



AKADÉMIAI KIADÓ

Acta Chromatographica

34 (2022) 2, 130–137

DOI:

10.1556/1326.2021.00893

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ORIGINAL RESEARCH
PAPER



Estimation of lipophilicity and design of new 17 β -carboxamide glucocorticoids using RP-HPLC and quantitative structure-retention relationships analysis

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Received: January 27, 2021 • Accepted: February 19, 2021

Published online: March 30, 2021

ABSTRACT

Eight 17 β -carboxamide glucocorticoids with local anti-inflammatory activity were selected and their retention behavior tested in six RP-HPLC systems (I–VI). $\log k_w$, a , and φ_0 parameters were calculated and correlation with previously determined $\log P_o/w$ values was examined. RP-HPLC system IV, which consisted of cyano column and methanol–water mobile phases (50:50, 60:40, 70:30, and 80:20, v/v), was selected as the most reliable for lipophilicity prediction and used for the analysis of chromatographic behavior of remaining fourteen 17 β -carboxamide glucocorticoids. Quantitative structure-retention relationships analysis was performed and PLS($\log k_w$) model was selected as the most statistically significant. On the basis of selected model and interpretation of corresponding descriptors, new derivatives with higher $\log k_w$ values and higher expected lipophilicity were designed.

KEYWORDS

17 β -carboxamide glucocorticoids, lipophilicity, QSRR, drug design

INTRODUCTION

The use of traditional glucocorticoids is followed by serious local and systemic side effects. The concept of soft drug represents one of the attempts to design safe drugs with fewer side effects. Soft drugs are isosteric and isoelectronic analogs of lead molecules, which undergo controlled and predictable metabolism after exerting therapeutic action at the target site. Metabolism of soft drugs prevents off-target or long-term toxicity by using easily accessible metabolic pathways and may eliminate the possibility of drug–drug interactions by avoiding saturable and highly used metabolic pathways [1].

Soft glucocorticoids are mostly esters of cortienic acid, a metabolite with no glucocorticoid activity. Several classes of 17 β -carboxamides of cortienic acids with different amines have also been synthesized so far [2, 3]. Twenty-two 17 β -carboxamide steroids have been introduced in our laboratory. These derivatives are amino acid amides of cortienic acids obtained from five corticosteroids (hydrocortisone, prednisolone, methylprednisolone, dexamethasone, and betamethasone) with local anti-inflammatory activity on skin comparable or higher than dexamethasone [4]. Quantitative structure-permeability relationships (QSPR) analysis of *in vitro* permeability results showed significant influence of lipophilicity on estimated skin permeability [5]. Therefore, lipophilicity should be considered when designing new derivatives with improved skin permeability and, consequently, better bioavailability at the site of action.

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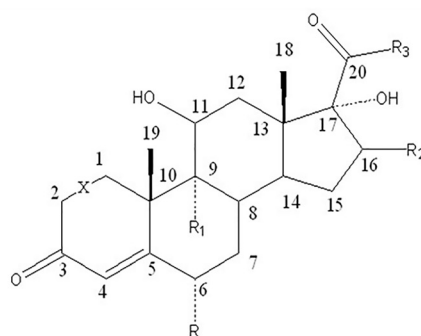
The reference method for lipophilicity ($\log P_{o/w}$) determination is the shake-flask method. Due to its disadvantages (e.g., relatively high amounts of analytes and octanol are required, the method is time-consuming and cannot be considered reliable for the compounds with $\log P_{o/w} > 4$), it has been replaced by several other methods for the lipophilicity evaluation – slow stirring method (SSM), capillary electrophoresis (CE), potentiometric titration, cyclic voltammetry (CV), capillary electrophoresis (CE), electrokinetic chromatography (EKC), thin-layer chromatography (TLC), and reversed-phase high-performance liquid chromatography (RP-HPLC). RP-HPLC represents a good alternative for the measurement of lipophilicity. It requires low amounts of tested compounds (1 mg or less) and enables reliable determination of wide range of $\log P_{o/w}$ values ($-3 < \log P_{o/w} < 8$) [6]. HPLC retention data were applied in lipophilicity estimation of various groups of drugs [7–9].

The aim of this study was to investigate the effects of different columns and mobile phase compositions on retention behavior of 17β -carboxamide glucocorticoids and select the most reliable RP-HPLC method for the estimation of their lipophilicity. The role of lipophilicity was discussed in terms of quantitative structure-retention relationships (QSRR) analysis and new derivatives with higher expected lipophilicity were designed.

EXPERIMENTAL

Chemicals and reagents

Chemical structures of tested 17β -carboxamide glucocorticoids are presented in Fig. 1. These derivatives were synthesized and structurally characterized in our laboratory [4, 5].



Compound	X	R	R ₁	R ₂	R ₃	$\log k_w$	a	φ_0
HG	-C=C-	-H	-H	-H	-NHCH ₂ COOCH ₃	1.61	-0.027	60.16
HA	-C=C-	-H	-H	-H	-NH(CH)CH ₃ COOCH ₃	1.87	-0.031	61.07
HEG	-C=C-	-H	-H	-H	-NHCH ₂ COOCH ₂ CH ₃	1.88	-0.031	61.15
HEA	-C=C-	-H	-H	-H	-NHCH ₂ CH ₂ COOCH ₂ CH ₃	2.05	-0.032	64.52
HF	-C=C-	-H	-H	-H	-NH(CH)C ₆ H ₅ COOCH ₃	2.95	-0.043	69.23
PG	-C=C-	-H	-H	-H	-NHCH ₂ COOCH ₃	1.84	-0.030	61.09
PA	-C=C-	-H	-H	-H	-NH(CH)CH ₃ COOCH ₃	2.04	-0.032	63.32
PEG	-C=C-	-H	-H	-H	-NHCH ₂ COOCH ₂ CH ₃	2.09	-0.033	63.39
PEA	-C=C-	-H	-H	-H	-NHCH ₂ CH ₂ COOCH ₂ CH ₃	2.23	-0.034	64.83
PF	-C=C-	-H	-H	-H	-NH(CH)C ₆ H ₅ COOCH ₃	3.01	-0.042	71.84
MPG	-C=C-	-CH ₃	-H	-H	-NHCH ₂ COOCH ₃	2.05	-0.032	62.97
MPA	-C=C-	-CH ₃	-H	-H	-NH(CH)CH ₃ COOCH ₃	2.25	-0.035	65.06
MPEG	-C=C-	-CH ₃	-H	-H	-NHCH ₂ COOCH ₂ CH ₃	2.29	-0.035	65.32
MPEA	-C=C-	-CH ₃	-H	-H	-NHCH ₂ CH ₂ COOCH ₂ CH ₃	2.47	-0.037	66.46
MPF	-C=C-	-CH ₃	-H	-H	-NH(CH)C ₆ H ₅ COOCH ₃	3.46	-0.047	73.09
DG	-C=C-	-H	-F	— CH ₃	-NHCH ₂ COOCH ₃	1.98	-0.031	63.94
DA	-C=C-	-H	-F	— CH ₃	-NH(CH)CH ₃ COOCH ₃	2.31	-0.036	64.76
DEG	-C=C-	-H	-F	— CH ₃	-NHCH ₂ COOCH ₂ CH ₃	2.27	-0.034	66.04
DEA	-C=C-	-H	-F	— CH ₃	-NHCH ₂ CH ₂ COOCH ₂ CH ₃	2.52	-0.038	66.25
DF	-C=C-	-H	-F	— CH ₃	-NH(CH)C ₆ H ₅ COOCH ₃	3.40	-0.047	72.51
BG	-C=C-	-H	-F	— CH ₃	-NHCH ₂ COOCH ₃	2.08	-0.033	62.19
BEG	-C=C-	-H	-F	— CH ₃	-NHCH ₂ COOCH ₂ CH ₃	2.33	-0.035	66.36

Fig. 1. Chemical structures and calculated chromatography parameters of tested compounds



Acetonitril and methanol HPLC purity (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), as well as deionized water (TKA water purification system, Niederelbert, Germany) were used in RP-HPLC analyses.

RP-HPLC analyses

RP-HPLC analyses were performed on HP1100 chromatograph using C8 (Zorbax Eclipse XDB-C8 150 × 4.6 mm; 5 μm particle size), phenyl (Zorbax Eclipse XDB-phenyl 150 × 4.6 mm; 5 μm particle size) and cyano columns (Zorbax SB-CN 150 × 4.6 mm, 5 μm particle size). Mobile phases were binary combinations of methanol and water, as well as of acetonitrile and water, in different ratios (Table 1). All compounds were firstly dissolved in dimethyl sulfoxide (1 mg/mL) and then diluted with the mobile phase to obtain final solutions (0.01 mg/mL), which were injected in triplicates. Logarithmic values of retention factors ($\log k$) were calculated for each compound and plotted with the percentage of organic solvent in the mobile phase. Chromatography parameters $\log kw$, a , and φ_0 were determined using Eq. (1) and Eq. (2) (S – percentage of organic solvent in the mobile phase).

$$\log k = \log kw + a \cdot S \quad (1)$$

$$\varphi_0 = -\log kw/a \quad (2)$$

Quantitative structure-retention relationships (QSRR) analysis

Descriptor selection as well as multiple linear regression (MLR), partial least squares (PLS), and support vector machine (SVM) modeling were performed in Statistica 13 [10].

Initial 3D structure optimization was performed using the “Fine build” 3D structure refinement procedure in MarvinSketch program [11]. Minimization of geometry of tested compounds was performed using Gaussian semi-empirical/PM3 method and molecular descriptors were calculated in Dragon software [12]. Finally, intercorrelation test was performed in order to prepare appropriate sets of molecular descriptors for the QSRR analysis. Pairs of descriptors with intercorrelation higher than 0.90 were examined and those with stronger influence on dependent variable ($\log kw$) were retained for final selection. Final selection of descriptors could be performed using several methods, such as genetic algorithm [13], principal component analysis [14] or stepwise MLR [5, 15]. In this study, STATISTICA’s algorithm implemented in feature selection

and variable screening (FSVS) was applied for the selection of variables for MLR and SVM modeling (number of cuts was set to 10 and following descriptors were selected: nBM, LOC, and F05[C–O]). For the PLS modeling, no further descriptor selection was performed (only refinement of those with intercorrelation higher than 0.90 was done) and 443 descriptors were used.

Test set for MLR($\log kw$), SVM($\log kw$), and PLS($\log kw$) model building consisted of DEA, DF, HEG, HF, MPEG, and PA, whereas other compounds were in the training set.

MLR have been applied to assess linear relationship between calculated molecular descriptors and $\log kw$. MLR($\log kw$) model was created using forward stepwise multiple regression analysis with following criteria: F to enter = 5 and F to remove = 1.

SVM was initially developed as a binary classification tool [16]. It can also be used as a method in QSAR and QSPR modeling [17, 18]. In this study, optimal SVM ($\log kw$) model was obtained using radial basis function (RBF) Kernel type and regression type 1 was also selected. Gamma value was optimized on the basis of S.D. ratio for training and test set values and was set to 0.25. Subsequently, capacity (C), and epsilon (\mathcal{E}), as parameters that control model building process to obtain the least regression error, were automatically optimized by the software and optimal values were: $C = 9$ and $\mathcal{E} = 0.1$. Finally, optimal SVM($\log kw$) consisted of 11 supported vectors (6 bounded).

PLS as a modeling tool is particularly useful when dealing with collinear, noisy, and numerous descriptors. Optimal number of components was selected on the basis of each component’s $R^2(Y)$ value and cumulative $R^2(Y)$ value, which takes into account contribution of all components to the PLS model. In this study, optimal PLS($\log kw$) model consisted of one component and the most influential descriptors (with highest scaled regression coefficient values) were analyzed.

Evaluation of quality of the created QSRR models was performed on the basis of following parameters: RMSEE (root mean squared error of estimation), RMSEP (root mean squared error of prediction), r , Q^2 , R^2_{pred} , F ratio, and P value.

RMSEE value was calculated for the training, whereas RMSEP for the test set. Q^2 is used to evaluate predictive capacity of the created model for compounds which are similar to those from the training set. This parameter is calculated using the leave-one-out (LOO) procedure. Briefly, each compound of the training set was deleted once and the remaining ones were used to create a model. Thus formed model was used to predict dependent variable ($\log kw$) value of the deleted compound. This procedure is repeated so all

Table 1. RP-HPLC systems tested in this study

System	Column and mobile phase
I	C8 column, mobile phases: acetonitrile–water = 30:70, 40:60, 50:50, and 60:40, v/v
II	C8 column, mobile phases: methanol–water = 50:50, 60:40, 70:30, and 80:20, v/v
III	Cyano column, mobile phases: acetonitrile–water = 30:70, 40:60, 50:50, and 60:40, v/v
IV	Cyano column, mobile phases: methanol–water = 50:50, 60:40, 70:30, and 80:20, v/v
V	Phenyl column, mobile phases: acetonitrile–water = 40:60, 50:50, 60:40, and 70:30, v/v
VI	Phenyl column, mobile phases: methanol–water = 60:40, 70:30, 80:20, and 90:10, v/v



the training set compounds were deleted once [19, 20]. Contrary to Q^2 , R^2_{pred} is an external validation parameter used to evaluate predictive capacity of the created model for compounds structurally different from those belonging to the training set [21]. Q^2 and R^2_{pred} values of models with good predictive capacity are higher than 0.5 [19, 22–24]. The F -test is based on the ratio MS Regression/MS Residual and represents significance of the created model. The P -value indicates probability level where a model with this F value may be the result of just chance. Statistically significant models are those with P -value lower than 0.05 [23].

RESULTS AND DISCUSSION

RP-HPLC analysis

Lipophilicity represents one of the key physico-chemical properties of drug molecule responsible for its solubility, permeability, binding to blood plasma proteins, interactions with structurally complement receptors and toxicity. In this study RP-HPLC method was applied, recognized as a mainstream experimental method for lipophilicity determination. The parameters that are the most frequently used for lipophilicity estimation are $\log k$ (determined using one ratio between organic and aqueous phase) [7, 9] and $\log kw$ (determined by extrapolation technique, when at least four different ratios between organic and aqueous phase are used) [8]. As lipophilicity descriptors, other RP-HPLC parameters can also be used – a and ϕ_0 [25–27]. Several papers have been published so far dealing with the utilization of chromatography techniques (TLC, HPTLC, and HPLC) for the estimation of lipophilicity of compounds having steroidal structure [28–30]. Maes et al. found good correlation between $\log Po/w$ and $\log k$, determined in an RP-HPLC system consisting of C18 column and phosphate buffer/methanol (35:65, v/v) and concluded that this system is suitable for the estimation of lipophilicity of 17 β -carboxamide steroids [28]. However, these authors did not test any other RP-HPLC system and there are no data on the influence of stationary phase and mobile phase composition on retention of this class of compounds.

Suitability of RP-HPLC systems I–VI (Table 1) for the estimation of lipophilicity of 17 β -carboxamide glucocorticoids was evaluated on the basis of correlation coefficients between chromatography parameters ($\log kw$, a , and ϕ_0) and $\log Po/w$ values, previously determined in our laboratory using shake-flask method [31]. Stationary phases used in this study (C8, cyano, and phenyl) were selected to enable investigation of various types of interactions with tested compounds (C8 was used instead of C18 to mimic hydrocarbon chain of octanol). For this purpose, representative set of eight 17 β -carboxamide glucocorticoids was formed, consisting of derivatives of all corticoid acids and amino acids used for the synthesis of these compounds (HG, DG, HEA, DEG, BEG, PF, MPF, and DF). Correlation coefficients (r) between chromatography parameters ($\log kw$, a , and ϕ_0) determined in systems IV, V, and VI, and $\log Po/w$

values were higher than 0.96. In systems I, II, and III, high correlation was observed between $\log kw$ and $\log Po/w$, as well as between ϕ_0 and $\log Po/w$ ($r > 0.96$), whereas correlation between a and $\log Po/w$ were low ($r < 0.76$). Due to the highest correlation coefficients between chromatography parameters ($\log kw$, a , and ϕ_0) determined in system IV and $\log Po/w$ ($r > 0.98$), this system (cyano column and methanol–water mobile phases (50:50, 60:40, 70:30, and 80:20, v/v)) was selected as the most reliable for lipophilicity prediction of 17 β -carboxamide glucocorticoids. Retention behavior of remaining fourteen 17 β -carboxamide glucocorticoids was also tested in system IV and chromatography parameters $\log kw$, a , and ϕ_0 were calculated (Fig. 1).

The highest values of chromatography parameters were observed for methyl ester L-phenylalanine derivatives (HF, PF, MPF, and DF), from which the highest lipophilicity can be expected. Compounds with the lowest values of chromatography parameters and lowest expected lipophilicity were methyl and ethyl ester glycine, as well as methyl ester L-alanine derivatives (HG, HEG, HA, PG, MPG, DG, and BG). These results show stronger influence of the structure of C17 β side chain than of the steroid ring and confirm the importance of careful selection of amino acids to be used for synthesis of derivatives with optimal lipophilicity for local application. Additionally, correlation between chromatography parameters was investigated and high correlation coefficients between pairs of parameters ($r > 0.95$) indicate that tested compounds possess high degree of structural similarity and similar mechanisms influence their chromatographic behavior.

Molecular descriptor interpretation and design of new compounds

In order to identify structural properties of tested molecules with the highest influence on their retention behavior, QSRR analysis was performed, including descriptor selection, MLR, PLS, and SVM modeling. Statistical parameters calculated for MLR($\log kw$), PLS($\log kw$), and SVM($\log kw$) are presented in Table 2.

All three models have low root mean squared errors of estimation and prediction, calculated for training and test set respectively (RMSEE and RMSEP), similar values of correlation coefficients r (calculated for test set), as well as high values of Q^2 and R^2_{pred} (higher than 0.5, indicating their potential for reliable prediction of $\log kw$ for compounds structurally similar or different than those from the training set). Although all three models could be considered reliable, PLS($\log kw$) was outlined due to the highest value of Q^2 and was used for the prediction of $\log kw$ of newly designed compounds. Low ratio between RMSEE and RMSEP (≤ 2), as well as similar Q^2 and R^2_{pred} values indicate that this model enables reliable prediction of $\log kw$ for training set compounds and compounds chemically different than them and also indicate that the model is not overfitted.

According to this model, descriptors with the highest positive influence on $\log k_w$ are MW, nBM, QXXm, RDF070m, H3m, and F05[C–O].



Table 2. Molecular descriptors and calculated statistical parameters of MLR($\log k_w$), PLS($\log k_w$) and SVM($\log k_w$) models

Model	Regression equations/selected descriptors	Training set					Test set		
		RMSEE	Q ²	R ²	F	P	RMSEP	r	R ² _{pred}
MLR($\log k_w$)	$\log k_w = -0.792457 + 0.286921 \cdot \text{nBM} + 0.976017 \cdot \text{LOC}^a$	0.16	0.57	0.85	43.45	0.000002	0.11	0.98	0.96
PLS($\log k_w$)	$\log k_w = f(\text{MW}, \text{QXXm}, \text{RDF070m}, \text{H3m}, \text{O}\%, \text{Mor08m}, \text{Mor08p}, \text{LLS_02}, \text{F05[C-O]}, \text{nBM})^b$	0.10	0.86	-	-	-	0.20	0.95	0.88
SVM($\log k_w$)	$\log k_w = f(\text{nBM}, \text{LOC}, \text{F05[C-O]})^a$	0.15	0.74	-	-	-	0.17	0.96	0.92

^a nBM - number of multiple bonds; LOC - lopping centric index; F05[C-O] - frequency of C-O at topological distance 5.

^b MW - molecular weight; QXXm - quadrupole x-component value/weighted by mass; RDF070m - Radial Distribution Function - 070/weighted by mass; H3m - H autocorrelation of lag 3/weighted by mass; O% - percentage of O atoms; Mor08m - signal 08/weighted by mass; Mor08p - signal 08/weighted by polarizability; LLS_02 - modified lead-like score from Monge et al. (8 rules); F05[C-O] - frequency of C-O at topological distance 5; nBM - number of multiple bonds.

nBM is number of multiple bonds, whereas MW is molecular weight. Both descriptors belong to constitutional indices. Therefore, the increase in $\log k_w$ could be expected if aromatic rings are introduced in the structure of 17 β -carboxamide glucocorticoids (synthesis of amides of cortienic acid and aromatic amino acids, such as L-tryptophan and L-histidine).

RDF070m is Radial Distribution Function - 070/weighted by mass and belongs to RDF descriptors. This group of descriptors are related to steric factors at specified radius from the geometrical center of the molecule [32]. In case of RDF070m, that distance is 7 Å, which corresponds to the space filled by C17 β side chain (Fig. 2). The highest values of this descriptor were calculated for HF, PF, MPF, DF, and HEA. These derivatives contain methyl ester of L-phenylalanine and ethyl ester of β -alanine in C17 β side chain. The lowest values of this descriptor were calculated for compounds with glycine and L-alanine in C17 β side chain. Therefore, it can be expected that introduction of voluminous or long C17 β side chain should increase $\log k_w$.

QXXm (quadrupole x-component value/weighted by mass) belongs to geometrical descriptors. It represents molecular shape including the second-order-based geometric moments of the property field density, obtained by mapping the atomic masses at the atomic position. QXXm values depend on conformational changes and this descriptor could be used for the differentiation of stereoisomers [33, 34]. The highest values of this descriptor were calculated for

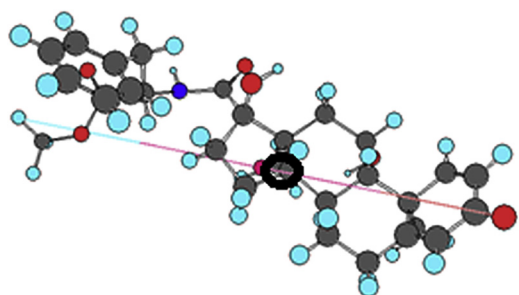


Fig. 2. 3D structure of PF with geometrical center and specified distance (7 Å) from it

derivatives of L-phenylalanine (HF, PF, MPF, and DF) and MPEA. When analyzing derivatives of different steroids, the highest values of QXXm were calculated for methylprednisolone derivatives. Therefore, designed derivatives with voluminous or long C17 β side chains should have high QXXm values, and consequently, $\log k_w$ should be increased.

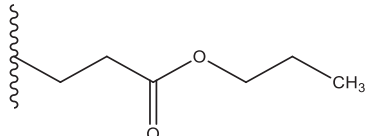
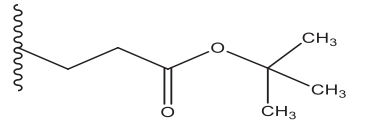
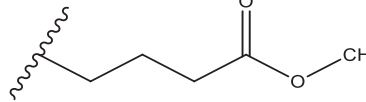
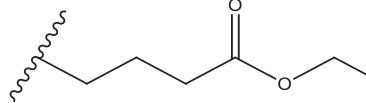
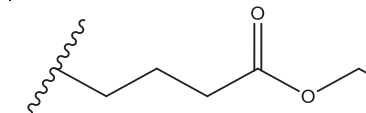
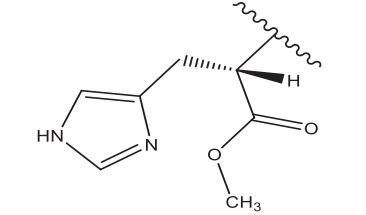
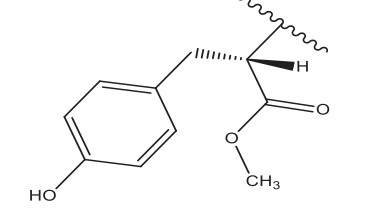
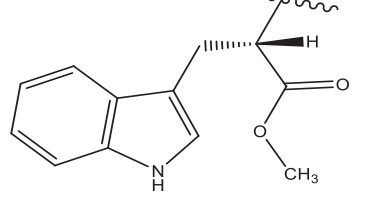
H3m (H autocorrelation of lag 3/weighted by mass) is a GETAWAY descriptor. These descriptors are related to the atomic masses of a molecule and calculated from the elements of the leverage matrix obtained by centered atomic coordinates. They are defined by applying some traditional matrix operators, concepts of the information theory and spatial autocorrelation formulas, weighting the molecule atoms in such a way as to account for atomic mass [35].

F05[C-O] is frequency of C-O at topological distance 5 (2D atom pairs descriptor). Topological distances are the shortest distances (expressed as number of chemical bonds) between C and O atoms [36]. The highest values of this descriptor are calculated for derivatives of methyl ester of L-phenylalanine (HF, PF, MPF, and DF). Detailed analysis of their structure indicates that high value of this descriptor is due to numerous C-O fragments with 5 bonds between these atoms. These fragments are formed between C atoms in L-phenylalanine ring and neighboring O atoms (such as -OH group at C17, as well as C-O and C=O groups in C17 β side chain). Therefore, introduction of other rings in C17 β side chain should increase F05[C-O] and $\log k_w$.

Descriptors with the highest negative influence on $\log k_w$ are O%, Mor08m, Mor08p, and LLS_02.

Mor08m and Mor08p are 3D-MorSE descriptors (Mor08m is signal 08/weighted by mass, whereas Mor08p is signal 08/weighted by polarizability). Although each 3D-MorSE descriptor captures the whole structure, its final value is usually result of short-distance atomic pairs (up to 3). Therefore, there is no need to analyze all possible atomic binary combinations - it is enough to check the closest neighbors [37]. Both Mor08m and Mor08p have negative values. Therefore, the lower descriptor value, the higher $\log k_w$ is calculated. The lowest values of these descriptors are calculated for methyl ester of L-phenylalanine derivatives. It can be assumed that this is due to numerous short C-C fragments that can be found in their C17 β side chains and

Table 3. Designed compounds and predicted \log_{kw} values

Compound	R	Predicted \log_{kw}
MPPA		2.46
MPTBA		2.63
MPMGB		2.34
MPEGB		2.43
MPPGB		2.54
MPHIS		2.88
MPTYR		3.27
MPTRP		3.43

introduction of similar groups should result in low descriptor values and high \logkw .

LLS_02 is modified lead-like score from Monge et al. [38]. This is one of drug-like indices descriptors and consists of 8 rules:

- H-bond donors ≤ 5 ;
- H-bond acceptors ≤ 9 ;
- molecular weight (MW) ≤ 460 ;
- Moriguchi's logP (MLogP) in the range from -4.0 to 4.2 ;
- rotatable bond number (RBN) ≤ 10 ;
- number of rings (nCIC) ≤ 4 ;
- number of halogens (nX) ≤ 7 ;
- total number of N + O ≥ 1 .

Molecules that obey all rules have LLS_02 = 1. The more rules are violated, the lower value of LLS_02 is calculated. Derivatives of methyl ester of L-phenylalanine violate two rules (MW ≤ 460 and number of rings ≤ 4). Therefore, in order to decrease LLS_02 and increase \logkw values, new derivatives should be designed to violate some of above-mentioned rules. However, attention is required since in this way drug likeness of designed compounds could be significantly impaired.

On the basis of PLS(\logkw) descriptor interpretations, new derivatives were designed and their \logkw values predicted using this model. Designed compounds are amides of methylprednisolone-derived corticoid acid and amino acids with aromatic rings, long, and voluminous side chains (Table 3).

Predicted \logkw values of designed derivatives are higher than experimentally obtained \logkw of majority of tested compounds. The highest values of \logkw were predicted for the derivatives with aromatic amino acids in C17 β side chain (methyl ester of L-histidine – MPHIS, methyl ester of L-tyrosine – MPYR, and methyl ester of L-tryptophan – MPTRP).

CONCLUSION

Eight 17 β -carboxamide glucocorticoids were selected and their retention behavior was tested in six RP-HPLC systems (I, II, III, IV, V, and VI). The most suitable system was IV (cyano column and methanol–water mobile phases), due to the highest correlation coefficients between chromatography parameters \logkw , a , and ϕ_0 and previously determined \logPo/w . Selected RP-HPLC system was then used for the investigation of retention behavior of remaining fourteen 17 β -carboxamide glucocorticoids. The highest values of chromatography parameters and highest expected lipophilicity were observed for methyl ester L-phenylalanine derivatives (HF, PF, MPF, and DF). Compounds with the lowest values of chromatography parameters and lowest expected lipophilicity were methyl and ethyl ester glycine, as well as methyl ester L-alanine derivatives (HG, HEG, HA, PG, MPG, DG, and BG). Quantitative structure-retention relationships analysis was performed in order to identify structural properties of tested molecules with highest

influence on their retention behavior. As the most reliable, PLS(\logkw) model was selected and descriptors with highest positive (MW, nBM, QXXm, RDF070m, H3m, and F05[C–O]) and negative influence (O%, Mor08m, Mor08p, and LLS_02) on \logkw were analyzed. Based on this model, new derivatives from which higher \logkw and lipophilicity can be expected were designed.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-9/2021-14/200161.

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