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A Comparative Study of Chromatographic Behavior and Lipophilicity of Selected Imidazoline Derivatives

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Abstract

Chromatographic behavior and lipophilicity of 20 selected imidazoline derivatives were examined by thin-layer chromatography using CN, RP-2, RP-8 and RP-18 as the stationary phases and a mixture of methanol, water and ammonia as the mobile phase. In all examined chromatographic systems, linear relationships were established between retention parameters and the volume fraction of methanol in the mobile phase ($r > 0.985$, 0.978, 0.981, 0.988 for the CN, RP-2, RP-8 and RP-18, respectively). The highest correlation between the obtained R_M^0 values was observed for RP-2 and RP-8 stationary phases. The experimental lipophilicity indices (R_M^0, m and C_0) obtained from the retention data were used in correlation study with the calculated logP values. Experimentally determined R_M^0 values for all investigated chromatographic systems exhibited the highest correlation with the calculated ClogP values (r: 0.880, 0.872, 0.897 and 0.889 for the CN, RP-2, RP-8 and RP-18 stationary phases, respectively). In addition, principal component analysis enables new information about similarity and differences between tested compounds as well as experimental lipophilicity indices and calculated logP values. Performed QSRR analysis showed that the frequency of C-C at topological distance 1 and CATS2D Lipophilic–Lipophilic at lag 01 were important descriptors with influence on the R_M^0 values in all the examined chromatographic systems, while the differences in the retention behavior of compounds on the examined stationary phases can be distinguished based on their specific geometrical, electronic and constitutional properties.

Introduction

Imidazoline derivatives are a family of biologically active compounds known for comprehensive therapeutic applications (e.g., antihypertensives, diuretics, analgetics, antiallergics, antidiabetics, antipsychotics). Several hypotheses connect their activity with three types of imidazoline receptors (I_1, I_2 and I_3) and α_2-adrenergic receptors located in different parts of CNS and the peripheral tissues (1–5). However, there is a growing evidence that 2-imidazoline scaffold could also be involved in pharmacological actions outside of the α/I receptor domain, being thus a privileged motif for drug discovery (6). All these findings have influenced the synthesis of numerous compounds containing an imidazoline chemical scaffold and imposed the need for understanding of their physicochemical properties, relevant to the pharmacokinetic behavior thereof.

Lipophilicity of a compound is one of the crucial physicochemical properties which influence drug absorption, distribution, metabolism and elimination. According to the International Union for Pure and Applied Chemistry (IUPAC), lipophilicity represents an affinity of a molecule or a moiety for a lipophilic environment and it is commonly measured by evaluating the distribution behavior of compounds in biphasic systems, either liquid–liquid (e.g., the partition coefficient in 1-octanol–water) or solid–liquid (retention in the reversed-phase high-performance liquid chromatography, RP-HPLC) or the thin-layer

chromatography (TLC) system (7). The most common method used to directly measure the lipophilicity of compounds is the so-called shake-flask method. Disadvantages of this method such as unsuitability for compounds with very high or very low logP-values and a limited inter-laboratory reproducibility led to its gradual replacement by indirect chromatographic techniques which well simulate octanol–water partitioning and enable working with small quantities of compounds as well as impure substances (8). In addition, an adequately chosen chromatographic system can be used for the simulation of biological conditions, because similar elementary intermolecular interactions are important for the behavior of chemical compounds in both biological and chromatographic environments (9). Therefore, the chromatographic retention parameters obtained directly from the retention data or extrapolated from linear relationships between the retention constant and the concentration of the organic modifier in the mobile phase have been successfully used for the evaluation of compounds lipophilicity (8) as well as in the quantitative structure–property relationship (QSPR), quantitative structure–retention relationship (QSRR) and quantitative structure–activity relationship (QSAR) studies (10, 11). Thus, the developed QSRR models revealed a lot of information on intermolecular interactions between the stationary and mobile phases, as well as on molecular structure that contributes to the lipophilicity of compounds and is responsible for higher affinity of compounds toward polar or nonpolar environments.

Selection of the stationary and mobile phase for the determination of lipophilicity can greatly affect the obtained values of chromatographic parameters. The most commonly used stationary phases for the evaluation of lipophilicity of compounds by the reversed-phase thin-layer chromatography (RP-TLC) are the nonpolar alkyl modified silica gels (RP-18, RP-8, RP-2), whereas some other types of chemically modified silica (such as the CN- and NH_2-modified stationary phases) have also gained an important position in the determination of lipophilicity (12–15). Recent studies have shown that the values of lipophilicity parameters determined experimentally by means of TLC are also dependent on the organic modifier used. Mixtures of an organic solvent with water (e.g., methanol/water, acetone/water and tetrahydrofurane/water) with an addition of an acid or base are most often used in RP-TLC as mobile phases (10, 12, 14). Finally, one should not underestimate a computational approach because it is fast and provides preliminary information about lipophilicity of compounds.

The main objective of this work was to perform a comprehensive study on lipophilicity and the retention behavior of 20 selected imidazoline derivatives by the thin-layer chromatography using different chemically bonded stationary phases.

Experimental

Instrumentation and reagents

Methanol of HPLC grade (J.T. Baker, Deventer, the Netherlands), de-ionized water (TKA water purification system, Niederelbert, Germany) and ammonium hydroxide, 25% (Merck, Darmstadt, Germany) were used throughout this study for the preparation of mobile phases.

All stationary phases used in the experiments, i.e., CN-modified silica plates (the HPTLC silica gel 60 CN F_{254s}, glass plates, 10 × 10 cm), octadecyl-modified silica plates (the TLC silica gel 60 RP-18 F_{254s} precoated aluminum sheets), octyl-modified silica plates (the TLC RP-8 F_{254s}, glass plates, 10 × 20 cm) and dimethyl-modified silica plates (the HPTLC silica gel 60 RP-2 F_{254s} glass plates, 10 × 10 cm) were purchased from Merck (Darmstadt, Germany).

The examined dataset consisting of 20 imidazoline derivatives and the related compounds, i.e., 2-benzylimidazoline (1), antazoline phosphate (2), benazoline oxalate (3), brimonidine hydrochloride (4), cirazoline hydrochloride (5), clonidine hydrochloride (6), detomidine hydrochloride (7), efaroxan hydrochloride (8), guanfacine hydrochloride (9), harman (10), harmine hydrochloride (11), idazoxan hydrochloride (12), moxonidine hydrochloride (13), oxymetazoline hydrochloride (14), phentolamine hydrochloride (15), RX 821002 hydrochloride (16), tetrahydrozoline hydrochloride (17), tizanidine hydrochloride (18) and xylometazoline hydrochloride (19) were purchased from Sigma-Aldrich (St. Louis, MO, USA) or provided by Zdravlje-Actavis (Leskovac, Serbia; trimazoline hydrochloride (20)).

Methods

Chromatography

All investigated compounds were dissolved in methanol (1 mg mL^{-1}), and the 2– 5-μL aliquots of each solute (depending on its UV absorption) were spotted on to the plates by Nanomat III applicator (Camag, Muttenz, Switzerland). The chromatography was performed in an ascending mode using the twin-trough chamber presaturated for 15 min with the mobile phase. The composition of mobile phase relating to the ratio of methanol and water was optimized for each stationary phase type to achieve a satisfactory migration of all examined compounds. Besides, taking into the account basic characteristics of imidazoline derivatives, an addition of ammonia in the mobile phase was necessary to prevent ionization of compounds and tailing of the spots (12–15). Finally, for each stationary phase, five different mobile phases were prepared with the volume fraction of methanol (ϕ) varying from 60 to 80% for CN and RP-2, and from 65 to 85% for RP-8 (15) and RP-18. The content of methanol was changed in 5% steps, while the content of ammonia was kept constant at 5%. All experiments were performed at room temperature. The developed plates were dried on air, and the detection of zones was performed under the UV lamp at 254 nm. Numerical values of the retardation factor, R_f, were obtained as an average from the three chromatograms and further used for the calculation of the retention parameter R_M, according to the Bate–Smith and Westall equation (16) and the R_M^0 and m using the Soczewiński–Wachtmeister equation (17). From equation (17), R_M^0 is the retention parameter corresponding to pure water and was obtained by extrapolation to 0% organic modifier in the mobile phase, while m corresponds to the specific hydrophobic surface area of the substance in contact with the stationary phase. In addition, another hydrophobicity parameter, C_0, which represents the volume fraction of the organic modifier in the mobile phase for $R_M = 0$ (18, 19) was used together with R_M^0 and m as chromatographic lipophilicity indices.

Computational methods

According to our earlier performed and detailed theoretical studies (20), the predominant imino tautomeric forms of clonidine, moxonidine, brimonidine and tizanidine were selected prior to optimization of the geometry. Selection of the predominant molecule/cation species at a given pH value of an aqueous phase was performed with the use of the MarvinSketch 6.1.0, ChemAxon program (21). All predominant forms of the tested compounds were preoptimized with the semiempirical PM3 method (22, 23) using the Gaussian 09 software (24) included in the ChemBio3D Ultra 13.0 program (25) and then refined by using a more precise Hartree-Fock/3-21G method for the energy minimization (26).

The Dragon program (27) was applied for the calculation of constitutional, physicochemical, thermodynamic and electronic properties of the examined compounds, while Virtual Computational Chemistry Laboratory (AlogPs, AClogP, AlogP, MlogP, XlogP2, XlogP3) (28), Molinspiration Cheminformatics (milogP) (29), MarvinSketch 6.1.0, ChemAxon program (ChemAxon logP) (21) and CS Chem Office, version 7.0 (ClogP and logP) (30) were used for the calculation of the lipophilicity parameter (logP) of the tested compounds.

Partial least squares modeling

Partial least squares (PLS) modeling was performed by the use of the Soft Independent Modeling of Class Analogy Simca P + 12.0 program (31). The retention parameter R_M^0 values obtained for the examined stationary phases (CN, RP-2, RP-8 and RP-18) were used as dependent variables, while the computed molecular descriptors were used as independent variables during the QSRR modeling. Principal component analysis (PCA) was performed on the examined data sets by the use of the Simca P 12+ program to divide the tested compounds into the training and the test sets in such a way that each chemical group (i.e., 2-aminoimidazoline, 2-arylmethylimidazoline, β-carboline) has one representative in the test set and the R_M^0 values (R_M^0 (CN), R_M^0 (RP-2), R_M^0 (RP-8) and R_M^0 (RP-18) of the compounds in the test set are homogeneously distributed in the whole range of the R_M^0 (CN), R_M^0 (RP-2), R_M^0 (RP-8) and R_M^0 (RP-18) values, respectively. The same training set consisting of 15 compounds (2-benzylimidazoline, antazoline, benazoline, brimonidine, cirazoline, detomidine, efaroxan, guanfacine, harman, idazoxan, moxonidine, oxymetazoline, tetrahydrozoline, tizanidine, xylometazoline) and the test set consisting of 5 compounds (clonidine, harmine, phentolamine, RX 821002, trimazoline) were used for all the examined mobile phase/stationary phase systems.

The variable importance in the projection (VIP) parameter was applied for the selection of molecular properties with the highest impact on the dependent variable during the PLS analysis (31, 32). In the course of the model building, descriptors with the lowest VIP values were successively removed from the model and each time a new PLS model was elaborated. For each new PLS model, statistical parameters such as regression factors R^2, Q^2, F ratio, P-value and the root mean square error of the estimation (RMSEE) were calculated and compared with the previous model. The procedure was repeated until the best models were established for each dependent variable (R_M^0 (CN), R_M^0 (RP-2), R_M^0 (RP-8), R_M^0 (RP-18)).

Internal validation of models was performed by calculating the cross-validated squared correlation coefficient (Q^2) (20). For a predictive QSRR model, the Q^2 value should be higher than 0.5 (33). In addition, the response permutation test (Y scrambling) was performed to examine an overfitting, due to the chance correlation and the statistical significance of R^2 and Q^2 (32). The Y-matrix was re-ordered randomly 100 times, whereas the X-matrix was left intact. The PLS model was fitted to the permuted Y-data, and the new R^2 and Q^2 parameters were calculated. All model selection steps were repeated on the scrambled Y response data. The regression lines were fitted through the R^2 and Q^2 values to obtain two separate intercepts. The values of the obtained intercepts for the valid QSRR models should be lower than 0.05 for the Q^2-intercept and should not exceed 0.4 for the R^2 intercept (32).

External validation of the developed models was performed by calculating a root mean squared error of the prediction (RMSEP) and R^2_{pred} (20). For a predictive QSRR model, the R^2_{pred} value should be higher than 0.5 (34). The quality of the QSRR model predictions was also examined by the calculated values of the r_m^2 metrics (r_m^2, r'^2_m, $\bar{r}^2_m$ and Δr^2_m) of the test set for the external validation (35, 36). Models were considered to be acceptable, if they fulfilled all following criteria:

(i) the values of r_m^2 and r'^2_m should be close to each other, and the value of avr_m^2 (average of r_m^2 and r'^2_m) should be higher than 0.5; (ii) the difference between r_m^2 and r'^2_m (Δr^2_m) should be lower than 0.2 (35, 36).

Results

Chromatographic behavior of imidazoline derivatives

Chromatographic behavior of 20 selected imidazoline derivatives (Figure 1) was examined on four stationary phases of different polarities (CN, RP-2, RP-8 and RP-18) using mixtures of methanol, water and ammonia (in different volume ratios) as mobile phases. In all the examined chromatographic systems, linear relationships were established between the retention parameters (the R_M values) and the volume fraction of methanol in mobile phase ($r > 0.985$ for CN; $r > 0.978$ for RP-2; $r > 0.981$ for RP-8; $r > 0.988$ for RP-18). Common retention behavior was observed in all systems, i.e., the R_f values increased with an increasing percent of an organic modifier in the mobile phase. The numerical values of the experimental lipophilicity indices obtained in four examined chromatographic systems are presented in Table I.

Good relationships between the slope m and the intercept R_M^0 value were obtained for all the examined systems (r: −0.860, −0.946, −0.831 and −0.949 for CN, RP-2, RP-8 and RP-18, respectively) (Table II). In addition, statistical evaluation of experimental results (Table II) showed high correlations between the lipophilicity indices obtained for the stationary phases of different polarities. Correlations between the R_M^0 values were in the range from 0.900 (between CN and RP-8) to 0.961 (between RP-2 and RP-8), between the C_0 values from 0.897 (between CN and RP-2) to 0.970 (between CN and RP-18) and between the m values from 0.638 (between CN and RP-8) to 0.859 (between RP-2 and RP-8).

According to the obtained experimental results (Table I), the most lipophilic compound is xylometazoline in the case of the CN and RP-8 stationary phases (R_M^0 (CN) = 3.395, R_M^0 (RP-8) = 3.353) and antazoline in the case of the RP-2 and RP-18 stationary phases (R_M^0 (RP-2) = 2.981, R_M^0 (RP-18) = 4.683). The least lipophilic character possesses moxonidine (R_M^0 (RP-2) = 0.249, R_M^0 (RP-8) = 0.695)) and brimonidine ((R_M^0 (CN) = 0.732, R_M^0 (RP-18) = 0.760)).

Correlation between experimental lipophilicity indices and calculated logP values

A computational approach to the calculation of the logP values can also be used for fast estimation of the lipophilicity of compounds. Numerous programs used for this purpose are now available. The calculated logP values obtained for 20 examined compounds using the programs based on different algorithms are presented in Table III.

The experimentally determined R_M^0 values for all investigated chromatographic systems exhibited the highest correlation with the calculated ClogP values (30) (r: 0.880, 0.872, 0.897 and 0.889 for the CN, RP-2, RP-8 and RP-18 stationary phases, respectively) (Table IV). Among all the calculated logP values, the ClogP revealed the most significant correlations with the C_0 values in the case of the CN, RP-8 and RP-18 stationary phases also, whereas milogP best correlates with C_0 obtained for the RP-2 stationary phase (Table IV). The lowest relationships were observed between slope m and the

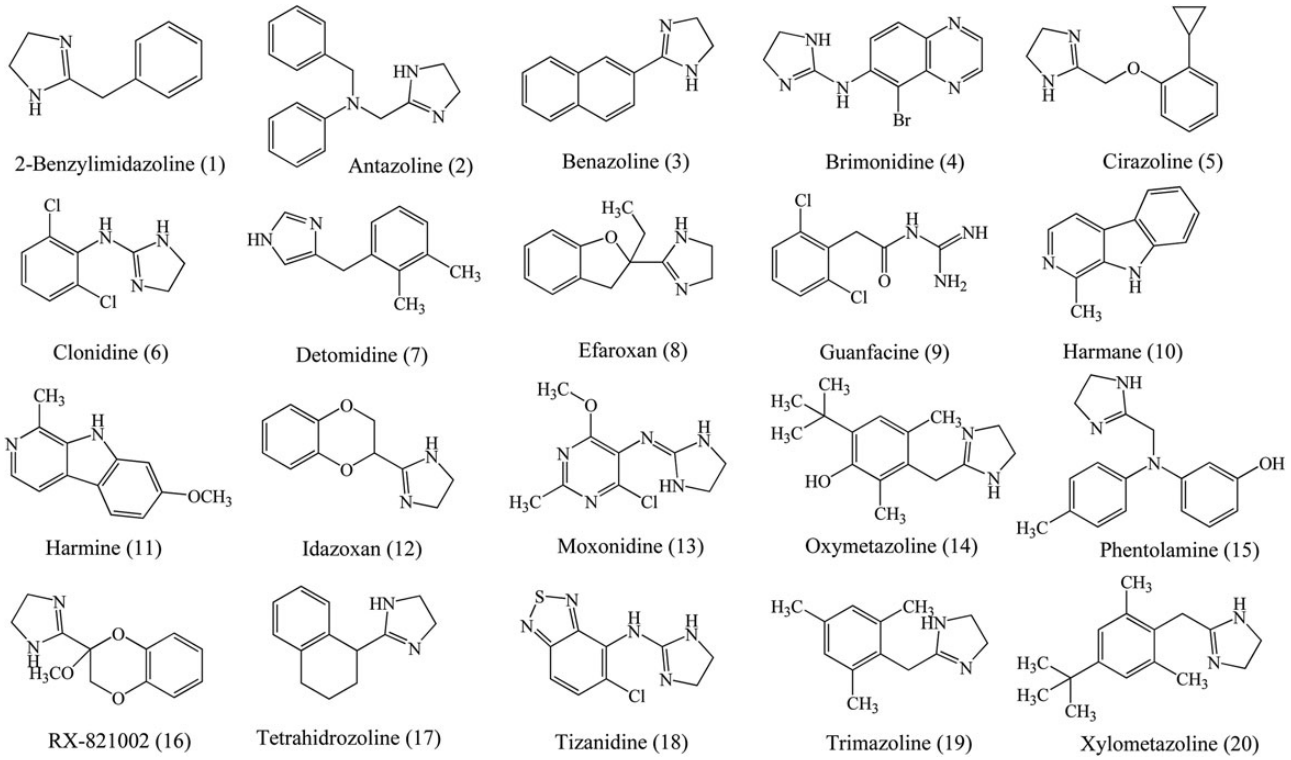

Fig 1. Chemical structures of the examined compounds.

Table I. Experimental Lipophilicity Indices Obtained for the CN, RP-2, RP-8 and RP-18 Stationary Phases

Compounds	CN			RP-2			RP-8			RP-18		
	R_M^0	m	C_0	R_M^0	m	C_0	R_M^0	m	C_0	R_M^0	m	C_0
2-Benzylimidazoline	1.640	−1.592	1.030	2.192	−2.870	0.764	2.511	−2.369	1.060	2.822	−2.435	1.159
Antazoline	3.117	−3.539	0.881	2.981	−3.892	0.766	3.319	−3.484	0.953	4.683	−4.651	1.007
Benazoline	2.329	−2.863	0.813	2.405	−3.351	0.718	2.884	−3.069	0.940	3.721	−3.785	0.983
Brimonidine	0.732	−1.746	0.419	0.773	−1.993	0.388	0.899	−1.533	0.586	0.760	−1.558	0.488
Cirazoline	2.491	−3.286	0.758	2.308	−3.178	0.726	2.466	−2.694	0.916	3.725	−3.883	0.959
Clonidine	1.124	−2.026	0.555	1.241	−2.204	0.563	1.327	−2.035	0.652	1.777	−2.447	0.726
Detomidine	1.375	−2.315	0.594	1.935	−2.853	0.678	2.090	−2.739	0.763	2.668	−3.221	0.828
Efaroxan	2.283	−3.164	0.721	2.300	−3.289	0.699	2.253	−2.483	0.907	3.273	−3.697	0.885
Guanficine	1.668	−2.850	0.585	1.455	−2.537	0.573	1.482	−2.125	0.697	1.809	−2.573	0.703
Harman	1.896	−2.889	0.656	2.244	−3.339	0.672	2.457	−3.008	0.817	2.705	−3.200	0.845
Harmine	1.894	−2.809	0.674	2.317	−3.405	0.680	2.882	−3.492	0.825	2.927	−3.420	0.856
Idazoxan	1.685	−2.671	0.631	1.570	−2.547	0.617	1.697	−2.129	0.797	2.325	−2.775	0.838
Moxonidine	0.822	−2.014	0.408	0.249	−1.202	0.207	0.695	−1.380	0.504	0.800	−1.550	0.516
Oxymetazoline	2.866	−2.912	0.985	2.484	−2.835	0.876	3.216	−2.749	1.170	4.625	−4.329	1.068
Phentolamine	2.630	−3.267	0.805	2.376	−3.289	0.722	2.210	−2.391	0.924	3.769	−4.014	0.939
RX 821002	1.242	−2.363	0.526	1.349	−2.363	0.571	1.364	−2.028	0.672	1.956	−2.589	0.756
Tetrahydrozoline	2.844	−3.000	0.948	2.699	−3.376	0.799	3.069	−2.660	1.154	3.577	−3.312	1.080
Tizanidine	0.895	−1.767	0.507	1.226	−2.421	0.506	1.239	−1.961	0.632	1.415	−2.179	0.649
Trimazoline	2.575	−2.579	0.998	2.588	−3.145	0.823	2.587	−1.992	1.299	4.101	−3.673	1.117
Xylometazoline	3.395	−3.537	0.960	2.892	−3.243	0.892	3.353	−2.672	1.255	4.071	−3.435	1.185

calculated logP values, where AlogPs have the highest correlation with the slopes obtained for the CN and RP-2 stationary phases (XlogP3 with m (RP-8) and logP), ChemAxon with m (RP-18) (Table IV).

In addition, PCA was performed in Simca P + 12.0 program (31) to get information about similarity and differences observed between tested compounds as well as experimentally determined and calculated lipophilicity parameters. Experimental lipophilicity indices obtained for the CN, RP-2, RP-8 and RP-18 stationary phases (Table I) and calculated logP values for the investigated compounds (Table III) were used for the PCA study. The score plot (Figure 2) visualizes the main differences between examined compounds, whereas the loading plot (Figure 3) shows main similarities between experimental

Table II. Correlation Matrix Between Lipophilicity Indices Obtained on Different Stationary Phases

		CN			RP-2			RP-8			RP-18		
		R_M^0	m	C_0	R_M^0	m	C_0	R_M^0	m	C_0	R_M^0	m	C_0
CN	R_M^0	1.000											
	m	−0.860	1.000										
	C_0	0.843	−0.476	1.000									
RP-2	R_M^0	0.914	−0.732	0.869	1.000								
	m	−0.801	0.726	−0.701	−0.946	1.000							
	C_0	0.857	−0.613	0.897	0.947	−0.837	1.000						
RP-8	R_M^0	0.900	−0.691	0.870	0.961	−0.875	0.907	1.000					
	m	−0.632	0.638	−0.501	−0.790	0.859	−0.665	−0.831	1.000				
	C_0	0.864	−0.523	0.961	0.864	−0.679	0.906	0.855	−0.440	1.000			
RP-18	R_M^0	0.944	−0.756	0.876	0.945	−0.841	0.909	0.928	−0.708	0.866	1.000		
	m	−0.870	0.820	−0.697	−0.885	0.857	−0.814	−0.845	0.773	−0.684	−0.949	1.000	
	C_0	0.849	−0.530	0.970	0.905	−0.750	0.939	0.893	−0.572	0.948	0.888	−0.724	1.000

Table III. The Calculated logP Values for the Investigated Compounds

Compound	AlogPs	ClogP	LogP (ChemAxon)	logP	AClogP	milogP	AlogP	MlogP	XlogP2	XlogP3
2-Benzylimidazoline	2.05	2.65	1.63	1.63	1.07	1.69	1.35	1.99	1.50	2.65
Antazoline	3.22	4.11	3.58	3.17	2.20	3.01	3.12	3.04	3.37	2.57
Benazoline	3.14	3.82	2.52	2.69	2.35	1.99	2.22	2.94	2.95	2.13
Brimonidine	1.40	1.51	0.77	1.66	1.30	1.17	1.63	1.05	1.55	0.96
Cirazoline	2.62	2.54	1.98	2.01	1.70	1.86	2.18	2.27	2.22	1.83
Clonidine	1.92	1.43	1.84	2.78	2.39	1.92	2.74	2.66	2.57	1.55
Detomidine	2.79	2.94	2.70	2.69	2.48	2.93	2.67	2.48	2.91	2.75
Efaroxan	2.98	2.84	2.14	2.28	1.77	2.49	2.19	2.27	1.79	1.71
Guanficine	2.28	1.37	1.57	1.86	1.21	1.56	2.00	2.30	2.20	2.24
Harman	3.36	3.06	2.10	2.00	2.78	2.59	2.45	1.59	2.59	3.59
Harmine	3.05	3.13	1.85	1.87	2.67	2.63	2.44	1.29	2.51	3.56
Idazoxan	1.01	1.81	1.06	1.06	0.77	1.03	1.19	1.18	1.18	0.70
Moxonidine	0.75	1.31	0.93	2.13	0.73	−0.05	0.99	1.63	0.70	0.93
Oxymetazoline	3.70	4.61	3.91	3.92	2.92	3.86	3.45	3.04	3.36	2.86
Phentolamine	2.91	3.81	3.22	3.20	1.66	3.25	3.35	2.76	3.26	2.64
RX 821002	1.01	1.61	1.86	1.82	1.21	1.07	1.21	1.08	0.81	0.47
Tetrahydrozoline	3.11	3.54	2.55	2.61	2.06	2.26	2.38	2.84	2.27	1.79
Tizanidine	1.69	2.14	1.40	3.22	1.98	1.33	2.21	1.23	2.13	1.48
Trimazoline	3.02	4.05	3.03	3.10	2.01	2.89	2.81	2.84	2.81	1.91
Xylometazoline	4.68	5.38	4.19	4.31	3.21	4.15	3.72	3.61	4.19	3.22

lipophilicity indices and calculated logP values. Because negative values of slope m correlation between slope and other chromatographic and calculated parameters were not observed in PCA although in correlation matrices (Table II), it was higher than 0.875 and 0.782 (Table IV). Therefore, absolute values of slope m were used in further analysis. According to the score plot (Figure 2), compounds were classified into different lipophilicity groups. Detomidine, harman, phentolamine, oxymetazoline and xylometazoline are grouped together based on the highest values of calculated logP and relatively high chromatographic lipophilicity indices. Harmine, benazoline, trimazoline, antazoline, efaroxan, cirazoline and tetrahydrozoline are grouped according to high chromatographic lipophilicity indices and relatively high calculated logP values. 2-Aminoimidazoline derivatives, moxonidine, brimonidine, tizanidine, clonidine and guanfacine are clustered on the basis of low chromatographic and relatively low calculated lipophilicity parameters, while the fourth group possesses low calculated logP and relatively low chromatographic parameters (Figure 2). The loading plot shows (Figure 3) that all chromatographic and calculated lipohilicity parameters according to PC1 that describes 74.32% of the data variance are grouped in the same cluster ($P[1] > 0.16$). They are distinguished based on PC2, which describes only 8.85% of data variance. Calculated logP values formed a cluster with $P[1] > 0$ while experimentally determined chromatographic parameters are in separated cluster with $P[2] < 0$. Considering that PC1 describes the highest percentage of variance in the PCA of examined parameters and chromatographic clusters and calculated lipophilicity indices in the same part of the loading plot, it can be concluded that there is a significant correlation between them.

QSRR analysis

Dataset of 20 examined imidazoline derivatives was subjected to the QSRR analysis. Experimental lipophilicity indices of the tested compounds expressed as the R_M^0 values covered a wide range of lipophilicity (R_M^0 (CN): 0.732–3.395; R_M^0 (RP-2): 0.249–2.981; R_M^0 (RP-8): 0.695–3.353; R_M^0 (RP-18): 0.760–4.683), ensuring a good quality and applicability of the developed QSRR models. The original dataset was divided into the training set of 15 compounds used for the

Table IV. Correlation Matrix Between Experimental Lipophilicity Indices Obtained on Different Stationary Phases and Calculated logP Values

	CN			RP-2			RP-8			RP-18		
	R_M^0	m	C_0	R_M^0	m	C_0	R_M^0	m	C_0	R_M^0	m	C_0
AlogPs	0.835	−0.703	0.709	0.863	−0.782	0.818	0.872	−0.712	0.758	0.810	−0.758	0.729
ClogP	0.880	−0.644	0.817	0.872	−0.738	0.832	0.897	−0.629	0.858	0.889	−0.786	0.820
LogP (ChemAxon)	0.853	−0.676	0.733	0.804	−0.654	0.805	0.799	−0.559	0.766	0.869	−0.810	0.749
logP	0.571	−0.376	0.471	0.468	−0.306	0.491	0.486	−0.232	0.527	0.559	−0.491	0.446
AClogP	0.515	−0.427	0.395	0.607	−0.559	0.600	0.662	−0.655	0.468	0.553	−0.555	0.447
milogP	0.784	−0.631	0.687	0.827	−0.728	0.841	0.803	−0.633	0.738	0.811	−0.779	0.709
AlogP	0.705	−0.595	0.538	0.685	−0.601	0.679	0.658	−0.519	0.589	0.705	−0.706	0.536
MlogP	0.778	−0.582	0.707	0.674	−0.507	0.677	0.671	−0.384	0.706	0.750	−0.659	0.690
XlogP2	0.717	−0.596	0.572	0.724	−0.652	0.704	0.718	−0.605	0.606	0.719	−0.701	0.583
XlogP3	0.520	−0.429	0.509	0.658	−0.645	0.599	0.700	−0.740	0.473	0.552	−0.540	0.527

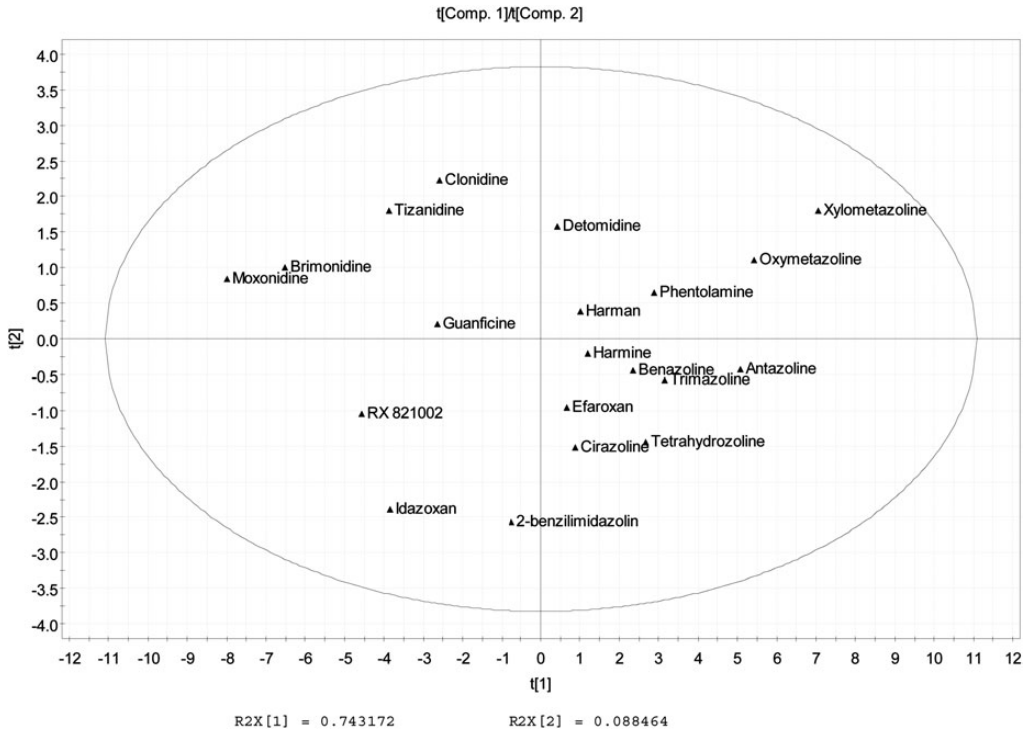

Fig 2. Score plot of PC1 and PC2 as a result of PCA for the experimental lipophilicity indices and calculated logP values.

building of models and the test set of 5 compounds used for an external validation of the developed QSRR models. The QSRR modeling was performed by the use of the PLS regression. Statistical parameters of internal and external validation for all developed QSRR models, along with the selected molecular descriptors describing the retention behavior of the tested compounds on stationary phases of different polarities are presented in Table V.

The obtained values of Q^2, R^2_{pred}, $\bar{r}^2_{\text{m}}$ and Δr^2_{m} as well as the low errors for the training and the test set (RMSEE and RMSEP) indicated that the models are predictive and robust, whereas the Y-scrambling test ($Q^2_{\text{intercept}}$ and $R^2_{\text{intercept}}$) showed that models were not obtained by chance (Table V). The optimal QSRR (RP-2), QSRR (RP-8) and QSRR (RP-18) models were developed with the three most significant descriptors, whereas QSRR (CN) was built with four molecular descriptors. The influence of the selected molecular properties on the dependent R_M^0 values is presented in Figure 4.

Discussion

Features of stationary phases and the presence of appropriate functional groups on their surfaces affect different behavior of compounds, when the CN, RP-2, RP-8 or RP-18 modified silica are used. A comparison between the lipophilicity indices obtained in different chromatographic systems with use of the same organic modifier revealed differences in the obtained R_M^0 values. It can be seen that the highest R_M^0 values were obtained using the most hydrophobic RP-18 stationary phase, while for the majority of the compounds the lowest R_M^0 values were noticed for the most polar CN stationary phase. In addition, by comparing the experimental R_M^0 values obtained on the RP-18 and CN stationary phases with methanol present in mobile phases and the results obtained on the same stationary phases but with tetrahydrofuran in mobile phases (14), an influence of organic modifier also becomes obvious. The majority of substances show higher R_M^0 values in methanol, which has lower elution strength and belongs to the

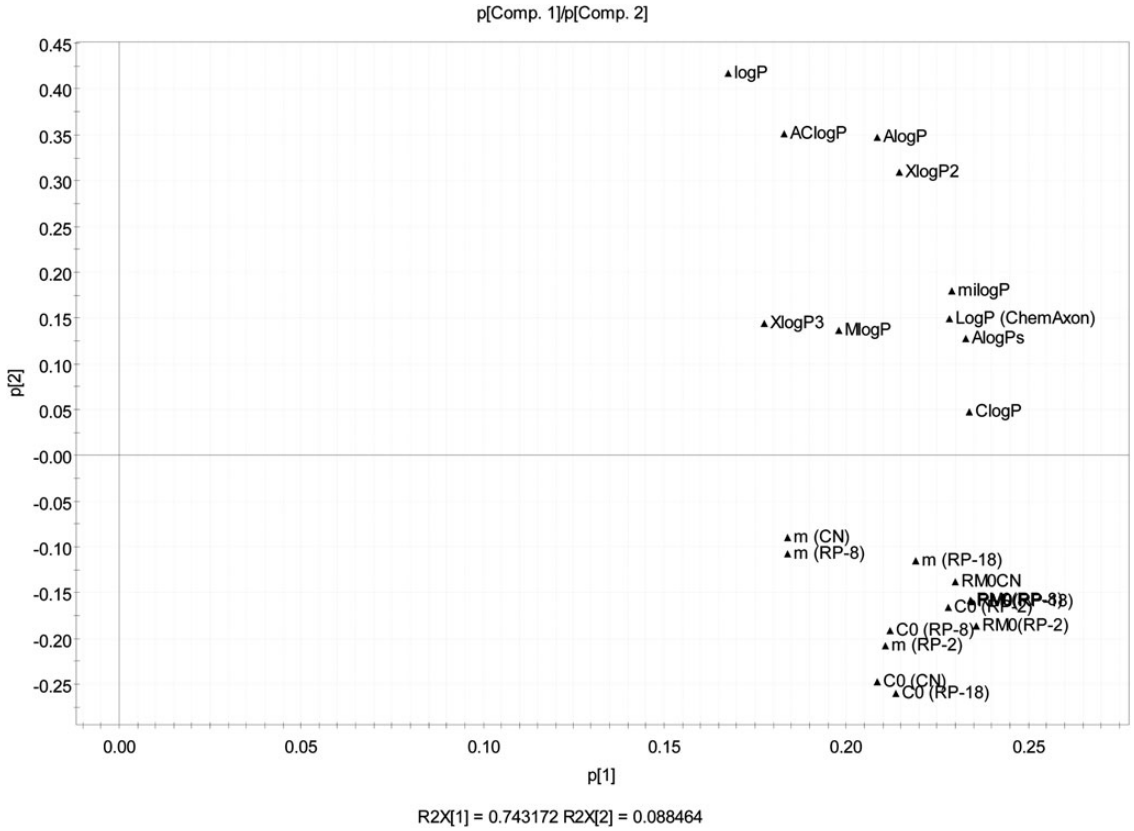

Fig 3. Loading plot as a result of PCA for the experimental lipophilicity indices and calculated logP values.

Table V. Statistical Results of the Developed QSRR Models

Chromatographic system	R^2Y	Q^2	F	P	RMSEE	RMSEP	r^2_{pred}	r^2_m	r'^2_m	avr^2_m	Δr^2_m	$R^2_{intercept}$	$Q^2_{intercept}$
QSRR (CN) = f (RDF025u, N-075, CATS2D_01_LL, F01[C-C])	0.928	0.913	63.2	4.26E−07	0.220	0.272	0.823	0.732	0.558	0.645	0.175	−0.075	−0.259
QSRR (RP-2) = f (DISPs, CATS2D_01_LL, F01[C-C])	0.934	0.911	61.8	4.81E−07	0.194	0.273	0.765	0.761	0.568	0.664	0.194	−0.102	−0.269
QSRR (RP-8) = f (Mor19s, CATS2D_01_LL, F01[C-C])	0.927	0.917	66.1	3.33E−07	0.228	0.342	0.727	0.665	0.542	0.603	0.124	−0.096	−0.258
QSRR (RP-18) = f (nC, CATS2D_01_LL, F01[C-C])	0.901	0.885	46.1	2.33E−06	0.383	0.401	0.815	0.719	0.817	0.768	0.097	−0.118	−0.268

F01[C-C], frequency of C-C at topological distance 1 (2D atom pairs); CATS2D_01_LL, CATS2D lipophilic–lipophilic at lag 01 (CATS 2D); N-075, R-N-R/R-N—X (atom-centered fragments); RDF025u, radial distribution function-025/unweighted (RDF descriptors); DISPs, displacement value/weighted by I-stat (geometrical descriptors); Mor19s, signal 19/weighted by I-state (3D-MoRSE descriptors); nC, number of carbon atoms (constitutional indices).

protonic solvents than in aprotonic tetrahydrofurane. These results can be attributed to different interactions that occur during the chromatographic process between the solvent and the examined compounds, as well as between the organic components of the mobile phase and the stationary phase.

It is also evident that among the tested imidazoline derivatives, 2-methylimidazolines (compounds 1, 2, 5, 14, 15, 19 and 20) exhibit significantly higher R^0_M values and stronger retention on all the examined stationary phases than 2-aminoimidazolines (compounds 4, 6, 13 and 18). An increased lipophilicity of these compounds and their stronger interactions with stationary phases can be attributed to the presence of a more hydrophobic aryl methyl scaffold attached to imidazoline in comparison with the aryl amino scaffold. Among arylimidazolines, an order of the increasing lipophilicity is the same for all stationary phases (16 < 12 < 8 < 17).

A similar order of an increasing lipophilicity was observed in the case of the logP values obtained using the available programs. The results given in Table IV show that the logP values calculated using different algorithms to various degrees correlate with the experimentally obtained R^0_M values. On the basis of these results, it is not possible to point out to the algorithm that is the best performing one for all systems and applicable to each chromatographic system, but it is possible to choose a logP value that best correlates with R^0_M obtained for a given system.

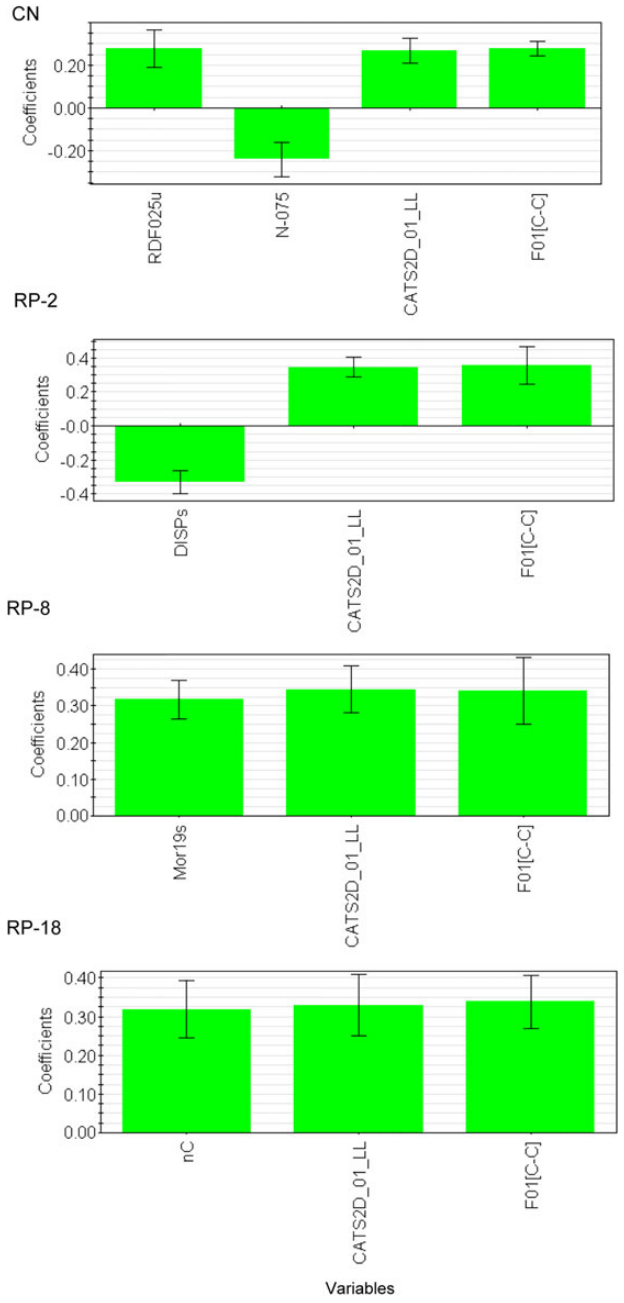

Fig 4. PLS coefficient plots of the developed QSRR models for different stationary phases. This figure is available in black and white in print and in color at *JCS* online.

In addition, the QSRR analysis was performed to define functional relationship between the retention/lipophilicity of the studied compounds and their structure. In all the devised QSRR models, frequency of C-C at the topological distance 1 (F01[C-C]) and CATS2D lipophilic–lipophilic at lag 01 (CATS2D_01_LL) are selected as important descriptors (27) with an influence on the R_M^0 values in the examined chromatographic systems. On the basis of positive correlation of F01 [C-C] with the R_M^0 values (Figure 4), it can be concluded that the presence of the functional groups with multiple C-C fragments, such as phenyl rings in antazoline, xylometazoline and oxymetazoline, leads to a significant increase in the values of this descriptor and consequently, to a stronger interaction with all the examined stationary phases. CATS2D_01_LL is related to the presence of the lipophilic pharmacophore point groups. Its positive correlation with the R_M^0 values (Figure 4) indicates that the molecules, which possess the pharmacophore groups that contribute to the lipophilic character of a molecule, demonstrate stronger interaction with all stationary phases. The differences in the retention behavior of the compounds can be distinguished based on the N-075, RDF025u, DISPs, Mor19s and nC descriptors (27). Descriptor N-075 negatively correlates, while RDF025u (Radial Distribution Function—025/unweighted) positively correlates with the R_M^0 (CN) values.

Negative correlation of DISPs (displacement value/weighted by I-state) with the R_M^0 (RP-2) values indicates that the compounds with the higher values of this descriptor (such as moxonidine and brimonidine) possess less lipophilic character and generate lesser interactions with the RP-2 stationary phase. Mor19s (signal 19/weighted by I-state) positively correlates with the R_M^0 (RP-8) values and belongs to the 3D-MoRSE descriptors. The 3D-MoRSE descriptors present the 3D structure of the molecule based on the electron diffraction. They are independent of the size of molecules and applicable to a large number of molecules with great structural variance (27). Positive correlation of nC (number of carbon atoms) descriptor with the R_M^0 (RP-18) values indicates that the compounds with a higher number of carbon atoms (such as xylomethazoline and antazoline) exhibit stronger interaction with the nonpolar RP-18 stationary phase and possess higher lipophilic character.

Conclusion

A comparative study of the retention behavior of 20 selected imidazoline derivatives performed on stationary phases of different polarities revealed similar chromatographic behavior of the examined compounds on the CN, RP-2, RP-8 and RP-18 stationary phases. In all the investigated chromatographic systems, 2-methylimidazolines exhibited significantly higher R_M^0 values and stronger retention compared with 2-aminoimidazolines. Different degrees of correlations between the experimentally obtained lipophilicity indices (R_M^0, m and C_0) and the calculated logP values revealed that R_M^0 is the most reliable parameter for the estimation of the lipophilicity of compounds, while all the investigated chromatographic systems including the CN, RP-2, RP-8 and RP-18 stationary phases and a mixture of methanol, water and ammonia as mobile phase are suitable systems for the evaluation of lipophilicity of the imidazoline derivatives. The performed PCA analysis confirmed significant correlation between experimentally determined chromatographic parameters and logP values calculated by different programs. Upon the results of the performed QSRR analysis, frequency of C-C at topological distance 1 (F01[C-C]) and CATS2D Lipophilic–Lipophilic at lag 01 (CATS2D_01_LL) represent important structural features that contribute to the retention mechanism on all the tested stationary phases, while differences in the retention behavior of compounds can be distinguished based on the N-075, RDF025u, DISPs, Mor19s and nC descriptors. Strict criteria of the internal and external validation that have been achieved for all the QSRR models indicate that the established models can be used as reliable tools for the prediction of the retention behavior in a wide range of lipophilicity of compounds.

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