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Determination of lipophilicity for antitumor acridinone derivatives supported by gradient high-performance liquid chromatography method

Research Article

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Abstract: The lipophilicity values of selected acridinone (imidazoacridinone and triazoloacridinone) derivatives were measured by gradient reversed-phase high-performance liquid chromatography (RP-HPLC) using a C18 stationary phase with a water/acetonitrile mixture as a mobile phase. The retention times obtained served as input data and appropriate log kw values (*i.e.*, the retention factor log k_w extrapolated to 0% organic modifier) as an alternative to log P were calculated using the DryLab program. The relationships between the lipophilicity (log k_w) and the chemical structure of the studied compounds, as well as correlation between experimentally determined lipophilicities (log k_w) and log P data calculated using some commonly available software, are discussed.

Keywords: *Lipophilicity • Gradient reversed-phase high-performance liquid chromatography (RP-HPLC)*

1. Introduction

Acridinones, as imidazoacridinone and triazoloacridinone derivatives, are new potent antitumor compounds that exhibit significant biological (cytotoxic and/or antitumor) activity towards many tumor cell lines, such as leukemia, melanoma, colon adenocarcinoma, lung carcinoma, breast carcinoma, and colon carcinoma and others. Additionally, the acridones are characterized by weak mutagenicity (for review see [1]). C-1305, one of the most active derivatives, was selected for extended preclinical trials, and C-1311, another active derivative, underwent phase II clinical trials for colon and breast cancer [2-3]. The mode of action of both imidazoacridinones and triazoloacridinones is believed to involve physicochemical interactions with DNA, influence of cell cycle progression, induction of cell death, and other pathways – in particular topoisomerase II inhibition and covalent DNA cross-linking preceded by metabolic activation [1]. It is therefore important to determine the lipophilicity (log P) (a physicochemical parameter) of these biologically active compounds in view of passive drug transport through biological barriers connected with membrane permeability.

It is important to note that log P provides a characterization of the affinity of a compound or its molecular fragment for a lipid environment. It is expressed as the logarithm of the partition coefficient (log P) of a solute between two essentially immiscible solvent phases (aqueous and non-aqueous solvents, in practice water and n-octanol). Log P is molecular physical property of high importance that impacts a wide range of systems. It can help to drive research forward in many industries and can also help to determine the ultimate fate of chemical substances in the environment, especially when it is used in conjunction with other parameters. The log P value

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also provides an indication as to the absorbance of a substance by plants, animals, humans or other living tissues [4]. In the pharmaceutical and biotechnological industries, log P is used to understand the behavior of drug molecules in the body. Along with other criteria, drug candidates are usually screened according to log P values to help drug selection and analog optimization. The importance placed on the log P value is a result of the fact that lipophilicity is considered as a major determining factor in a compound's absorption, distribution in the body, penetration across vital membranes and biological barriers, metabolism and excretion (collectively referred to as ADME properties) [5].

High-performance liquid chromatography (HPLC) is most widely applied in medicinal chemistry to determine lipophilicity parameters (as log k or log k_w) of biologically active compounds, for which crossing biological barriers strongly depend on lipophilicity. The fundamental processes of drug action and the processes that are the basis of chromatographic separations have much in common, and this provides unique advantages of using HPLC retention parameters in modeling pharmacokinetic properties of drugs. Biological processes of absorption, distribution, excretion and receptor binding are dynamic in nature, as are the processes of analyte distribution in chromatography. None of the essential pharmacological and chromatographic processes (except metabolism) implies the breaking of existing bonds in a drug (analyte) molecule or the formation of new bonds. The same basic intermolecular interactions determine the behavior of chemical compounds both in biological and chromatographic environments [6,7].

These facts encouraged us to study the hydrophobic properties of acridinone (imidazoacridinone and triazoloacridinone) derivatives for which lipophilicity index (log k_w) has been determined using a HPLC method, and to discuss the results obtained with calculated lipophilicity parameters (log P).

2. Experimental procedure

2.1. Chemicals

HPLC-grade acetonitrile and water as well as analytical-grade formic acid were from POCH (Gliwice, Poland). All analyzed acridinone derivatives, as 0.1 mg mL^{-1} solutions in 0.2% lactic acid, were available from the compound collection of the Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk. Moreover, the acridinone derivatives examined in this study were selected to include analogue compounds differing in chemical structures (Fig. 1).

2.2. Lipophilicity experimental determination

A Shimadzu High-Performance Liquid Chromatography set (Kyoto, Japan) equipped with vacuum degasser (DGU-20A5), solvent pump (LC-20AD), autosampler (SIL-20AD), diode array detector (SPD-M20A), column oven (CTO-20AC), communications bus module (CBM-20A) and LC Solution software (*vs*. 1.0.0.1 Kyoto, Japan) were used for complex HPLC analysis. The following HPLC columns were employed: (a) Luna 5u C18(2) 100 A column, 150×4.6 mm i.d. (Phenomenex, Torrance, CA, USA) packed with hybrid stationary phase on the basis silica gel and silicaorganic compounds, with chemically bounded octadecylsilane, with particles size 5 µm; (b) Candeza CD-C18 column, 150×4.6 mm i.d. (Imtakt Corporation, Kyoto, Japan) packed with hybrid stationary phase on the basis silica gel and silicaorganic compounds, with chemically bounded octadecylsilane, with particles size 3 µm; (c) TSK-gel ODS-80TS column, 150×4.6 mm i.d. (Tosoh Corporation, Tokyo, Japan) packed with hybrid stationary phase on the basis silica gel and silicaorganic compounds, with chemically bounded octadecylsilane, with particles size 5 µm; (d) Ascentis C18 column, 150×4.6 mm i.d. (Supelco, Bellefonte, PA, USA) packed with hybrid stationary phase on the basis silica gel and silicaorganic compounds, with chemically bounded octadecylsilane, with particles size 5 µm; (e) Unison UK-C18 column, 150×4.6 mm i.d. (Imtakt Corporation, Kyoto, Japan) packed with hybrid stationary phase on the basis silica gel and silicaorganic compounds, with chemically bounded octadecylsilane, with particles size 3 µm; (f) Zorbax SB-C8 column, 75×4.6 mm i.d. (Agilent Technologies, Santa Clara, CA, USA) packed with hybrid stationary phase on the basis silica gel and silicaorganic compounds, with chemically bounded octadecylsilane, with particles size 3.5 µm.

Gradient HPLC elution was 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 acridinones were 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_w values (*i.e*., the retention factor log k extrapolated to 0% organic modifier, as an alternative to log P) were calculated using the DryLab 6.0 program (Molnar-Institute, Berlin, Germany) according to the theory elaborated by Snyder and co-workers [8,9]. The log k_w parameters of acridinone derivatives determined in six RP-HPLC columns, are presented in Table 1.

Moreover, all the chromatographic measurements were performed at a flow rate 1 ml/min and the UV

Table 1. Comparison of the determined lipophilicities (log k_w) with calculated lipophilicities (log P) of studied acridinones.

	Compound	log k_w						log P							
		log k_w – Luna	log k_w – Candeza	log k_w – TSK	log k_w – Ascentis	log k_w – Unison	log k_w – Zorbax	AC_logP	AB/LogP	miLOGP	ALOGP	MLOGP	KOWWIN	XLOGP2	XLOGP3
imidazoacridinones	C-1176	0.98	1.10	1.02	1.00	1.17	1.29	2.57	1.69	2.13	-0.21	2.40	3.22	2.22	1.98
	C-1212	1.05	1.17	1.09	1.08	1.24	1.35	3.04	2.44	2.40	-0.15	2.64	3.71	2.58	2.34
	C-1266	1.22	1.35	1.27	1.23	1.39	1.48	3.96	3.19	3.17	0.88	3.09	4.69	3.29	3.05
	C-1310	0.93	1.06	0.99	0.96	1.13	1.28	3.20	2.69	2.46	0.26	2.31	4.27	2.47	2.35
	C-1311	0.92	1.04	0.95	0.91	1.13	1.26	3.15	2.56	2.38	0.22	2.09	3.72	2.24	2.36
	C-1330	1.14	1.30	1.16	1.14	1.32	1.39	3.34	2.79	2.91	0.47	2.31	4.28	2.98	2.69
	C-1335	1.00	1.14	1.04	1.21	1.20	1.35	3.61	3.31	2.65	0.28	2.31	4.21	2.59	2.72
	C-1336	0.94	1.10	0.98	0.95	1.16	1.26	3.83	3.67	2.89	0.81	2.53	4.76	3.58	2.30
	C-1371	0.93	1.06	0.97	0.96	1.13	1.30	2.74	2.34	1.89	-0.42	1.86	3.23	1.75	1.98
	C-1415	1.01	1.19	1.01	1.04	1.25	1.30	3.45	2.66	2.88	0.48	2.86	4.20	3.06	2.71
	C-1419	0.97	1.08	1.00	0.95	1.17	1.28	3.15	2.76	2.38	0.22	2.09	3.72	2.23	2.36
	C-1492	1.09	1.21	1.14	1.11	1.27	1.44	3.67	3.09	2.67	0.62	2.31	4.21	2.46	2.70
	C-1554	1.20	1.33	1.23	1.21	1.36	1.43	3.76	3.07	3.30	0.07	3.09	4.75	3.50	3.08
	C-1558	1.51	1.66	1.49	1.50	1.62	1.53	4.96	4.28	4.56	1.88	3.73	6.11	4.88	4.39
triazoloacridinones	C-1296	1.23	1.35	1.22	1.23	1.39	1.36	3.85	2.19	2.26	0.41	2.44	3.07	1.90	3.02
	C-1303	0.98	1.08	1.00	0.98	1.18	1.22	3.08	1.35	1.48	0.08	1.45	2.09	0.71	2.31
	C-1305	1.06	1.16	1.07	1.06	1.24	1.30	3.55	2.10	1.75	0.14	1.68	2.59	1.07	2.66
	C-1410	1.01	1.14	1.03	1.04	1.19	1.26	3.30	2.06	1.26	-0.11	1.45	2.37	0.90	2.21
	C-1531	0.75	0.92	0.81	0.76	1.02	1.03	2.55	1.45	0.92	-0.32	1.17	1.58	0.33	1.88
	C-1533	1.02	1.13	1.05	1.05	1.20	1.27	3.30	1.80	1.73	0.61	1.68	2.64	1.70	1.89
	C-1544	1.12	1.24	1.11	1.11	1.31	1.29	3.76	3.07	3.30	0.97	3.09	4.75	3.50	3.08

detection wavelength at 254 nm. Dwell volume for HPLC system was measured at 0.745 mL. The dead time for all HPLC columns was determined by injection of solvent B and was equal 2.123 min. for Luna 5u C18(2) 100 A column, 1.837 min. for Candeza CD-C18 column, 2.621 min. for TSK-gel ODS-80TS column, 1.918 min. for Ascentis C18 column, 1.754 min. for Unison UK-C18 column, and 1.005 min. for Zorbax SB-C8 column, respectively.

2.3. Lipophilicity calculations

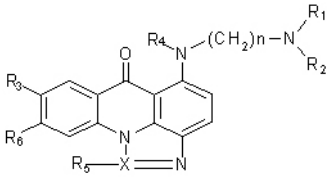
Parameters characterizing lipophilicity (log P) of the studied acridinones were calculated with the usage of the on-line Virtual Computational Chemistry Laboratory, providing ability for interactive prediction of log P utilizing algorithms such as Pharma Algorithm (AC_logP parameter), Actelion algorithm (AB/LogP parameter), Molinspiration algorithm implemented in the DragonX software (miLOGP, KONWWIN, ALOGP and MLOGP parameters) [10,11], XLOGP2 and XLOGP3 programs (XLOGP2 and XLOGP3 parameters) [12].

2.4. Statistical analysis

The statistical correlation analysis has been performed with the use of Statistica 9.0 software (StatSoft, Tulsa, OK, USA).

3. Results and discussion

The experimental determination of lipophilicity can be difficult, especially for zwitterionic and very lipophilic or polar compounds [13]. There is therefore huge interest in the development of methods for predicting log P values. Besides the Shake Flask Method and high-performance liquid chromatography (HPLC), classically used for log P measurement, recently various software programs have been used to calculate the log P of a compound by the sum of its fragments (log P values for individual fragments are obtained from experimental data and/or determined statistically). Moreover, this log P prediction is a powerful resource because it can be performed in the absence of a sample of a compound. It is also noteworthy that to date “whole-molecule” approaches to determining log P from structure alone have been unsuccessful and therefore “molecular fragmentation” methods have been employed [14]. In fragment-based methods, each structure is broken down into a set of sub-structural fragments. The contribution of each fragment in each structure to the log P value of the entire set is assessed. All of these fragment-based approaches depend on a list of certain criteria [15]. First, the fragment definition should take into account every atom in every molecule. If not, there are missing values. Second, each



	Compound	X	n	R_1	R_2	R_3	R_4	R_5	R_6
imidazoacridinones	C-1176	C	2	CH_3	CH_3	H	H	H	H
	C-1212	C	3	CH_3	CH_3	H	H	H	H
	C-1266	C	5	CH_3	CH_3	H	H	H	H
	C-1310	C	2	CH_2CH_3	CH_2CH_3	OH	H	CH_3	H
	C-1311	C	2	CH_2CH_3	CH_2CH_3	OH	H	H	H
	C-1330	C	2	CH_2CH_3	CH_2CH_3	OCH_3	H	H	H
	C-1335	C	3	CH_2CH_3	CH_2CH_3	OH	H	H	H
	C-1336	C	3	CH_2CH_3	CH_2CH_3	OH	H	CH_3	H
	C-1371	C	3	CH_3	CH_3	OH	H	H	H
	C-1415	C	2	CH_2CH_3	CH_2CH_3	H	H	H	H
	C-1419	C	2	CH_2CH_3	CH_2CH_3	H	H	H	OH
	C-1492	C	5	CH_3	CH_3	OH	H	H	H
	C-1554	C	2	CH_2CH_3	CH_2CH_3	CH_3	H	H	H
	C-1558	C	2	CH_2CH_3	CH_2CH_3	$C(CH_3)_3$	H	H	H
triazoloacridinones	C-1296	N	3	CH_3	CH_3	CH3	H	-	H
	C-1303	N	2	CH_3	CH_3	OH	H	-	H
	C-1305	N	3	CH_3	CH_3	OH	H	-	H
	C-1410	N	2	H	CH_2CH_3	OH	H	-	H
	C-1531	N	2	H	$(CH_2)_2N(CH_3)_2$	OH	H	-	H
	C-1533	N	2	CH_3	CH_3	OH	CH_3	-	H
	C-1544	N	2	CH_3	CH_3	H	CH_3	-	H

Figure 1. Chemical structure of studied acridinones.

atom must be included in only one fragment definition. Otherwise, the interpretation of results is difficult. Third, there may be only one or two examples in the database, for a particular fragment type as some of the fragment definitions in the training set will have few representatives. On these occasions, there will be more uncertainty in the fragment contribution to the log P. On the other hand, calculation of log P can be done with the use of the atom-based methods, in which calculation of the log P of a molecule is connected with breaking the structure down into atomic fragments and using multiple regression to obtain the average contribution of each atom across the set [15]. Moreover, methods based on molecular properties are also proposed for log P calculation. These methods work by calculating molecular properties (*i.e.* various solvation-associated properties as the solute bipolarity, the solute hydrogen-bond acidity, the solute hydrogen-bond basicity, and others) for each structure and relating these to LSER (Linear Solvation Energy Relationship)-type equations required for log P calculation. A disadvantage with this approach is that not all parameters necessary for log P calculation are available for all structures. However, there is currently a large amount of research focusing on replacing experimental parameters with calculated parameters for the LSER equation. One approach has developed a set of descriptors based on surface area with several parameters derived from the electrostatic potential on the surface [15].

Reversed-phase high-performance liquid chromatography (RP-HPLC) methods are popular and widely used for lipophilicity measurements [16,17]. The general procedure involves the measurement of the directly accessible retention time under isocratic conditions with varying amounts of an organic modifier in the mobile phase. The chromatographic lipophilicity index, log k, can be derived from the retention factor k. On the other hand, the log k_w as the retention factor extrapolated to 0% organic modifier is obtained by performing several measurements varying the ratio of water to organic solvent. However, determination of log k_w using isocratic RP-HPLC method has some disadvantages, because determination of log k_w is time consuming due to the requirement of obtaining a number of measurements before the calculation of log k_w can be performed [18]. For this reason, a gradient HPLC elution method connected with the use of the DryLab program have become more popular for log k_w determination [19,20].

In the present study, the last strategy described above for lipophilicity determination of acridinone (imidazoacridinone and triazoloacridinone) derivatives was applied. Our latest studies [21,22] concerning QSAR analysis of acridinones revealed that parameters describing lipophilicity possessed the most significant influence on the antitumor activity strictly connected with interaction with DNA of these compounds. It is also worth noting that this observation is in agreement with the earlier data obtained by Mazerska *et al.* [23], for which antitumor activity of imidazoacridinones was dependent on lipophilicity, as well as with the data obtained by Składanowski *et al.* [24], which indicated that hydrophobic properties of acridinones can play important role in transport and accumulation of these compounds in cells.

The chemical structures of the 21 acridinones considered in the present work are presented in Fig. 1. The values of all lipophilicity parameters, both experimentally determined from six HPLC columns (log k_w_Luna-log k_w_Zorbax), as well as eight calculated (AC_logP-XLOGP3) parameters for the compounds are

depicted in Table 1. The results obtained for six RP-HPLC columns showed that experimentally determined lipophilicities (log k_w) are lower than those calculated (log P) using various algorithms.

In the case of log k_w values (Table 1) some relationships connected with properties of the HPLC columns (dimension and particles size) have been observed. Similar log k_w values for all analyzed acridinones have been determined for columns (Luna 5u C18(2) 100A, TSK-gel ODS-80TS and Ascentis C18) characterized by the same dimension as 150×4.6 mm with particles size 5 μm, compared to columns (Candeza CD-C18 and Unison UK-C18) characterized by the same dimension as for columns presented above but with lower particle size (3 μm), and for which higher log k_w values have been observed. Besides the influence of particle size on log k_w values, the dimensions of the column also have a great importance because the highest log k_w values have been determined on the column (Zorbax SB-C8) with the lowest dimension (75×4.6 mm with particles size 3.5 μm) from all of those examined with RP-HPLC analysis. These observations indicated that the distribution process of analytes in chromatography is dynamic in nature, as in the biological processes of absorption, distribution and others. Indirectly it also supports the belief that lipophilicity is considered as a major determining factor in the penetration of a compound across vital membranes and biological barriers.

In the case of calculated lipophilicities (log P) (Table 1), differences between their values are probably caused because of usage of different methods (algorithms) in the calculations. The ALOGP, MLOGP, XLOGP2 and XLOGP3 belong to the indexes of log P calculated with the use of the atom-based methods of log P calculations, compared to log P parameters as AC_logP, AB/LogP and miLOGP calculated using different fragment-based methods of log P calculations. On the other hand, the KOWWIN was calculated using both atom/fragment contribution methods of log P calculation [15].

The most valuable information is related to the differences in lipophilicity values within the group of analyzed acridinones, which are related via their diversity in chemical structures (see Fig. 1 and Table 1). The greatest differences of all determined log k_w parameters, as well as calculated log P parameters, was observed with imidazoacridinone C-1558, a compound possessing a very hydrophobic *t*-butyl moiety linked to the acridinone core, whereas the values with the best agreement of all log k_w and log P parameters was obtained with triazoloacridinone C-1531, a compound with a more hydrophilic triaminoalkyl side chain attached to the acridinone core compared to other acridinones which had only a diaminoalkyl side chain in their structure. This observation indicated that the character of the group linked to the acridinone chromophore has a significant role in lipophilic properties of all of these compounds. Moreover, log k_w measured on all HPLC columns was characterized by the lower values for acridinones hydroxyl-substituted in position 8 (R_3) and those that possessed 2-3 methylene group in diaminoalkyl side chain, compared to derivatives with the same number of methylene groups but unsubstituted, methyl-substituted or methoxy-substituted in position 8. Additionally, some influence of the nature of the substituent in acridinone ring on log k_w values was observed. Firstly, an increase in the number of methylene groups on the diaminoalkyl sidechain from 2-3 (compound C-1371) to 5 (compounds 1492 and C-1266) caused an increase of log k_w values. Second, replacement of methyl group with an ethyl group on the aminoalkyl side chain (substituent R_1 and R_2) caused an increase in log k_w values of 8-hydroxyl-substituted (C-1371 *vs*. C-1335) as well as 8-unsubstituted (C-1176 *vs*. C-1415) acridinone derivatives, respectively. Third, the position of the hydroxyl group attached to the acridinone core (substituent R_3 and R_6) influences the log k_w (9-OH derivative as C-1419 characterized by higher values of log k_w compared to 8-OH derivative as C-1311). Fourth, derivatives with an attachment of a methyl group to diaminoalkyl side chain (substituent R_4), such as C-1533 and C-1544, were characterized by generally higher values of log k_w compared to derivatives (C-1310 and C-1336) with methyl group linked to the imidazo- (and probably triazolo-) moiety of the acridinone ring (substituent R_5). Moreover, methyl-substituted derivatives in position R_4 or R_5 were characterized by an increase in log k_w values compared to unsubstituted derivatives in these positions (C-1533 *vs*. C-1303 and C-1310 *vs*. C-1311, respectively).

On the other hand, the correlation analysis between experimentally determined lipophilicities (log k_w) and calculated log P parameters for both imidazoacridinones and triazoloacridinones individually (for correlation matrix see Tables 2 and 3, respectively) as well as for all studied acridinones (correlation matrix presented in Table 4) were also performed. The correlation coefficients obtained showed that generally log k_w correlates relatively poorly (R below 0.85 for the most cases) with calculated lipophilicity (log P) of compounds from each series (Tables 2-4). Moreover, statistical relevance of particular correlation coefficients was established at significance level $p \leq 0.05$ and all determined R values that fulfill this criterion are presented in bold type (for detail see Tables 2-4).

Table 2. Correlation coefficients (R) between determined (log k_w) and calculated (log P) lipophilicity for imidazoacridinones. Values of R with $p \leq 0.05$ are bolded and greater than 0.84 are underlined.

	AC_logP	AB/LogP	miLOGP	ALOGP	MLOGP	KOWWIN	XLOGP2	XLOGP3
log k_w_Luna	0.81	0.64	**0.91**	0.74	**0.86**	0.81	0.82	**0.94**
log k_w_Candeza	0.83	0.67	**0.93**	0.76	**0.88**	0.84	**0.86**	**0.94**
log k_w_TSK	0.80	0.63	**0.88**	0.72	**0.85**	0.80	0.80	**0.92**
log k_w_Ascentis	0.81	0.67	**0.86**	0.68	0.81	0.78	0.77	**0.92**
log k_w_Unison	0.83	0.66	**0.92**	0.76	**0.88**	0.82	**0.85**	**0.94**
log k_w_Zorbax	0.74	0.58	0.77	0.62	0.73	0.69	0.65	0.83

Table 3. Correlation coefficients (R) between determined (log k_w) and calculated (log P) lipophilicity for triazoloacridinones. Values of R with $p \leq 0.05$ are bolded and greater than 0.84 are underlined.

	AC_logP	AB/LogP	miLOGP	ALOGP	MLOGP	KOWWIN	XLOGP2	XLOGP3
log k_w_Luna	**0.98**	0.64	0.73	0.68	**0.75**	0.68	0.67	**0.81**
log k_w_Candeza	**0.97**	0.68	0.74	0.67	**0.78**	0.70	0.69	**0.82**
log k_w_TSK	**0.97**	0.61	0.70	0.67	0.72	0.65	0.64	**0.77**
log k_w_Ascentis	**0.96**	0.62	0.68	0.66	0.70	0.65	0.64	**0.75**
log k_w_Unison	**0.97**	0.68	**0.78**	0.70	**0.80**	0.72	0.71	**0.84**
log k_w_Zorbax	**0.95**	0.57	0.62	0.63	0.61	0.60	0.57	0.69

Table 4. Correlation coefficients (R) between determined (log k_w) and calculated (log P) lipophilicity for all acridinones studied. Values of R with $p \leq 0.05$ are bolded and greater than 0.84 are underlined.

	AC_logP	AB/LogP	miLOGP	ALOGP	MLOGP	KOWWIN	XLOGP2	XLOGP3
log k_w_Luna	**0.85**	**0.59**	**0.76**	**0.72**	**0.74**	**0.64**	**0.66**	**0.90**
log k_w_Candeza	**0.86**	**0.63**	**0.79**	**0.75**	**0.78**	**0.68**	**0.71**	**0.92**
log k_w_TSK	**0.84**	**0.61**	**0.76**	**0.71**	**0.75**	**0.66**	**0.68**	**0.89**
log k_w_Ascentis	**0.84**	**0.63**	**0.75**	**0.68**	**0.73**	**0.65**	**0.66**	**0.88**
log k_w_Unison	**0.86**	**0.61**	**0.78**	**0.75**	**0.77**	**0.66**	**0.69**	**0.92**
log k_w_Zorbax	**0.73**	**0.69**	**0.79**	**0.59**	**0.76**	**0.75**	**0.72**	**0.75**

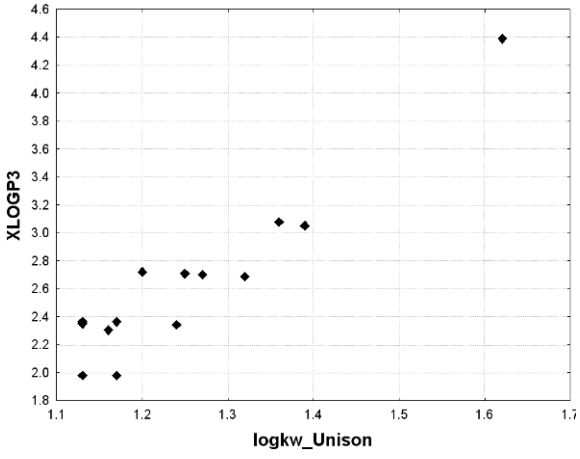


Figure 2. Example of correlation between determined (logkw_Unison) and calculated lipophilicity (XLOGP3) for imidazoacridinones with statistical significance level $p = 1 \times 10^{-7}$.

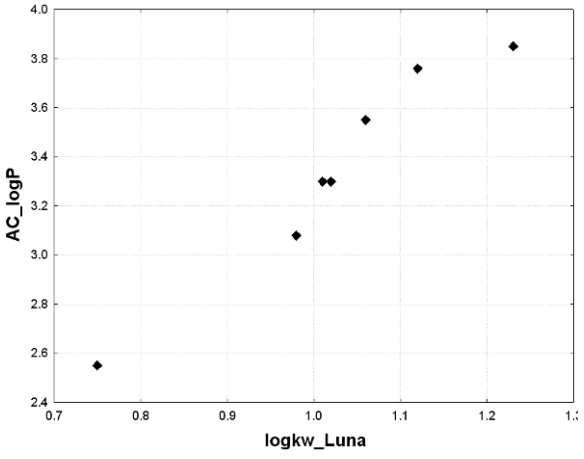


Figure 3. Example of correlation between determined (logkw_Luna) and calculated lipophilicity (AC_logP) for triazoloacridinones with statistical significance level $p = 2 \times 10^{-4}$.

However, in the group of imidazoacridinones some parameters of log P as miLOGP, MLOGP and XLOGP3 correlated reasonably well (R from 0.85 to 0.94) with log k_w parameters determined on all studied HPLC columns (excluding log k_w_Zorbax) (Table 2) (for example of graphical presentation of correlation see Fig. 2). In the case of the triazoloacridinone group, only one parameter, as AC_logP, highly correlated (R over 0.95) with log k_w obtained using all HPLC columns (Table 3) (for example of graphical

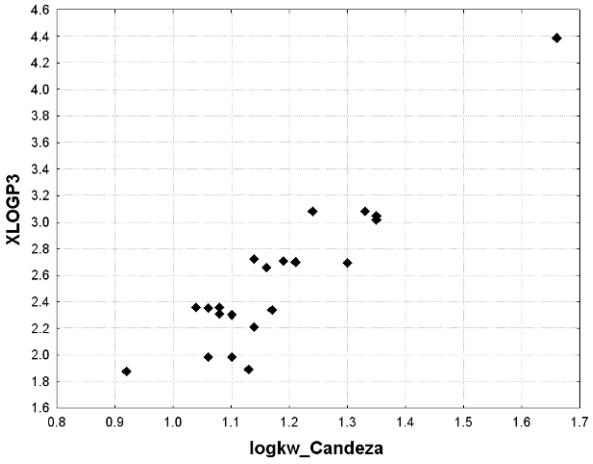


Figure 4. Example of correlation between determined (logkw_Candeza) and calculated lipophilicity (XLOGP3) for acridinones with statistical significance level $p = 1 \times 10^{-7}$.

presentation of correlation see Fig. 3). The correlation coefficients determined for all analyzed acridinones (imidazoacridinones together with triazoloacridinones) showed that only two parameters of calculated lipophilicity (log P) such as AC_log P (correlated the best with log k_w in the case of triazoloacridinones) and XLOGP3 (correlated the best with log k_w in the case of imidazoacridinones) correlated with log k_w measured experimentally for the group of studied acridinones (for example of graphical presentation of correlation see Fig. 4). These results are probably connected with the fact that the structure of the acridinone chromophore with an attached imidazo- or triazolo- ring (AC_log P calculated using fragment-based method) as well as the structure and location of substituents attached to the acridinone ring (XLOGP3 calculated using the atom-based method) have an influence on the lipophilicityof the acridinones.

4. Conclusions

The compounds studied can be divided into two groups according to their structure. The correlation coefficients obtained showed that generally log k_w correlates relatively poorly (R below 0.85 for most cases) with calculated lipophilicity (log P) of compounds from each series. However, in the group of imidazoacridinones, some parameters of log P as miLOGP, MLOGP and XLOGP3 correlated reasonably well (R from 0.85 to 0.94) with log k_w parameters determined from almost all of the studied HPLC columns. In the case of the triazoloacridinone group, one parameter, namely AC_logP correlated in high manner (R over 0.95) with log k_w obtained using all of the HPLC columns.

It can be concluded that experimentally determined log k_w data can describe lipophilicity within the series of the tested compounds. The chromatographically established hydrophobicity parameter values (log k_w) of all the target compounds, has been determined by means of RP-HPLC methods for lipophilicity measurement. Gradient HPLC can be used for rapid screening of hydrophobicity of analytes including drugs and drug candidates. Moreover, two gradient runs suffice to get reliable measure of analyte lipophilicity.

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We are grateful to Prof. Andrzej Składanowski from the Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk for making the acridinones studied available for this work. The work was also partially supported by the Polish State Committee for Scientific Research Project N N405 024340.

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