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Short communication

Importance of retention data from affinity and reverse-phase high-performance liquid chromatography on antitumor activity prediction of imidazoacridinones using QSAR strategy

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ABSTRACT

Quantitative structure–activity relationships (QSAR) studies for prediction of cytotoxic and antitumor activity of imidazoacridinones (IA) based on experimentally obtained high-performance liquid chromatography (HPLC) retention data and calculated parameters using computational (molecular modeling) medicinal chemistry methods were proposed. The RP-HPLC and affinity-HPLC chromatographic techniques with four diversified HPLC systems applying columns with octadecylsilanes (C18), phosphatidylcholine (IAM), as well as α_1-glycoprotein (AGP) and albumin (HSA) were used for the determination of the retention constants $\log k$ and $\log k_w$ which characterize lipophilicity and protein affinity of IA. Moreover, molecular modeling studies were performed using HyperChem and Dragon software's, and structural descriptors were calculated and subsequently used. The QSAR equations using multiple linear regression (MLR) analysis method were derived which indicated that in vivo antileukemia activity of IA depends on cytotoxic activity against leukemia cells, whereas this cytotoxic activity depends on $\log k$ and $\log k_w$ parameters obtained on all HPLC systems. Moreover, the QSRR equations were derived and indicated that $\log k$ and $\log k_w$ parameters depend on calculated non-empirical structural parameters. The predictive power of obtained QSAR and QSRR equations allowed the prediction of cytotoxic and antitumor activity of IA and also their HPLC retention parameters. Finally, the equations can be used for prediction of antileukemia activity of IA without the necessity of carrying out experimental measurements.

1. Introduction

Imidazoacridinones (IA) are acridinone derivatives exhibited significant antitumor activity towards many tumors in animals and display potent cytotoxicity against a number of tumor cell lines of human and animal origin in tissue cultures. The most active imidazoacridinone derivative C-1311 underwent phase II clinical for colon and breast cancer. The proposed imidazoacridinones' mechanisms of action being crucial for their cytotoxic and antitumor activity are strictly associated with the interactions of these compounds with nucleic acids (for review see [1]).

The fundamental processes of drug action (also antitumor drugs action) are connected with its absorption into the general circulation followed with reversible binding to blood proteins, pharmacodynamic and pharmacokinetic related distribution to various tissues (also tumor tissues), biotransformation of the drug to its metabolites as well as excretion of the unaltered drug and/or its biotransformation products [2,3]. Moreover, it is considered that the dynamic and fast equilibrium processes of drug action have much in common with the processes that are basis of the chromatographic separations [3]. Therefore, chromatographic data are often used to model pharmacodynamics and pharmacokinetics of drugs. It is believed that the most important physicochemical phenomenon determining absorption, distribution and excretion processes is the passive diffusion of drugs and their metabolites through biological membranes. Hence, the partition coefficient of the drug between aqueous intra- and extracellular environment as well as phospholipid membranes is important for pharmacokinetic transport within living system (also tumor cells). On the other hand, lipophilicity may also influence the interaction between a drug compound and a pharmacological receptor (cellular DNA in the case of IA). The most convenient method of assessment of partition parameter (lipophilicity) of compounds (analytes) is reversed-phase high-performance liquid chromatography (RP-HPLC) [4,5]. However, if the goal is to model processes in biophase mimicked processes in living system, the active components of the chromatographic and the biological system must be similar. This gives the immobilized artificial membrane (IAM) stationary phases in which a phospholipid moiety is covalently bound to aminopropylsilica [6].

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Although lipophilicity strongly affects the pharmacodynamic and pharmacokinetic actions of drugs it is not the sole property to determine pharmacological (also cytotoxic and antitumor) effects. For the majority of drugs (also for IA) the specific interactions with biomolecules (as serum proteins) in the biophase are also important. There are two main serum protein stationary phases commonly using in affinity HPLC for study of drug binding. Acidic and neutral drugs are mostly bound by human serum albumin (HSA) protein stationary phase, whereas basic drugs have a higher affinity to α_1-acid glycoprotein (AGP) stationary phase [7,8].

The experimentally obtained chromatographic retention data as well as molecular descriptors obtained by computational (molecular modeling) medicinal chemistry methods are a powerful tools in the QSAR studies containing diverse sources of chemical information useful for better understanding between molecular structure and experimental evidence [9,10]. Through the use of statistical chemometric techniques, biological (pharmacological) activity of analyzed compounds could be characterized in terms of HPLC retention data and/or various descriptors, and also retention parameters could be characterized in terms various descriptors of the analytes. Among various chemometric methods, MLR analysis is the most often performed to QSAR analysis as well as to process retention data in the quantitative structure–retention relationship (QSRR) analysis. If statistically significant and physically meaningful QSAR and QSRR are obtained, they can be applied to predict pharmacological (cytotoxic and antitumor) activity and retention for an analytes, respectively, and also to identify the most informative structural descriptors regarding analyte properties [9,11].

The objective of this study was determining the importance of these retention data on antitumor activity prediction of IA using QSRR and QSAR studies.

2. Experimental

2.1. Antitumor and cytotoxic activity of imidazoacridinones

The IA examined in this study has been selected for twelve compounds differing in chemical structures as well as in cytotoxic and anticancer activities (Table 1). The data of cytotoxic activity of IA against mouse leukemia L1210 cells line expressed as the compound concentration inhibiting growth of cells by 50% after 72 h incubation ($IC50_{L1210}$) as well as antitumor activity of IA against P388 leukemia in mice in vivo and expressed as the percentage of increase in survival time of treated to control mice with P388 leukemia at optimal dose (ILS_{P388}) both have been taken from the literature [12].

2.2. Chemicals

HPLC-grade acetonitrile, water and 2-propanol as well as analytical-grade formic acid and other reagents were from POCH (Gliwice, Poland). All analyzed imidazoacridinone derivatives were available from the collection of the Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Gdańsk, Poland.

2.3. HPLC retention data parameters' determination

A Shimadzu High-Performance Liquid Chromatography set (Kyoto, Japan) has been used for complex HPLC analysis. The following HPLC columns have been employed: (a) octadecylsilane (C18) column Unison UK-C18 (Imtakt Corporation, Kyoto, Japan), (b) immobilized artificial membrane column IAM.PC.DD 2 (Regis Chemical Company, Morton Grove, IL, USA), (c) AGP column (Chiral Technologies Europe/Daicel, Illkirch, France), and (d) HSA column (Chiral Technologies Europe/Daicel, Illkirch, France). In the case of all experiments the IA stock solutions (0.1 mg/mL solutions of IA in 0.2% lactic acid) were diluted in methanol to concentration 10 μg/mL and then samples were injected into columns in volume 10 μL. In the case of reversed-phase HPLC analysis performed on Unison UK-C18 column, the gradient HPLC elution has been carried out with solvent A (water with 0.1% (v/v) formic acid) and solvent B (acetonitrile with 0.1% (v/v) formic acid). Gradient experimental retention times (t_R) of the series of IA have been measured with linear gradient of 5–100% of solvent B in two gradient runs differing in gradient time (t_G equal to 10 min and 30 min). These retention times served as input data and appropriate $\log k$ and $\log k_w$ values (i.e. the retention factor $\log k$ extrapolated to 0% organic modifier) have been calculated using DryLab 6.0 program (LC Resources, Walnut Creek, CA, USA). Moreover, all the chromatographic measurements were performed at a flow rate 1 mL/min and the UV detection wavelength at 254 nm. In the case of affinity HPLC analysis IA were chromatographed in isocratic conditions. For the HSA and AGP columns the chromatographic conditions were optimized with 0.025 M phosphate buffer of pH 6.8 and 2-propanol with ratio of 94:6 (v/v) or 88:12 (v/v), respectively. For IAM.PC.DD 2 column the mobile phase was 0.025 M phosphate buffer of pH 6.8 and acetonitrile with ratio of 80:20 (v/v). The flow rate of mobile phase was 0.8 mL/min and the detection UV wavelength at 254 nm. The values of imidazoacridinones' HPLC retention data as $\log k$ ($\log k$_C18) as well as $\log k_w$ ($\log k_w$_C18) parameters obtained on Unison UK-C18 column, and $\log k$ parameters ($\log k$_HSA, $\log k$_AGP and $\log k$_IAM) obtained on Chiral HSA, AGP, IAM.PC.DD 2 columns were calculated and subjected further to QSAR analysis.

2.4. Structural parameters

The structures of the tested IA were studied by molecular modeling with the use of HyperChem 7.5 Release software (HyperCube Inc., Gainesville, FL, USA) and Dragon 5.0 software (Talete, Milano, Italy). The structures of the compounds were firstly pre-optimized with the Molecular Mechanics Force Field (MM+) procedure and the resulting geometries were further refined by means of the Semi-Empirical Molecular Orbitals Method AM1 using the Polak-Ribiere algorithm. In the next step, resulting geometrical structures were studied with the use of Dragon software and over 1300 molecular descriptors were calculated and taken for QSAR studies [13]. Moreover, parameters characterized lipophilicity ($\log P$) and aqueous solubility ($\log S$) of studied IA were calculated using on-line Virtual Computational Chemistry Laboratory (VCCLab) and also taken for QSAR analysis [14].

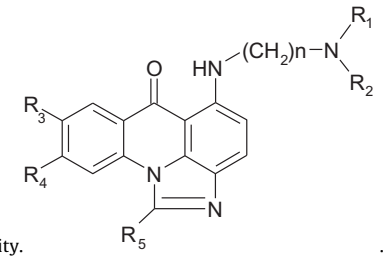
2.5. QSAR analysis

The statistical QSAR analysis was performed using stepwise multiple linear regression (MLR) approach with the use of Statistica 9.0 software (StatSoft, Tulsa, OK, USA).

3. Results and discussion

There are two main objectives for the development of QSAR. The development of predictive and robust QSAR for the prediction of activity of molecules acts as an informative tool by extracting significant patterns in descriptors related to the measured biological activity leading to understanding of mechanisms of given biological activity, and could help in suggesting design of novel molecules with improved activity profile. The proposed QSAR studies were performed to develop predictive and robust QSAR models for prediction of antileukemia activity of selected imidazoacridinones' molecules (for activity see Table 1).

Table 1



Chemical structure of IA and their cytotoxic and antitumor activity.

Compound	*n*	R_1	R_2	R_3	R_4	R_5	ILS_{P388} (%)[a]	$EC50_{1210}$ (μM)[b]
C-1176	2	CH_3	CH_3	H	H	H	90	1.19
C-1212	3	CH_3	CH_3	H	H	H	25	1.95
C-1266	5	CH_3	CH_3	H	H	H	10	3.03
C-1310	2	CH_2CH_3	CH_2CH_3	OH	H	CH_3	185	0.25
C-1311	2	CH_2CH_3	CH_2CH_3	OH	H	H	93	0.031
C-1330	2	CH_2CH_3	CH_2CH_3	OCH_3	H	H	96	1.78
C-1371	3	CH_3	CH_3	OH	H	H	120	0.034
C-1415	2	CH_2CH_3	CH_2CH_3	H	H	H	55	1.72
C-1419	2	CH_2CH_3	CH_2CH_3	H	OH	H	27	1.96
C-1492	5	CH_3	CH_3	OH	H	H	85	1.12
C-1554	2	CH_2CH_3	CH_2CH_3	CH_3	H	H	18	1.42
C-1558	2	CH_2CH_3	CH_2CH_3	$C(CH_3)_3$	H	H	0	3.44

[a] The increase in survival time of treated to control mice with P388 leukemia at optimal dose.
[b] The concentration of the compound inhibiting growth of mouse leukemia L1210 cells by 50% after 72 h incubation.

3.1. QSAR analysis using experimentally obtained data

In the first stage of the proposed QSAR studies, using MLR method the QSAR model was performed for imidazoacridinones' experimental antitumor activity in vivo against P388 leukemia (ILS_{P388}). The calculated QSAR equation (1) characterized by two statistically significant independent variables as experimental value of cytotoxic activity $EC50_{L1210}$ and calculated value of highest positive electron charge on the atoms MAX_POS, and also characterized by good correlation ($R = 0.9030$) and statistical significance ($p < 0.05$) level for of each term and of whole equation (see Table 2). Generally, the obtained data indicated that in vitro cytotoxic activity and electronic properties of molecule of IA had the influence upon studied antitumor (antileukemia) activity of these compounds.

However, the QSAR strategy recommend calculation of QSAR model on the basis non-empirical structural descriptors as independent variables giving ability to predict the relative biological activities or other experimental properties without a set of drugs and necessity of carry out of experimental measurements. For these reasons, in the next part of studies the QSAR models were performed for experimentally obtained in vitro cytotoxic activity of IA against leukemia L1210 cells ($EC50_{L1210}$). It is important to note, that our latest study [10] concerning QSAR analysis of acridinones (imidazoacridinones and triazoloacridinones) revealed that parameters describing lipophilicity possessed the most significant influence on the antitumor activity indicated that hydrophobic properties of acridinones can play important role in transport and accumulation of these compounds in the cells. On the other hand, lipophilicity besides influences a drug's permeation ability (cellular matrix-nucleus as well as cellular matrix-mitochondrium barrier crossing in the case of drugs interacted with genome or mitochondrial DNA, respectively), also influences a drug's distribution properties as degree of plasma proteins binding. Hence, specific interactions of IA with serum proteins as HSA and AGP are very important for biological (cytotoxic and/or antitumor) activity of these compounds. Therefore, the retention times of studied IA were measured in the given chromatographic systems and the values of the logarithm of the HPLC retention factors ($\log k$ and/or $\log k_w$) were calculated (see Table 3) and subjected further to QSAR analysis. Next, using these data as well as non-empirical descriptors and MLR method the QSAR models were built for experimental in vitro cytotoxic activity of IA against leukemia L1210 cells ($EC50_{L1210}$), and the four statistically significant QSAR equations (2)–(5) were obtained and characterized by two statistically significant independent variables (see Table 2). Eqs. (2)–(4) relating $EC50_{L1210}$ on experimentally obtained HPLC retention data on studied columns ($\log k_w$_C18, $\log k$_C18, $\log k$_HSA and $\log k$_AGP) compared to Eq. (5) relating $EC50_{L1210}$ on molecular descriptors log_*P* and E_LUMO (the calculated lipophilicity parameter $\log P$ and energy of the lowest unoccupied molecular orbitals, respectively). Moreover, the obtained QSAR models indicated that in vitro cytotoxic activity against leukemia L1210 cells ($EC50_{L1210}$) is strictly connected with all lipophilic-related properties of IA influenced on their biological (cytotoxic and antitumor) activity.

As the next step, the ability to make use of the predicted values of in vitro cytotoxic activity of IA against leukemia L1210 cells to prediction of in vivo antitumor activity of IA against P388 leukemia (ILS_{P388}) using QSAR equation (1), was carried out. The plots of the experimental data (ILS_{P388}) versus the predicted data (ILS_{P388}pred) (Fig. 1A–D) proved good correlation (values of correlation coefficient *R* are generally on the similar level ($R = 0.8456$–0.9281) or even better ($R = 0.9602$) compared to *R* value equal 0.9030 and obtained on the basis original data according to Eq. (1)). These results confirmed very good usefulness of non-empirically obtained $EC50_{L1210}$ values (obtained on the basis experimentally obtained HPLC retention data) on prediction of in vivo activity of IA against P388 leukemia, and indicated also that in vivo antitumor activity of IA could be performed without necessity to perform biological experiments of cytotoxicity measurements in tissues culture.

3.2. QSRR analysis of HPLC retention data

As was mentioned above in QSAR strategy preferable is using non-empirically obtained parameters for build QSAR model for prediction of biological (pharmacological) activity not only for model data set, but first of all for test data set (new compounds data set). For this reason QSAR analysis named quantitative

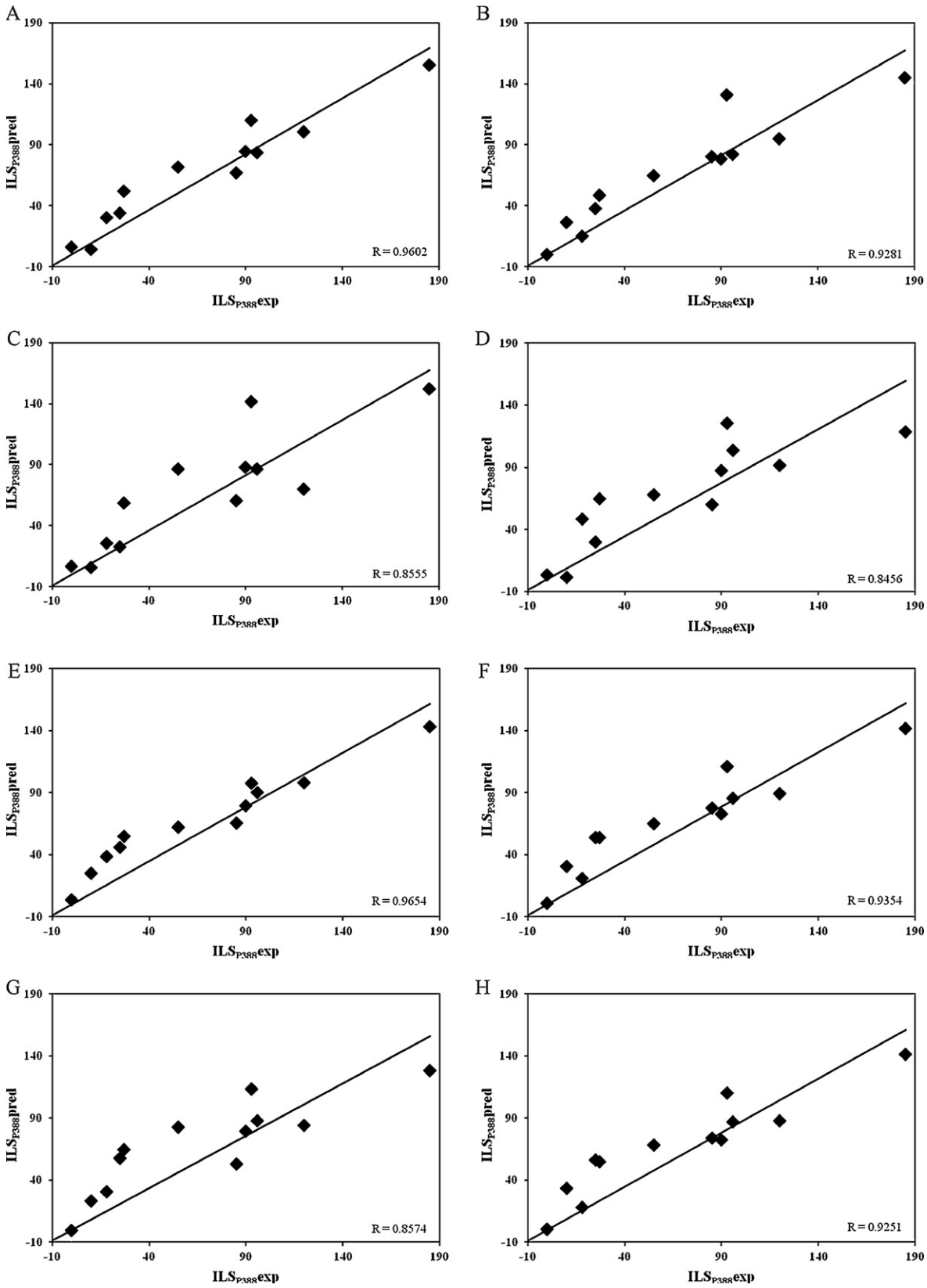


Fig. 1. Correlations between the experimental data and the predicted data from derived multiple regression QSAR equation (1) from Table 2 for antitumor activity of IA (ILS_{P388}) on the basis calculated cytotoxic activity ($EC50_{L1210}$) obtained using experimental HPLC data and: (A) Eq. (2) from Table 2; (B) Eq. (3) from Table 2; (C) Eq. (4) from Table 2; (D) Eq. (5) from Table 2; or obtained using QSAR strategy obtained HPLC data and: (E) Eq. (2) from Table 2; (F) Eq. (3) from Table 2 (log k_AGP taken to Eq. (3) was calculated on the basis experimental value of log k_IAM); (G) Eq. (4) from Table 2; (H) Eq. (3) from Table 2 (log_AGP taken to Eq. (3) was calculated on the basis QSAR strategy obtained value of log_IAM).

Table 2
Multiple regression QSAR equations derived for antitumor and cytotoxic activities as well as HPLC retention parameters of IA (dependent variable = $k_0 + k_1A + k_2B$).

Eq. no.	Dependent variable	Coefficients and statistically significant parameters					Statistical parameters of equation			
		k_0	k_1	A	k_2	B	R (R^2)[a]	S[b]	F[c]	p[d]
(1)	ILS_{P388}	2799.10 ± 949.64 $p = 1 \times 10^{-2}$	-35.12 ± 7.54 $p = 1 \times 10^{-3}$	$IC50_{L1210}$	-8238.08 ± 2928.24 $p = 2 \times 10^{-2}$	MAX_POS[e]	0.9030 (0.8154)	25.92	19.88	5×10^{-4}
(2)	$IC50_{L1210}$	-3.82 ± 1.40 $p = 2 \times 10^{-2}$	7.56 ± 1.00 $p = 3 \times 10^{-5}$	$\log k_w$_C18	-2.90 ± 0.87 $p = 9 \times 10^{-3}$	log k_HSA	0.9300 (0.8649)	0.44	28.80	1×10^{-4}
(3)	$IC50_{L1210}$	-5.82 ± 1.32 $p = 2 \times 10^{-3}$	7.68 ± 1.16 $p = 9 \times 10^{-5}$	$\log k_w$_C18	-1.68 ± 0.63 $p = 9 \times 10^{-3}$	log k_AGP	0.9189 (0.8316)	0.49	22.21	3×10^{-4}
(4)	$IC50_{L1210}$	-11.32 ± 2.25 $p = 7 \times 10^{-4}$	84.68 ± 29.38 $p = 2 \times 10^{-2}$	$\log k_w$_C18	-81.01 ± 30.33 $p = 3 \times 10^{-2}$	log k_C18	0.9116 (0.8310)	0.49	22.13	7×10^{-4}
(5)	$IC50_{L1210}$	12.41 ± 3.61 $p = 7 \times 10^{-3}$	0.952 ± 0.222 $p = 2 \times 10^{-3}$	log_P[e]	10.05 ± 3.93 $p = 3 \times 10^{-2}$	E_LUMO[e]	0.8781 (0.7710)	0.57	15.15	1×10^{-3}
(1a)	log k_C18	1.48 ± 0.43 $p = 8 \times 10^{-3}$	-0.298 ± 0.019 $p = 1 \times 10^{-7}$	AC_log S[f]	0.158 ± 0.054 $p = 2 \times 10^{-2}$	E_HOMO[e]	0.9832 (0.9668)	0.03	130.84	2×10^{-7}
(2a)	log k_w_C18	1.68 ± 0.50 $p = 8 \times 10^{-3}$	-0.308 ± 0.022 $p = 1 \times 10^{-7}$	AC_log S	0.175 ± 0.062 $p = 2 \times 10^{-2}$	E_HOMO	0.9794 (0.9591)	0.03	105.58	6×10^{-7}
(3a)	log k_HSA	-5.83 ± 1.11 $p = 5 \times 10^{-4}$	2.55 ± 0.39 $p = 1 \times 10^{-4}$	EEig06r[g]	-0.035 ± 0.011 $p = 1 \times 10^{-2}$	T(O···O)[g]	0.9132 (0.8339)	0.07	22.58	3×10^{-4}
(4a)	log k_AGP	-3.89 ± 1.09 $p = 6 \times 10^{-3}$	0.821 ± 0.218 $p = 4 \times 10^{-3}$	log k_IAM	1.98 ± 0.58 $p = 8 \times 10^{-3}$	EEig09r[g]	0.9184 (0.8435)	0.12	24.25	2×10^{-4}
(5a)	log k_IAM	0.659 ± 0.150 $p = 2 \times 10^{-3}$	-0.622 ± 0.055 $p = 1 \times 10^{-6}$	ALOGpS[f]	0.316 ± 0.051 $p = 2 \times 10^{-4}$	AB/log S[f]	0.9681 (0.9372)	0.05	67.18	4×10^{-6}

[a] Multiple correlation coefficient (determination coefficient).
[b] Standard error of estimate.
[c] Value of the F-test of significance.
[d] Significance level.
[e] Parameter calculated using HyperChem software.
[f] Parameter calculated using VCCLab software.
[g] Parameter calculated using Dragon software.

Table 3
Values of experimental and predicted data for HPLC retention data of IA.

Compound	log k_C18exp[a]	log k_C18pred[b]	log k_w_C18exp	log k_w_C18pred	log k_HSAexp	log k_HSApred	log k_AGPexp	log k_AGPpred	log k_AGPpred(I)[c]	log k_IAMexp	log k_IAMpred
C-1176	1.07	1.07	1.17	1.18	1.32	1.29	1.08	1.02	1.02	1.27	1.26
C-1212	1.13	1.10	1.24	1.20	1.28	1.29	1.18	1.27	1.32	1.39	1.45
C-1266	1.28	1.26	1.39	1.37	1.36	1.50	1.64	1.61	1.65	1.77	1.82
C-1310	1.04	1.05	1.13	1.15	1.62	1.55	1.51	1.55	1.54	1.43	1.42
C-1311	1.04	1.09	1.13	1.19	1.27	1.30	1.44	1.37	1.35	1.36	1.33
C-1330	1.22	1.19	1.32	1.29	1.53	1.51	1.53	1.43	1.45	1.54	1.56
C-1371	1.03	1.01	1.13	1.10	1.64	1.51	1.57	1.36	1.33	1.36	1.32
C-1415	1.15	1.17	1.25	1.27	1.32	1.29	1.06	1.18	1.23	1.36	1.42
C-1419	1.07	1.09	1.17	1.18	1.29	1.36	1.07	1.23	1.24	1.34	1.35
C-1492	1.17	1.18	1.27	1.28	1.56	1.56	1.82	1.81	1.75	1.79	1.72
C-1554	1.26	1.24	1.36	1.34	1.66	1.68	1.51	1.51	1.46	1.64	1.59
C-1558	1.51	1.52	1.62	1.64	1.68	1.69	1.72	1.81	1.80	1.65	1.64

[a] Experimentally obtained values of HPLC retention parameters.
[b] Predicted values of HPLC retention parameters on the basis derived QSAR models according to Eqs. (1a)–(5a) from Table 2.
[c] Predicted values of HPLC parameter log k_AGP on the basis calculated values of log k_IAM using QSAR Eq. (5a) from Table 2.

structure–retention relationship (QSRR) in the case to process HPLC retention data was used to build QSRR models with predictive potency for all experimental obtained $\log k$ and $\log k_w$ parameters presented above ($\log k$_C18, $\log k_w$_C18, $\log k$_HSA, $\log k$_AGP and $\log k$_IAM parameters). The multiple regression QSRR analysis for these HPLC parameters was performed and the five statistically significant QSAR equations (1a)–(5a) were obtained and characterized by two statistically significant independent variables (see Table 2). Eqs. (1a) and (2a) relating $\log k$_C18 and $\log k_w$_C18, respectively, on molecular descriptor AC_log$S$ characterized aqueous solubility of IA and molecular descriptor E_HOMO that characterize the energy of the highest occupied molecular orbitals in imidazoacridinone molecule, Eq. (3a) relating $\log k$_HSA on molecular descriptors EEig06r and T(O···O) which are calculated parameters characterized eigenvalue from edge adjacency matrix weighted by resonance integrals and sum of topological distances between O···O atoms in imidazoacridinone molecule, respectively, Eq. (4a) relating $\log k$_AGP on experimentally obtained HPLC retention data on IAM column ($\log k$_IAM) and molecular descriptor EEig09r characterized eigenvalue from edge adjacency matrix weighted by resonance integrals in imidazoacridinone molecule, and Eq. (5a) relating $\log k$_IAM on molecular descriptors ALOGpS and AB/log$S$ characterized aqueous solubility of IA (Table 2). Moreover, the obtained QSAR models indicated that the aqueous solubility of IA as well as electronic, steric and electrostatic properties of imidazoacridinone molecule have crucial influence on the values of HPLC retention data parameters of all studied IA. It is important to note, that aqueous solubility ($\log S$) have strongly effect the lipophilicity properties of compounds (lipophilicity parameter $\log P$ value is the logarithm of the ratio of the concentrations of the un-ionized compound in the two immiscible solvents as water and *n*-octanol, hence solubility level of compound in aqueous is very important factor for value of $\log P$ parameter) as was observed in QSAR models derived for C18 and IAM columns which are commonly using for HPLC-base lipophilicity determination. It is also demonstrated that hydrophobic drugs with high partition coefficients are preferentially distributed to hydrophobic compartments such as lipid bilayers of cells while hydrophilic drugs with low partition coefficients preferentially bound to hydrophilic compartments such as blood serum proteins. Additionally, electronic properties of IA characterized by E_HOMO value are also important for lipophilicity of these drugs. On the other hand, the QSAR models for IA obtained for $\log k$_HSA and $\log k$_AGP parameters as dependent variables are in some agreement with data obtained by Kaliszan [6–9] for which HSA binds drugs generally through lipophilicity related interactions limited by steric volumes of drugs (EEig06r and T(O···O) parameters obtained in the case of IA) whereas AGP binds drugs though hydrophobic and electrostatic interactions ($\log k$_IAM and EEig09r parameters, respectively, in the case of considered imidazoacridinone derivatives). Additionally, calculated values of all considered HPLC retention parameters ($\log k$_C18pred–$\log k$_IAMpred) obtained for all IA (Table 3) on the basis derived QSAR (QSRR) equations (1a)–(5a) proved very good predictive potency of proposed QSAR models.

3.3. QSAR analysis using predicted data

In the final stage of the proposed QSAR analysis, the ability to make use of the calculated values of HPLC retention data parameters ($\log k$_C18pred–$\log k$_IAMpred) on the basis QSRR equations (1a)–(5a) in order to predict the in vitro cytotoxic activity of IA against leukemia L1210 cells using appropriate QSAR equations (2)–(4), was carried out. The values of $EC50_{L1210}$ obtained with the use of the QSAR strategy were incorporated into QSAR equation (1) and derived earlier for the prediction of IA in vivo antitumor activity against P388 leukemia (ILS_{P388}) (see Table 2). The correlation plots between experimental (ILS_{P388}) and predicted data (ILS_{P388}pred) (see Fig. 1E-H) proved good correlation (values of correlation coefficient R are on the similar or even better level (R = 0.8574–0.9654) compared to R values and obtained on the basis experimental data (see Fig. 1A–D). Most importantly, the obtained data indicated that using imidazoacridinones' cytotoxic activity $EC50_{L1210}$ received on the basis QSAR together with QSRR analysis allowed to prediction of in vivo activity of IA against P388 leukemia (ILS_{P388}) with very high predictive potency. Summarized, these results indicated that in vivo antitumor activity of IA may be determined without necessity of experimentally obtained data characterized biological as well as physicochemical properties of considered compounds, but with the use QSAR analysis performed on the basis only non-empirical molecular descriptors obtained by computational (molecular modeling) medicinal chemistry methods.

4. Conclusions

Data presented in this report demonstrate that QSAR as well as QSRR analysis could be applied successfully for the prediction of antitumor and cytotoxic activity of IA. The affinity-HPLC and RP-HPLC using chromatographic methods established parameters values ($\log k$ and $\log k_w$) of all the target compounds, is very important for cytotoxic activity of considered compounds. Proposed QSAR strategy could be considered as helpful supporting tool for rational search for imidazoacridinone derivatives most promising as potential antitumor agents against P388 leukemia (and others type of tumors), and allowed to reduce the number of routine biological assays in the search for new imidazoacridinone (acridinone) core based antitumor drugs.

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