Study of Retention Parameters Obtained in RP-TLC System and Their Application on QSAR/QSPR of Some Alpha Adrenergic and Imidazoline Receptor Ligands

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Abstract

The retention constant (R_m^0) is determined for 11 selected adrenergic and imidazoline receptor ligands by reverse-phase-thin layer chromatography. It is established that the retention behavior of investigated compounds mostly depends on geometrical, electrostatic, and hydrogen bonding properties. Good correlations among hydrophobic parameters R_m^0 versus log P for all eleven tested compounds are obtained. The satisfactory correlations are found between R_m^0 versus apparent partition coefficient octanol–buffer pH 7.4 (log P') or apparent partition coefficient in four liposome systems (log K'_M) and hypotensive activity (pC_{25}) for five imidazolines. The results confirm the suitability of this parameter in quantitative structure-property and structure-activity relationships studies of these drugs.

Introduction

The role of lipophilicity in drug–biomacromolecule interactions has been extensively discussed in terms of quantitative structure-property relationships (QSPR) and quantitative structure-activity relationships (QSAR). The distribution of a compound in a living system may be viewed as a series of partitioning steps, in conjunction with diffusion through several regions. The affinity of a compound for biological membranes may be represented by its lipophilicity or hydrophobicity as one of the most important intrinsic physicochemical properties of the compound. Hydrophobicity is usually related with polarity, molecular size, and hydrogen bonding of the molecules. In most cases, it governs the ability of drugs to enter and pass through biological membranes and, hence, determines absorption, distribution, transport, and storage. Receptor binding may also be a hydrophobic intereaction and hence affect biological activity. Therefore, hydrophobic factors are commonly observed in QSARs.

Usually, the lipophilicity of a compound is quantitatively characterized by log P, the logarithm of its *n*-octanol–water partition coefficient. Nowadays, separation techniques, such as reversed-phase (RP) high-performance liquid chromatography (HPLC) or RP thin-layer chromatography (TLC) are alternative techniques because the measured retention indices can be correlated with log P and other lipophilicity parameters of the compounds. The tediousness of determinations and limited interlaboratory reproducibility of log P, on one hand, and the observations of linear relationship between log P and chromatographic retention parameters, on the other hand, gave rise to the substitution of the former by the readily available chromatographic data (1).

The predictive and interpretable capability of quantitative chromatographic retention-biological activity models is supported by the fact that in adequate experimental conditions the solute partitioning into the chromatographic system can emulate the solute partitioning into lipid bilayers of biological membranes (2). On the other hand, not only the calculated lipophilicity, but also various sterical and polarity parameters significantly influence the retention, indicating the involvement of factors other than hydrophobic forces in the retention mechanism (3,4).

A central issue of this study was to investigate hydrophobic parameters and then to discuss the main driving factors determining the chromatographic retention phenomena in order to use this result in subsequent QSAR analysis of the drugs acting as modulators on alpha$_1$, alpha$_2$, and imidazoline I_1/I_2 receptors. They have been introduced generally as partial agonist or antagonist of alpha adrenergic receptors. Chemically, most of these drugs are derivatives of amidine, imidazoline, imidazolidine, or guanidine. The 2-(arylmethyl)-imidazoline, such as tetrahydrazoline, naphazoline, oxymethazoline, and xylomethazoline are selective alpha$_1$ agonists and, therefore, are used as vasoconstrictors in the treatment of hypotension, shock, and as topical nonprescripton drugs for treating nasal congestion and bloodshot eyes. On the other hand, 2-(arylamino)-imidazolines, such as clonidine and moxonidine, represent powerful agonists at alpha$_2$ adrenoceptors and imidazoline I_1 receptors (5). The oxa-izoster of imidazoline-rilmenidine is I_1 agonist, and amiloride is supposed to be agonist on I_2 receptors (6,7). In this group, this makes the imidazoline ring a part of

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guanidino group, and the uncharged form could exist as a part of tautomers. They are used as antihypertensive drugs. Amidino and cyclic guanidino groups are part of structure of amiloride and doxazosin, drugs widely used in therapy of hypertension.

The QSAR and QSPR approach of imidazolines has already been applied (8,9). Affinity of these drugs for the receptors and the ability to reach active sites depends on many factors, including molecular features, pK_a, and lipid solubility of the drugs. The retention parameters of derivatives of imidazoline have been investigated using the HPLC system (10–14).

The object of this work was to investigate the hydrophobicity parameters obtained in the RP-TLC system and their possible application in better rationalization of drug action of some imidazolines, amidines, and related drugs and, therefore, their applicability in QSAR studies of these drugs.

Experimental

Reagents

All reagents used were of analytical grade purity. The following standards were used: tramazoline hydrochloride and doxazosin (Zdravlje, Leskovac, Serbia and Montenegro); moxonidine (Solvay pharmaceuticals, Hanover, Germany); naphazoline hydrochloride (Panfarma, Belgrade, Serbia and Montenegro); oxymetazoline (Lek, Ljubljana, Slovenia); xylometazoline (Dolder AG, Basel, Switzerland); tetrahydrozoline hydrochloride (Hemomont, Podgorica, Serbia and Montenegro); hydrochlortiazide and amiloride (Galenika, Zemun-Belgrade, Serbia and Montenegro); and clonidine hydrochloride and rilmenidine (Sigma-Aldrich, Taufkirchen, Germany).

Chromatography

Solutions for chromatographic investigation were prepared by dissolving 1 mg/mL of compounds in methanol and rilmenidine in water. Aliquots of 2 µL of each solute were spotted on the TLC aluminium sheets RP-18 F_{254s} (Merck, Darmstadt, Germany) by Nanomat III (Camag, Muttenz, Switzerland). The chromatograms were developed by the ascending technique using methanol–dioxane as the mobile phase (φ = 0.05 – 0.5, the volume fraction of methanol in dioxane). The detection of the spots was performed under UV light at 254 nm. Three chromatograms were developed for each percentage of methanol, and retardation factor (R_f) values were calculated as average values. Retention factor (R_m) values were calculated from the equation: $R_m = \log (1/R_f - 1)$.

Calculations of molecular descriptors

The chemical structures of investigated compounds used in this study are presented in Figure 1. The structures were constructed using the Spartan program package (15). Geometry optimization was performed using the AM1 method. Calculation of the surface area and electrostatic potential were also performed in the Spartan program.

Codessa software (16) was used for the calculation of a large number of molecular descriptors, including constitutional, topological, geometrical, electrostatic, and quantum-chemical. The same software was used for selection of the best descriptors using heuristic methods, as well as for the definition of QSAR equations using multiple linear regression (MLR) analysis.

Results and Discussion

The impact of different solvents for the estimation of hydrophobicity parameter R_m^0 and correlation with lipophilicity of several classes of drugs using TLC have widely been investigated (17,18). The influence of the mobile phase composition on mobility and resolution of investigated substances have been performed using a binary system, such as methanol–water and acetonitrile–water. Because the better mobility of investigated substances was obtained in the methanol–water system, and because of the higher selectivity of methanol by comparison with water, the former was further used as the organic modifier. In order to optimize the com-

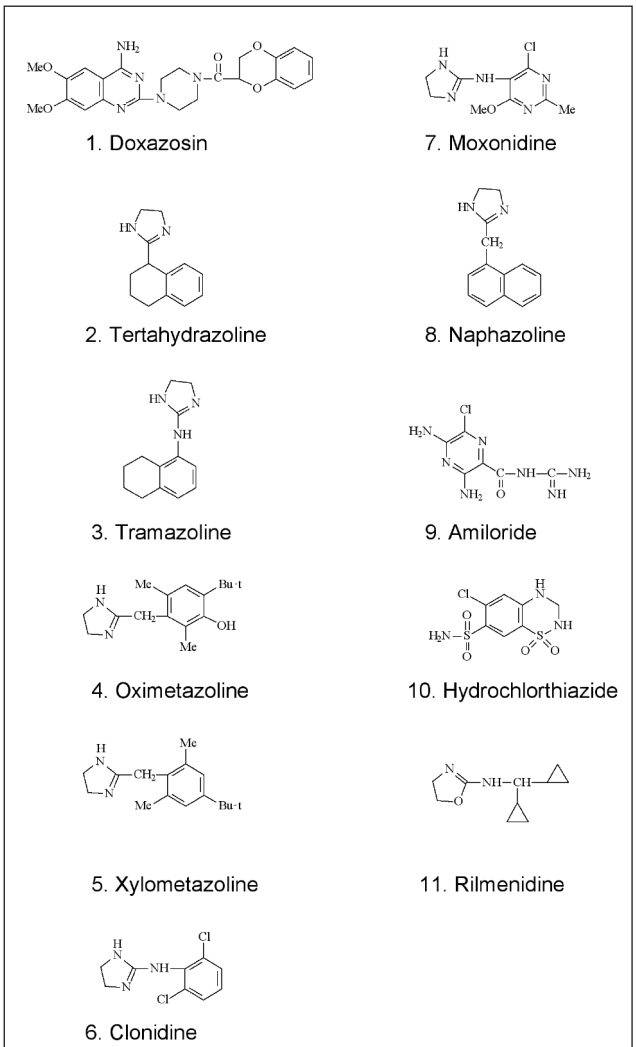

Figure 1. The chemical structures of investigated compounds.

position of the mobile phase and considering proton–donor, proton–acceptor, and dipole–dipole interactions of solvents, acetonitrile and dioxane were selected as diluents. The weaker interaction between compounds and mobile phase and compounds and the surface of the adsorbent were observed using dioxane as the organic diluent.

The hydrophobicity parameter R_m^0 was determined from R_m values for each percentage of methanol using the following linear equation: $R_m = R_m^0 + m \times \log \varphi$, where φ is the volume fraction of methanol in the mobile phase (0.05–0.5). The obtained slopes, m, and intercept values, R_m^0, are presented in Table I.

From the data in Table I, the linear relationship between R_m^0 and m is given by the following equation:

$$R_m^0 = -0.926 - 1.98\ m \qquad \text{Eq. 1}$$

where $r = 0.996$. The corresponding linear relationship is presented in Figure 2. The very good correlation indicated the suitability of the examined system for estimating the lipophilicity of the compounds.

Partition coefficient (P) is usually used as an expression of the lipophilic character of compounds. In order to avoid the practical difficulties that often arise from the direct determination of the partition coefficient, the R_m^0 value can also be used because it is related to the logarithm of the partition coefficient between the polar mobile phase and non-polar stationary phase of the partition TLC system.

The linear plot R_m^0 versus log P obtained from the literature, given in Table I, of the 11 compounds investigated shows that satisfactory correlation has been obtained:

$$R_m^0 = -1.1436 + 0.69444 \log P \qquad \text{Eq. 2}$$

where ($n = 11$, $r^2 = 0.6121$, F = 14.20)

Timmermans et al. (8) found that lipophilicity plays a significant role in hypotensive activity, and it does not show a significant influence on hypertensive activity of selected imidazolines.

The other molecular properties, apart from lipophilicity, could be of interest to investigate, and the success of QSPR depends mainly on the selection of meaningful molecular descriptors among numerous electronic, geometric, topological, and molecular size-related descriptors. The retention mechanism operating in individual chromatographic systems varies with the nature of the interactions among the analytes, the stationary phase, and the mobile phase (13). There was an attempt to identify the type of these interactions, applying the heuristic method by which the most significant descriptors, besides log P, are classified according to r^2 values (see Table II), in one-parameter correlations.

By applying MLR in two and three parameter analysis, including log P in any equation as the most significant parameter for retention behavior in this experimental conditions, the following results were obtained:

$$R_m^0 = -7.6255 + 0.4118 \log P + 0.78182\ Eex\ (C–N) \qquad \text{Eq. 3}$$

where $n = 11$, $r^2 = 0.7205$, and F = 10.31

$$R_m^0 = 1.0248 + 0.6941 \log P - 0.00876\ MSA \qquad \text{Eq. 4}$$

where $n = 11$, $r^2 = 0.7636$, and F = 12.92

$$R_m^0 = 64.611 + 0.0601 \log P - 4.8904\ Er'(HN) \qquad \text{Eq. 5}$$

where $n = 11$, $r^2 = 0.8073$, and F =16.76

$$R_m^0 = -21.093 + 0.5457 \log P - 0.07054\ MSA + 4.027\ Eex(C–N) \qquad \text{Eq. 6}$$

where $n = 11$, $r^2 = 0.8184$, and F = 10.52

$$R_m^0 = 9.0021 - 0.15831 \log P - 9.3836\ Er'(HN) - 340.25\ (PC'_N) \qquad \text{Eq. 7}$$

where $n = 11$, $r^2 = 0.9525$, and F = 46.74

The results of the heuristic method show that in this set of chemically diverse drugs there is a higher influence of electrostatic and quantum-chemical reactions of nitrogen respon-

Table I. Hydrophobicity Parameters Obtained From Equation $R_m = R_m^0 + m \log \varphi$; Mobile Phase Methanol–Dioxane (φ = 0.05–0.5)

	Compound	R_m^0	m	log P*
1.	Doxazosin	–2.006	0.6249	0.649
2.	Tetrahydrazoline	1.719	–1.366	3.313
3.	Tramazoline	1.422	–1.227	2.069
4.	Oxymetazoline	1.239	–1.039	4.169
5.	Xylometazoline	1.092	–0.9608	4.905
6.	Clonidine	0.0868	–0.5771	1.412
7.	Moxonidine	0.5154	–0.6550	0.914
8.	Naphazoline	1.948	–1.426	3.527
9.	Amiloride	0.9554	–1.009	1.896
10.	Hydrochlorthiazide	–1.654	0.2737	–0.071
11.	Rilmenidine	–1.676	0.3693	0.575

* SciFinder Scholar 2004 Edition.

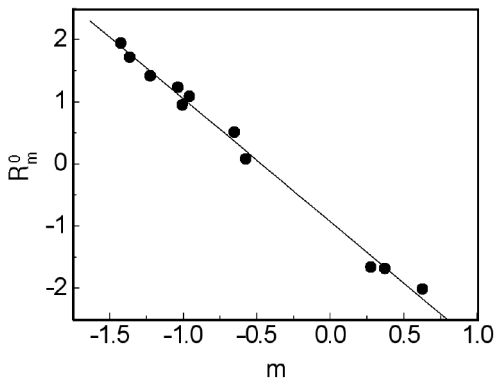

Figure 2. Relationship between retention constants (R_m^0) and the slope (m) of TLC equations.

sible for the binding site rather than hydrogen bonding capability and the shape of molecules (equation 7 and Figure 3). It is interesting to note that the hydrogen-bonding charged surface area of hydrochlorothiazide and amiloride, drugs that are used to treat hypertension, shows very high values.

Table II. Calculated Descriptors Using Codessa Software

Compound	HBCA*	Eex(C–N)†	Er'(HN)‡	PC'$_N$§	MV**	MSA††
Doxazosin	38.705	8.0897	13.7039	–0.1084	403.0915	420.9763
Tetrahydrazoline	6.0518	9.5787	13.4231	–0.1112	202.3218	205.7895
Tramazoline	9.4274	9.1939	13.3033	–0.1058	215.2906	227.9458
Oxymetazoline	12.9804	9.5703	13.4161	–0.1114	268.2695	276.7855
Xylometazoline	6.3709	9.5860	13.4121	–0.1115	278.3121	290.1884
Clonidine	13.4425	9.2722	13.3163	–0.1049	186.2746	204.0698
Moxonidine	19.4795	9.2630	13.3123	–0.1045	206.6834	223.6665
Naphazoline	7.9888	9.6040	13.3945	–0.1115	209.7338	217.9874
Amiloride	79.7442	9.5957	13.2174	–0.1047	179.6623	219.2672
Hydrochlorthiazide	170.6309	6.8722	13.5754	–0.1046	206.4716	236.5443
Rilmenidine	11.9568	9.0178	13.3833	–0.0993	183.2043	198.5107
r^2	0.2598	0.5229	0.3727	0.3537	0.1028	0.1521
F	–1.7774	3.1404	–2.3125	–2.2194	–1.0158	–1.2705

* HBCA = H-bonding charged surface area (Semi-Mo).
† Eex(C–N) = Max exchange energy for a C–N bond.
‡ Er'(HN) = Min resonance energy for H–N bond.
§ PC'$_N$ = Min partial charge for a N atom.
** MV = Molecular volume.
†† MSA = Molecular surface area.

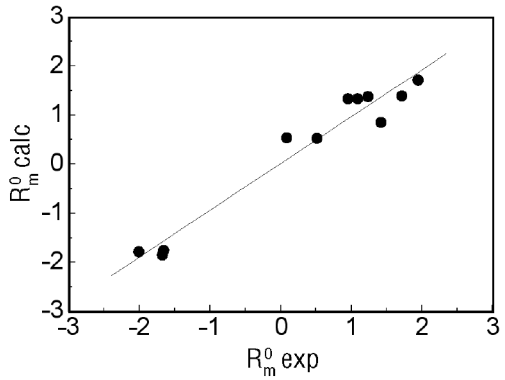

Figure 3. Correlation between experimental and calculated R_m^0 values using equation 7.

The activity of alpha agonists depends on various factors, including the lipid solubility of these drugs, although the partitioning of solutes into bilayer membranes might occur by a different mechanism other than partitioning into a bulk oil phase (19). The hydrophobic interaction is one of the most important, but least understood non-covalent structural effects in ligand binding (20).

Z.W. Choi and J.A. Rogers (19) investigated the relationship of apparent partition coefficients of a group of imidazoline alpha adrenoceptor agonists in the liposome–buffer system, as well as the *n*-octanol–buffer system in QSAR employing biological activities and receptor binding affinities. The values of log P' (apparent partition coefficient octanol–buffer pH 7.4) and K'$_M$ (apparent partitioning coefficient in four liposome systems), used in QSAR studies of imidazolines, are taken from their paper and are presented in Table III. Their results showed that the liposome represents a more selective model membrane system than a bulk oil phase for predicting the biological activities of imidazolines; therefore, the parameters obtained in liposome systems are more useful in correlation with activities of these drugs. In this work, the applicability of hydrophobic parameters R_m^0 obtained in RP-TLC in QSAR studies of imidazolines was investigated.

The good linear correlation of R_m^0 values for five imidazolines, experimentally determined in this work, and log P', as well as parabolic correlations of R_m^0 and K'$_M$ values in four systems, are presented by the following equations:

$$\log P' = 0.919 - 0.83\ R_m^0 \qquad \text{Eq. 8}$$

where $r = 0.885$

$$\log K'_M(1) = 1.043 + 1.505\ R_m^0 - 0.71\ (R_m^0)^2 \qquad \text{Eq. 9}$$

Table III. Apparent Partition Coefficients of Alpha Adrenoceptor Agonists in the *n*-Octanol–Buffer pH 7.4 (log P') and Liposome–Buffer (log K'$_M$) Systems; Hypertensive (pC_{60}) and Hypotensive (pC_{25}) Activities*

		log K'$_M$*				Activities	
Compounds	Log P'	DMCP	DMCP–CHOL–DCP (7:1:2 mol ratio)	DMCP–PS (3:5:1 mol ratio)	DMCP–STA (3:1 mol ratio)	pC_{60}	pC_{25}
Oxymetazoline	–0.32	1.94	2.50	2.96	1.16	2.24	ND
Xylometazoline	0.40	1.94	2.40	2.80	1.30	1.12	0.26
Tramazoline	–0.62	1.48	2.17	2.59	0.77	1.80	0.55
Naphazoline	–0.52	1.34	2.12	2.45	0.70	1.83	0.95
Clonidine	0.85	1.15	1.61	2.01	1.17	1.78	2.04

*See the literature (19).

where $r^2 = 0.804$

$$\log K'_M(2) = 1.501 + 1.42\ R_m^0 - 0.576\ (R_m^0)^2 \quad \text{Eq. 10}$$

where $r^2 = 0.890$

$$\log K'_M(3) = 1.888 + 1.559\ R_m^0 - 0.661\ (R_m^0)^2 \quad \text{Eq. 11}$$

where $r^2 = 0.893$

$$\log K'_M(4) = 1.163 + 0.334\ R_m^0 - 0.309\ (R_m^0)^2 \quad \text{Eq. 12}$$

where $r^2 = 0.698$

It was also indicated previously that the hypotensive activity of imidazoline mostly depends on the lipophilic solute–membrane interactions, as well as electrostatic interactions at negatively charged sites (19). Therefore, it was supposed that the hydrophobic parameter obtained in the RP-TLC system can be useful in QSAR studies, which was confirmed by very good correlations of R_m^0 and hypotensive activity (pC_{25}) of five imidazolines shown in equation 13.

$$pC_{25} = 2.287 - 3.12\ R_m{}^0 + 1.26\ (R_m^0)^2 \quad \text{Eq. 13}$$

where $r^2 = 0.978$

Correlation with hypertensive activity (pC_{60}) did not give satisfactory results [$r = 0.1147$ (linear) and $r^2 = 0.047$ (polynomial)], which is in accordance with the supposition that hypertensive activity, besides the lipophilicity, depends on other factors, such as the pK_a of compounds, composition and surface charge, and membrane structure.

Several molecular descriptors have been calculated in Spartan software, such as electrostatic potential (EP) and surface area (SA), in order to build better QSAR equations for the prediction of hypertensive activity (Table IV). Obtained results confirm that apart from lipophilicity, other molecular descriptor have greater impact on activity:

$$pC_{60} = -8.324 - 0.1702R_m^0 * + 10.01EP* \quad \text{Eq. 14}$$

where $r^2 = 0.4749$

$$pC_{60} = -8.259 - 0.1380R_m^0 * + 10.98\ EP* - 1.307SA* \quad \text{Eq. 15}$$

where $r^2 = 0.6206$

Table IV. Hypotensive (pC_{25}) and Hypertensive (pC_{60}) Activity Taken From Literature and the Calculated Descriptors: Electrostatic Potential (EP) and Surface Area (SA) (Spartan software) of the Compounds Investigated*

Compound	pC_{60}	pC_{25}	EP	SA	EP†	SA†	$R_m{}^{0}$†
Oxymethazoline	2.24	–	212.31	310.03	1.060	0.9526	1.135
Xylomethazoline	1.12	0.26	200.31	325.47	1	1	1
Tramazoline	1.80	0.55	211.17	253.49	1.054	0.7788	1.302
Naphazoline	1.83	0.95	203.58	245.5	1.016	0.7543	1.784
Clonidine	1.78	2.04	198.58	225.44	0.9914	0.6927	0.079

*See the literature (19).

Conclusion

The hydrophobicity parameter R_m^0 plotted versus log P of the 11 compounds investigated shows satisfactory linear correlation using MLR. The retention behavior of drugs investigated depends on geometrical properties (size and shape), electrostatic properties (energy for H–N bonds and partial charges of N atom), and hydrogen bonding. Correlation between hydrophobic parameter R_m^0 and liposome–buffer systems used in permeability studies of five imidazolines indicate the suitability of this parameter in QSPR studies of these drugs. Good quantitative correlation of R_m^0 with pC_{25} confirms that hypotensive activity mainly depends on hydrophobic interactions of ligands with receptors. Correlation with pC_{60} indicate that hypertensive activity of drugs, besides hydrophobicity, depends on other factors, such as pK_a, surface charges, and other electronic and quantum-chemical properties.

Acknowledgments

This work was supported by the Ministry of Science and Enviroment Protection of Republic of Serbia, Grant #142071B.

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Manuscript received February 26, 2006;
revision received September 18, 2006.