significant equations describing structure–antitumor activity relationships and structure–ability to physicochemical (noncovalent) interaction with DNA relationships in acridinone derivatives group were derived. It has been found that hydrophobic and total molecular symmetry properties are important for antitumor activity of acridinone derivatives, and electronic and topological properties are important for physicochemical (noncovalent) DNA-duplexes stabilization of these compounds.

Table 5 Values of the cross-validated root-mean-square error RMSECV test

QSAR model for dependent variable	RMSECV test							
	Leave-one-out method				Leave-ten-out method			
	1 ^a	2	3	4	1	2	3	4
ΔT_m	3.36	2.53	2.56	2.39	3.44	2.63	2.64	2.41
ILS	53.39	42.10	28.48	22.79	54.23	42.35	28.74	22.27

^a 1–4 represents RMSECV test performed only for one, combined two and three, and for all the four significance descriptors in QSAR models, respectively. In the case of QSAR model, for ΔT_m as dependent-variable values, 1–4 were obtained for only GATS7e, GATS7e combined with μ_i , GATS7e combined with μ_i and H-047, GATS7e combined with μ_i , H-047, and Mp descriptors. In the case of QSAR model for ILS as dependent-variable values, 1–4 were obtained for only G3m, G3m combined with logP, G3m combined with logP and G2p, and G3m combined with logP, G2p and G3p descriptors

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