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Estimation of Angiotensin-Converting Enzyme Inhibitors Protein Binding Degree Using Chromatographic Hydrophobicity Data

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SUMMARY

Introduction Angiotensin-converting enzyme (ACE) inhibitors represent a significant group of drugs primarily used in the treatment of hypertension and congestive heart failure.

Objective Selected ACE inhibitors (enalapril, quinapril, fosinopril, lisinopril, cilazapril) were studied in order to establish a fast and easy estimation method of their plasma protein binding degree based on their lipophilicity data.

Methods Chromatographic hydrophobicity data (parameter C_0) were obtained on cellulose layers under conditions of normal-phase thin-layer chromatography (NPTLC), using different binary solvent systems. The ACE inhibitors lipophilicity descriptors (log*P*) values were calculated using the software package Virtual Computational Chemistry Laboratory. The ACE inhibitors plasma protein binding data were collected from relevant literature.

Results ACE inhibitors protein binding data varied from negligible (lisinopril) to 99% (fosinopril). The calculated lipophilicity descriptors, $\log P_{KOWWIN}$ values ranged from -0.94 (lisinopril) to 6.61 (fosinopril). Good correlations were established between plasma protein binding values and calculated $\log P_{KOWWIN}$ values (R^2=0.8026) as well as chromatographic hydrophobicity data, C_0 parameters (R^2=0.7662). Even though good correlation coefficients (R^2) were obtained in both relations, unacceptable probability value with $p>0.05$ was found in relation between protein binding data and calculated $\log P_{KOWWIN}$ values. Subsequently, taking into consideration the request for probability value lower than 0.05, a better relationship was observed between protein binding data and chromatographically obtained hydrophobicity parameters C_0 values.

Conclusion Cellulose layers are easily available and cost effective sorbent to assess hydrophobicity. Experimentally obtained data on ACE inhibitors hydrophobicity and plasma protein binding estimation are important parameters in evaluating bioavailability of these drugs.

Keywords: angiotensin-converting enzyme inhibitors; plasma protein binding; hydrophobicity

INTRODUCTION

High-throughput evaluation of drug's properties - absorption, distribution, metabolism and elimination (ADME), is crucial in its discovery and design process. The number of molecular physicochemical properties (lipophilicity, solubility, molecular weight, volume of drug molecule, polar surface area) plays important role in drug's ADME characteristics, as well as in plasma protein binding (PPB) degree [1, 2, 3]. Lipophilicity is one of the most important properties, since lipophilic molecules exhibit better absorption, penetration into tissues and a higher degree of distribution. Also, it is well-known that more lipophilic drugs exert a higher degree of protein binding in comparison to less lipophilic ones with similar properties [1, 2, 3].

Drug molecules are *in vivo* either bound to plasma proteins and lipids, to proteins and lipids in tissues, or they are free, that is, unbound, and diffuse among the aqueous environment of blood and tissues. Depending on the specific affinity for plasma protein, the portion of the bound and unbound drug may differ. The PPB degree significantly influences drug's efficiency. The less bound drug passes through cell membranes or diffuses and exhibits pharmacologic effects more efficiently. Also, PPB can influence the drug's biological half-life in the body since bound portion may act as a reservoir from which the drug is slowly released as the unbound form [4, 5].

Angiotensin-converting enzyme (ACE) inhibitors are widely used for treating hypertension, congestive heart failure and renal failure [5, 6, 7]. They exert antihypertensive effect by blocking the conversion of angiotensin I to angiotensin II, lowering arteriolar resistance, increasing venous capacity, increasing natriuresis and downregulating sympathetic adrenergic activity. They inhibit cardiac and vascular remodeling associated with chronic hypertension, heart failure, and myocardial infarction, reduce ventricular preload and afterload, cardiac output, cardiac index, stroke work and volume. Furthermore, they cause selective dilatation of efferent renal arterioles lowering renovascular resistance. In addition to antihypertensive, ACE inhibitors also exhibit antiproliferative, antiatherosclerotic and fibrinolytic effects. In hypertensive patients with renal failure, particularly of diabetic etiology, ACE

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inhibitors are used as drugs of choice because, in addition to antihypertensive effects, they attenuate the progression of microalbuminuria and proteinuria [8-11].

According to the available literature, a number of authors investigated the relationship between lipophilicity and ACE inhibitors pharmacological activity, duration of action and absorption [12, 13, 14]. In our previous studies of ACE inhibitors we reported their lipophilic properties under different chromatographic conditions [15, 16, 17]. Also, in our recently published studies we presented correlations between reversed-phase chromatographic hydrophobicity data and ACE inhibitors absorption values [18], as well as their plasma protein binding data [19].

OBJECTIVE

In continuation of our previous investigations the aim of this study was to assess relationship between ACE inhibitors lipophilicity data, experimentally obtained under conditions of normal-phase thin-layer chromatography (NP-TLC) on cellulose layers, and their plasma protein binding properties. The main topic was to establish the fast, easy, cost-effective approach enabling estimation of protein binding degree of ACE inhibitors.

METHODS

Based on the differences in chemical structure, ACE inhibitors can be distributed into three groups: with sulfhydryl group (represented by captopril), with carboxyl group (represented by enalapril) and with phosphinic acid group (represented by fosinopril) [5].

In this study the following ACE inhibitors were investigated:

1. enalapril maleate, (*S*)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-*L*-alanyl]-*L*-proline maleate;

2. quinapril hydrochloride, [3*S*-[2[*R**(*R**)],3*R**]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid hydrochloride;

3. fosinopril sodium, (4*S*)-4-Cyclohexyl–1-[[(*R*)-[(1*S*)-2-methyl-1-(1-oxopropoxy)- propoxy](4-phenylbutyl) phosphinyl]acetyl]-*L*-proline, sodium salt;

4. lisinopril dihydrate, (*S*)-1-[N^2-(1-carboxy-3-phenylpropyl)-*L*-lysyl]-*L*-proline dihydrate;

5. cilazapril monohydrate, [1*S*-[1α,9α (*R**)]]-9-[[1-(ethoxycarbonyl)-3-phenylpropyl] amino]octahydro-10-oxo-6*H*-pyridazino[1,2-a][1,2]diazepine-1-carboxylic acid monohydrate.

Aiming to provide a fast, high-throughput technique for modeling of PPB with lipophilicity, we selected these compounds as representative ACE inhibitors according to their PPB values collected from relevant references [5, 20], ranging from negligible (lisinopril) to 99% (fosinopril), and their lipophilicity, logP_{KOWWIN} values, calculated using software package Virtual Computational Chemistry Laboratory [21] ranging from -0.94 (lisinopril) to 6.61 (fosinopril). The values of PPB and lipophilicity of ACE inhibitors which were not included in this study are within these ranges. Additionally, captopril was excluded from the proposed model since it belongs to the sulfhydryl group of ACE inhibitors, with a notably different structure which leads to significant differences in behavior under chromatographic conditions.

The normal-phase thin-layer chromatography experiments were performed on cellulose, 10×10 cm, (Art. 105552, Merck, Germany) layers. The plates were spotted with 2 µL aliquots of freshly prepared ethanolic solutions of enalapril, quinapril, fosinopril and cilazapril and aqueous solution of lisinopril (2 mg/mL) and developed by the ascending technique. Several non-aqueous binary solvent systems were used with varying quantities (volume fraction) of components (Table 1). All components of mobile phases were of the analytical grade of purity. After development, the detection was performed by exposing the plates to iodine vapor. The ratio between the distance that each compound travelled and the distance that solvent front travelled presented the R_F values. All investigations were performed at room temperature (22±2°C).

The R_M values, representing the measure of compounds chromatographic behavior, were calculated for each solute in each mobile phase according to the Bate-Smith and Westall equation $R_M=\log(1/R_F - 1)$ [22]. The retention behavior of investigated substances in TLC can be presented as the relationship between R_M values and content of more polar component in mobile phase by the linear equation: $R_M=R_M^0 + m\,C$; where *C* represents the volume fraction (% V/V) of the more polar component in mobile phase, *m* is slope of the linear plot and R_M^0 (intercept) the extrapolated value R_M obtained at *C*=1%. The value of the intercept, R_M^0 represents the lipophilicity of the examined substance. Another hydrophobicity parameter, the C_0, can be calculated

Table 1. Chromatographic hydrophobicity parameters of the investigated compounds

Compounds		Investigated ACE inhibitors				
		1	2	3	4	5
Cyclohexan – carbon tetrachloride	R_M^0	1.786±0.097	1.683±0.038	1.843±0.044	2.417±0.032	1.766±0.095
	C_0	1.413	1.074	0.831	1.489	1.320
Cyclohexan – toluene	R_M^0	1.520±0.067	1.344±0.061	1.469±0.079	2.204±0.111	1.314±0.073
	C_0	1.293	0.918	0.704	1.397	1.236
Cyclohexan - benzene	R_M^0	1.319±0.053	1.304±0.034	1.397±0.144	2.615±0.111	1.327±0.085
	C_0	0.983	0.707	0.551	1.132	0.858

The numbers 1–5 denote the substances (Figure 1). R_M^0 and C_o are chromatographically obtained hydrophobicity parameters.

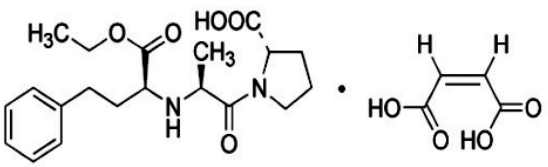
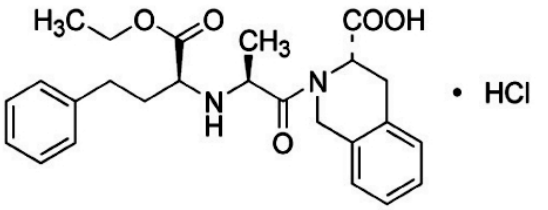
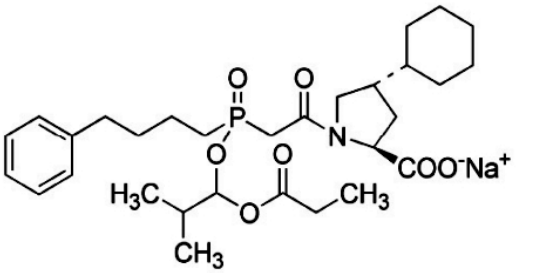
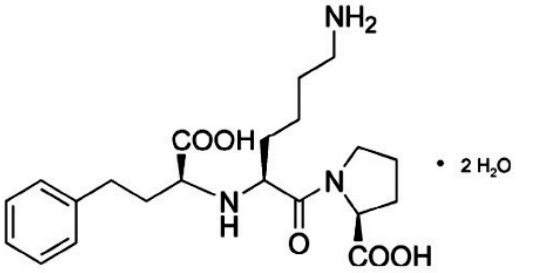
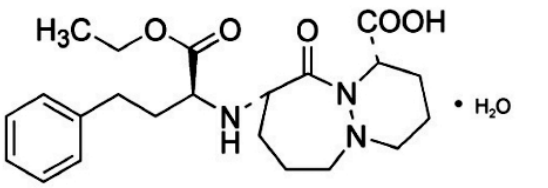
ACE inhibitors		$logP_{O/W}$	$logP_{KOWWIN}$
	**1. enalapril maleate**, (*S*)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-*L*-alanyl]-*L*-proline maleate,	2.45	2.45
	**2. quinapril hydrochloride,** [3*S*-[2[*R**(*R**)],3*R**]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid hydrochloride ,	3.72	3.72
	**3. fosinopril sodium,** (4*S*)-4-Cyclohexyl–1-[[(*R*)-[(1*S*)-2-methyl-1-(1-oxopropoxy)-propoxy](4-phenylbutyl)phosphinyl] acetyl]-*L*-proline, sodium salt,	6.61	6.61
	**4. lisinopril dihydrate**, (*S*)-1-[N^2-(1-carboxy-3-phenylpropyl)-*L*-lysyl]-*L*-proline dihydrate,	-1.22	-0.94
	**5. cilazapril monohydrate,** [1*S*-[1*α*,9*α*(*R**)]]-9-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]octahydro-10-oxo-6*H*-pyridazino[1,2-a][1,2]diazepine-1-carboxylic acid monohydrate.	-	2.27

Figure 1. Investigated ACE inhibitors

as: $C_0=-R_M^0/m$ [23, 24]. All experiments were carried out in triplicate, according to the general standards specified for this method. The relative standard deviations (RSD) for acquired absolute values were calculated and they all were under 1.00%. The average values of hydrophobicity parameters (R_M^0, C_0) obtained in these investigations are presented in Table 1.

The ACE inhibitors molecular lipophilicity descriptors – log*P* values were calculated using software package Virtual Computational Chemistry Laboratory [21]. Experimentally determined $logP_{O/W}$ ($logP_{Octanol/Water}$) values of examined ACE inhibitors were obtained from the Clarke's Analysis of Drugs and Poisons [20]. The different log*P* values of investigated ACE inhibitors were calculated using the software package Virtual Computational Chemistry Laboratory. In our previously published study [18] the selection of $logP_{KOWWIN}$ values was evaluated on the basis of its best agreement with experimental, from literature obtained $logP_{O/W}$ values (R^2=0,999). The ACE inhibitors PPB data were collected from relevant references [5, 20]. The $logP_{KOWWIN}$ and $logP_{O/W}$ values of investigated ACE inhibitors are presented in Figure 1.

The Microsoft Excel 2003 was used to perform the statistical analysis of regression.

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RESULTS

The protein binding data of investigated ACE inhibitors varied from negligible (lisinopril), through 24% (cilazapril), 55% (enalapril), 97% (quinapril) to 99% (fosinopril). The calculated logP_{KOWWIN} values ranged from -0.94 (lisinopril) to 6.61 (fosinopril).

In the first stage of the study, chromatographically established hydrophobicity parameters C_0, which are generally accepted as a reliable measure of lipophilicity were correlated with calculated logP values. The relations established between chromatographic hydrophobicity parameters (C_0) and logP_{KOWWIN} values are shown in Table 2.

Very good correlations (R^2>0.86) were obtained for all used solvent systems (as proposed: the range of R^2>0.79 in literature [25]) confirming hydrophobicity parameters C_0 obtained on cellulose layers under conditions of normal-phase thin-layer chromatography, as suitable measure of ACE inhibitors lipophilicity. Since the best correlation (R^2=0.916) was observed for cyclohexan – benzene mobile phase, hydrophobicity parameters C_0 obtained with this solvent system will be considered in further correlation.

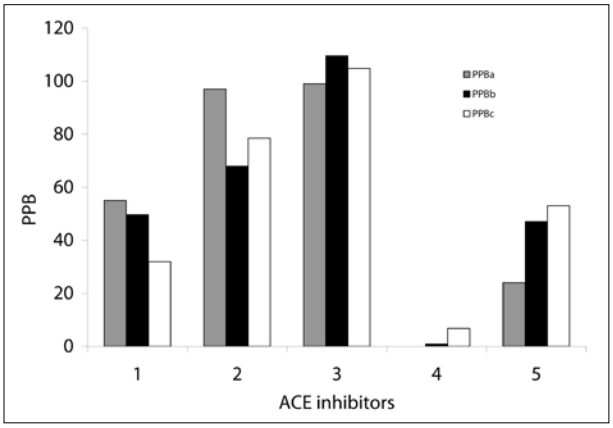
Table 2. Equations and correlation coefficients for C_0 *vs.* logP_{KOWWIN} values

Solvent system	Equation	R^2
Cyclohexan – carbon tetrachloride	$C_0 = 1.486 – 0.092 \log P_{KOWWIN}$	0.867
Cyclohexan – toluene	$C_0 = 1.387 – 0.098 \log P_{KOWWIN}$	0.864
Cyclohexan - benzene	$C_0 = 1.072 – 0.080 \log P_{KOWWIN}$	0.916

Table 3. Data of plasma protein binding (PPB) collected from relevant literature and predicted from relations with calculated logP_{KOWWIN} and chromatographic hydrophobicity parameters (C_0 values)

ACE inhibitors	PPB[a]	$PPB_{predicted}$ [b]	$PPB_{predicted}$ [c]
1	55	49.6	31.9
2	97	67.9	78.5
3	99	109.5	104.8
4	0	0.8	6.8
5	24	47.0	53.0

[a]PPB – collected from relevant literature; [b]PPB – predicted from relation with logP_{KOWWIN} values (eq. 1); [c]PPB – predicted from relation with C_0 values (eq. 2)



Graph 1. Values of plasma protein binding (PPB) collected from relevant literature (PPBa) [4] and predicted from relation (eq. 1) with calculated logP_{KOWWIN} (PPBb) or relation (eq. 2) with chromatographic hydrophobicity parameters, C_0 (PPBc)

In the next stage of this study the relationship between calculated lipophilicity (logP_{KOWWIN} values) as well as chromatographically obtained hydrophobicity parameters (C_0) and PPB data of examined ACE inhibitors was investigated. The following correlations were obtained:

PPB=14.400(±4.123) logP_{KOWWIN} + 14.364 (±15.378) (1)
n=5, R^2=0.803, F=12.200

PPB=-168.651 (±53.782) C_0 + 197.712 (±46.808) (2)
n=5, R^2=0.7662, F=9.833

The presented correlations can be considered as good, with high correlation coefficients (R^2 higher than 0.75) and acceptable F values due to a limited number of compounds. Next, the ACE inhibitors lipophilicity data, both calculated as well as chromatographically obtained could be considered as high-throughput screening techniques for the evaluation of selected compounds protein binding degree.

Even though good correlation coefficients (R^2) were obtained in both relations, unacceptable probability value with p>0.05 was found in relation between protein binding data and calculated logP_{KOWWIN} values. Subsequently, although the correlation coefficient obtained in equation 2 (R^2=0.7662) was slightly lower than R^2 obtained in equation 1 (R^2=0.803), better relationship with acceptable probability value (p<0.05) was observed between protein binding data and chromatographically obtained hydrophobicity parameters C_0.

Values of PPB degree, collected from the relevant literature as well as predicted from relations with calculated logP_{KOWWIN} (eq. 1) and hydrophobicity parameters, C_0 values (obtained under conditions of NP-TLC) (eq. 2) are presented in Table 3 and at Graph 1.

DISCUSSION

The investigation of protein binding parameters has received significant attention since its importance was recognized at the beginning of the 20th century. A number of authors suggest several *in vitro* assays that can be employed in determination of different drugs plasma protein binding degree. There are examples of separation techniques including equilibrium dialysis, ultrafiltration, ultracentrifugation and gel filtration [26, 27, 28]; recently developed chromatographic methods based on columns with immobilized human serum albumin [26, 29, 30] and capillary electrophoretic (CE) methods [27, 31, 32]. In a recently published study Ghafourian and Amin suggested *in silico* model for predicting PPB degree of compounds based on correlations with their computed molecular descriptors. They established positive effect of lipophilicity measured by calculated logP descriptor on plasma protein binding [33].

Still, most of these methods have certain limitations and a new approach for a fast, reliable and cost-effective determination of plasma protein binding should be developed.

In this research several selected, most frequently prescribed, ACE inhibitors (enalapril, quinapril, fosinopril,

lisinopril, cilazapril) (Figure 1) were studied to establish the correlation between their plasma protein binding degree and lipophilicity data, calculated logP values or experimentally obtained hydrophobicity parameters C_0 with normal-phase thin-layer chromatography on cellulose sorbent. In the first stage of this study, chromatographically obtained C_0 parameter was verified as a good measure of ACE inhibitors lipophilicity. Moreover, it was established that ACE inhibitors lipophilicity data correlate well with their plasma protein binding values. The good correlation (R^2=0.7662) with acceptable probability value ($p<0.05$) was found between hydrophobicity parameters C_0 and ACE inhibitors plasma protein binding data. The main advantage of cellulose – sorbent compared to silica gel – sorbent usually used in TLC, is that cellulose is easily available and cost-effective sorbent.

Present study can be considered as effective experimental assay which could be used as a fast, easy, cost-effective screening technique beside other previously proposed methodologies for PPB prediction. The proposed methodology has confirmed that lipophilicity is essential in drug's PPB and could be regarded as new, additional, in vitro approach appropriate for modeling of PPB with lipophilicity of the investigated group of ACE inhibitors. In addition, besides simplicity and speed, this method could be considered as the economical high-throughput technique which does not require either expensive equipment investment or extensive analytical staff training.

CONCLUSION

High-quality correlation obtained between hydrophobicity parameters C_0 and protein binding data indicate that experimental techniques such as NP-TLC method can be suitable for the estimation of ACE inhibitors PPB degree.

The proposed approach based on hydrophobicity data experimentally obtained with a fast and easy chromatographic technique is capable of screening ACE inhibitors PPB as well as new synthesized drugs and can be of great importance in drug research and development.

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Процена степена везивања инхибитора ангиотензин-конвертујућег ензима за протеине плазме применом хроматографски добијених параметара хидрофобности

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КРАТАК САДРЖАЈ

Увод Инхибитори ангиотензин-конвертујућег ензима (*ACE*) су велика група лекова изузетно значајна у лечењу хипертензије.

Циљ рада Анализирани су изабрани *ACE*-инхибитори (еналаприл, квинаприл, фозиноприл, лизиноприл, цилазаприл) ради постављања новог приступа погодног за брзу и једноставну процену везивања за протеине плазме на основу њихових параметара липофилности.

Методе рада Хроматографски параметри хидрофобности (вредности C_0) добијени су у условима нормалнофазне хроматографије (*NPTLC*) на танком слоју целулозе, уз коришћење двокомпонентних мобилних фаза. Вредности параметара липофилности *ACE*-инхибитора (*logP*) израчунате су помоћу софтверског пакета *Virtual Computational Chemistry Laboratory*. Подаци о проценту везивања *ACE*-инхибитора за протеине плазме преузети су из одговарајуће литературе.

Резултати Проценат везивања за протеине плазме испитиваних *ACE*-инхибитора био је у опсегу од 0% (лизиноприл) до 99% (фозиноприл), док су вредности израчунатих параметара липофилности (вредности $logP_{KOWWIN}$) биле од -0,94 (лизиноприл) до 6,61 (фозиноприл). Добијене су задовољавајуће корелације између вредности везивања *ACE*-инхибитора за протеине плазме и израчунатих $logP_{KOWWIN}$ вредности (коефицијент корелације R^2 био је 0,8026), као и хроматографски добијених параметара хидрофобности, C_0 (R^2=0,7662). Иако су задовољавајући коефицијенти корелације добијени у обе релације, неприхватљиве вредности вероватноће (p>0,05) добијене су за зависност између вредности везивања *ACE*-инхибитора за протеине плазме и израчунатих $logP_{KOWWIN}$ вредности. Стога се, узимајући у обзир захтев да вредности вероватноће буду ниже од 0,05, бољом може сматрати зависност између вредности везивања *ACE*-инхибитора за протеине плазме и хроматографски добијених параметара хидрофобности.

Закључак Примена хидрофобних параметара *ACE*-инхибитора експериментално добијених у условима нормалнофазне хроматографије на танком слоју целулозе за процену степена њиховог везивања за протеине плазме значајна је за развој и испитивање лекова ове групе и процену њихове биорасположивости.

Кључне речи: инхибитори ангиотензин-конвертујућег ензима (*ACE*-инхибитори); везивање за протеине плазме; липофилност

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