Effect of the Lipophilicity of Endocannabinoid Peptides on QSAR Studies in the Modulation at CB1 Receptors

Juan Sebastián Hernández[1], Silvana Pinheiro[1,2], William J. Zamora[1,2,3*]

1 CBio3 Laboratory, School of Chemistry, University of Costa Rica, San Pedro, San José, Costa Rica.
2 Laboratory of Computational Toxicology, Biological Testing Laboratory (LEBi), University of Costa Rica, San Pedro, San José, Costa Rica
3 Advanced Computing Lab (CNCA), National High Technology Center (CeNAT-CONARE), Pavas, San José, Costa Rica.

Abstract

The endocannabinoid (EC) system is a neuromodulatory pathway that consists of two G protein-coupled receptors, CB1 and CB2 which can be activated by a distinct and diverse number of compounds such as *N*-arachidonoylglycerol (2-AG), anandamide, phytocannabinoids, and a series of peptides derived from hemoglobin known as endocababionod peptides (pepcans). Here, we analyzed the impact of several hydrophobicity scales of amino acids for describing the lipophilicity of seven endocannabinoid peptides in order to perform QSAR studies that correlate the lipophilicity of peptides with the experimental inhibitor constants to CB1 human receptors. The results indicate that the use of a state-of-the-art lipophilicity scale (*ProtL* scale) for describing the lipophilicity of peptides which takes into account the local-context dependency of the conformations of peptides but also the pH of the environment outperforms the results obtained with other experimental scales which only consider sequence-based information. Finally, our findings point out that is more valuable to know when and where to use a lipophilicity scale, instead of evaluating which is better or not, as they all have useful information on particle conditions and environments.

Keywords

Lipophilicity, Hydrophobicity, Endocannabinoid Peptides, Hemopressin Peptides, Pepcans, Amino Acids, QSAR, Solvation

Introduction

Neuronal communication is the foundation of complex brain function where synapses transmit specific signaling patterns through the release of neurotransmitters and neuromodulators, thus, leading to changes in the membrane potentials of neurons. Signaling mechanisms are primarily located on the synapse, and are mediated by the molecular components in the synaptic cleft [1], [2]. The endocannabinoid (EC) system is a neuromodulatory pathway that consists of two G protein-coupled receptors, CB1 and CB2 [3] which are activated by two endogenous ligands: *N*-arachidonoylglycerol (2-AG), and 2-arachidonoyl phosphatidylethanolamine (AEA), also known as anandamide[4], [5]. In addition, this system is regulated by two enzymes, fatty acid amide hydrolase (FAAH), and monoacylglyceride lipase (MAGL) [6]. Unlike other neuromodulators, the EC system works through retrograde signaling, in which messengers are released from the postsynaptic neuron to the presynaptic neuron. As a result, the postsynaptic neuron can exert both short-term and long-term control over the presynaptic neuron[7] .The EC system has received significant attention due to its role in regulating emotional state, metabolism, learning, brain plasticity, and brain development[8]–[12].

Since the isolation and identification of the phytocannabinoid ∆9-THC in 1964, over 113 related structures have been found in Cannabis sativa extracts. The lipophilic properties of these molecules led to the discovery of endogenous ligands with similar mechanisms of action as those found in phytocannabinoids[13]. As endogenous ligands, AEA has a higher affinity for the CB1 receptor as a partial agonist, while 2-AG has higher potency for both receptors as a full agonist[14]. It has been demonstrated that both, endogenous and exogenous cannabinoid-like structures can affect receptors other than CB1 and CB2, such as ion channels and others [15]. Therefore, developing structures with high selectivity is a challenge in cannabinoid research. As a result, a growing number of cannabinoid-like molecules have been discovered, including strong agonists and

inverse agonists such as rimonabant[16], [17], although their mechanism remains to be explored.

Pepcans are endocannabinoid peptides derived from hemoglobin that mostly act as allosteric modulators of endocannabinoid receptors (see Figure 1). Hemopressin Hp (PVNFKFLSH) or Pepcan-9 is a nonapeptide discovered in mouse brain extracts This peptide is an inverse agonist of the CB1 receptor with antinociceptive properties. Interestingly, the activity of pepcans varies greatly depending on their length[17].

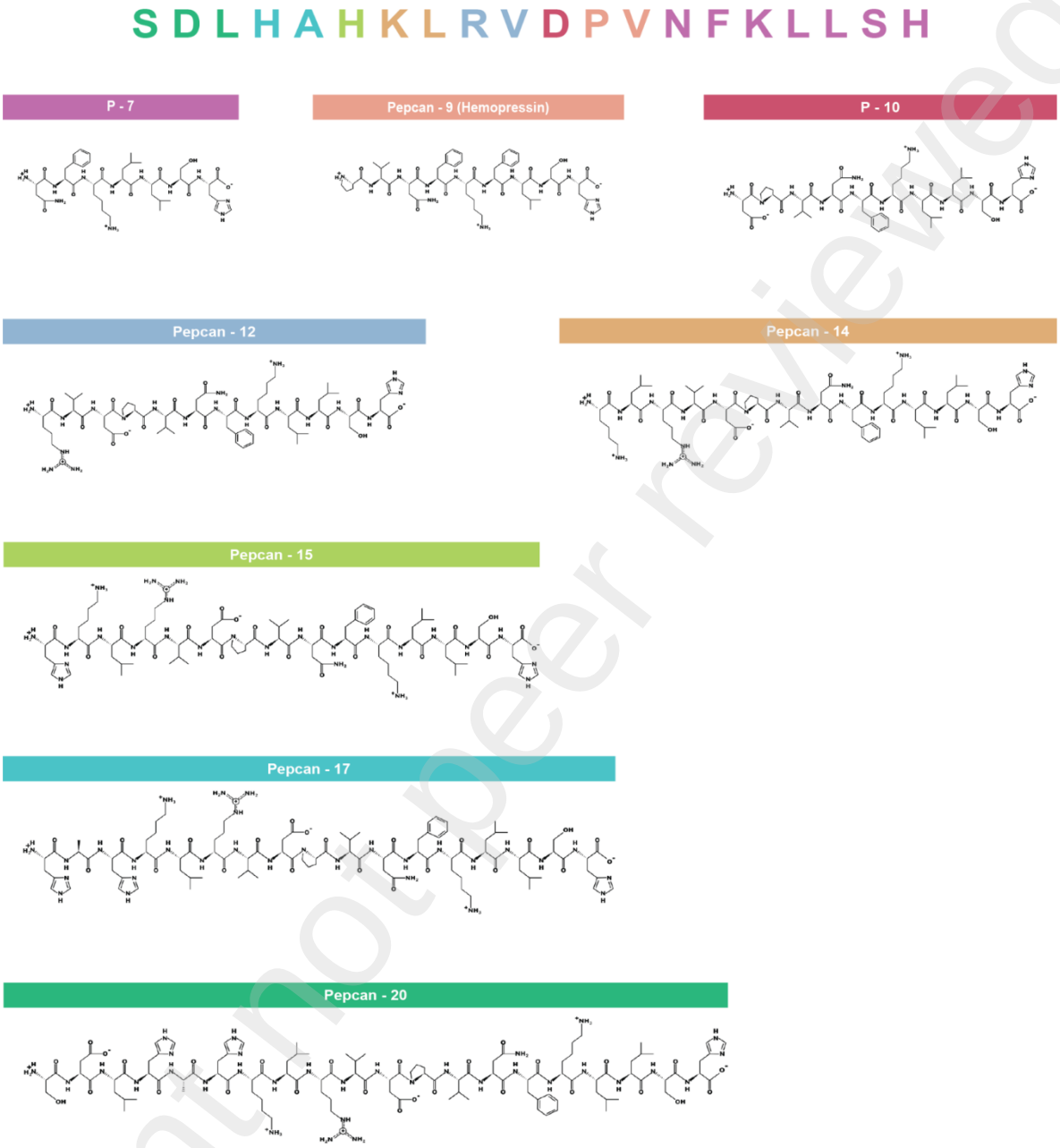


Figure 1. Structures and sequences of hemopressin peptides also called endocannabinoid peptides (pepcans).

In addition, their ability to work as negative allosteric modulators may overcome the selectivity issues observed with orthosteric ligands like cannabinoid-like molecules. Therefore, the discovery of pepcans has contributed to the research of new mechanisms of action of chemical structures different from the core found in phytocannabinoids, and endogenous ligands [18], [19]. Importantly, these new structures may hold the potential to modulate the EC system without the psychoactive effects.

The results reported by Emendato, et al.[20] provided the structural basis to understand the mechanisms by which both Pepcan-9 (Hemopressin) and Pepcan-12 act as allosteric modulators of the cannabinoid receptors in aqueous environments. Using NMR, the conformation of these endocannabinoid peptides were analyzed to evaluate their biological activity and tendency to aggregate. It was found that in low polarity environments, they can adopt α-helical structures that are similar to the native conformation of the N-terminal region of the helix G of hemoglobin. At acidic pH, both peptides have a helical region at the C-terminus, while at neutral pH, the helical region is longer in Pepcan-12 but shorter in Pepcan-9. Docking studies have shown that Pepcan-9 can bind efficiently to CB1 in a bioactive conformation, similar to the rimonabant [18]. There are identified two possible binding sites on the receptor surface, however, the precise mode of interaction between Pepcan-12 and CB1 remains to be analyzed. In contrast, no aggregation or precipitation was observed under any conditions previously tested, highlighting the importance of modifying Pepcan-9 to stabilize the active conformation [21], [22]. This information is useful to further advance the development of selective peptide-based ligands for CB1.

Lipophilicity is a fundamental descriptor for the evaluation of biomolecular interactions [23], [24] and its contribution in improving the predictions of molecular docking in small molecules [25], binding of peptides to receptors [26], and binding protein interfaces [27] has been evidenced in the literature. However, to the best of our knowledge, there is no study on the hydrophobicity of pepcans and its influence on their ability to act as inhibitors of CB1 receptors. In this study, our aim is to evaluate impact of several hydrophobicity scales of amino acids for describing the lipophilicity of endocannabinoid peptides in order

to perform QSAR studies that correlate the lipophilicity of peptides with the experimental inhibitor constants to CB1 human receptors.

Methods

Dataset

The peptides used in this study are listed in Table 1 together with their experimental inhibitor constants - expressed as logK_i - to CB_1 receptors. Five peptides correspond to hemopressin peptides derived from the cleave of hemoglobin α-chain (RVD-Hp or pepcan-12, pepcan-14, pepcan-15, pepcan-17, and pepcan-20) whereas two peptides are C-terminally truncated versions of the RVD-Hp peptide (p-7 and p-10). The experimental inhibitor constants were obtained from pepcan-induced pepcan-F4 displacement from human CB_1 receptors previously determined by Bauer et al [14].

Table 1. Sequence and Experimental Pepcan-induced Pepcan-F4 Displacement from Human CB_1 Receptors of the Seven Model Peptides.

Peptide	Sequence	logK_i
p-7	RVDPVNF	4.00
p-10	RVDPVNFKLL	3.43
pepcan-12	RVDPVNFKLLSH	1.40
pepcan-14	KLRVDPVNFKLLSH	1.26
pepcan-15	HKLRVDPVNFKLLSH	0.30
pepcan-17	HAHKLRVDPVNFKLLSH	1.46
pepcan-20	SDLHAHKLRVDPVNFKLLSH	4.00

Structure Modelling

Peptide structures were modelled using the ColabFold Server which gives accelerated prediction of peptide structures by combining the homology search of MMseqs2 with AlphaFold2 [28]. The resulting structural models - rank 1 model for each peptide- were saved in their PDB formats for physicochemical properties calculations.

In order to compare the results with the previous models, the seven peptides were extracted from the helix G of human haemoglobin in the deoxy form (PDB code: 2DN2) in this biological environment.

Property Predictions

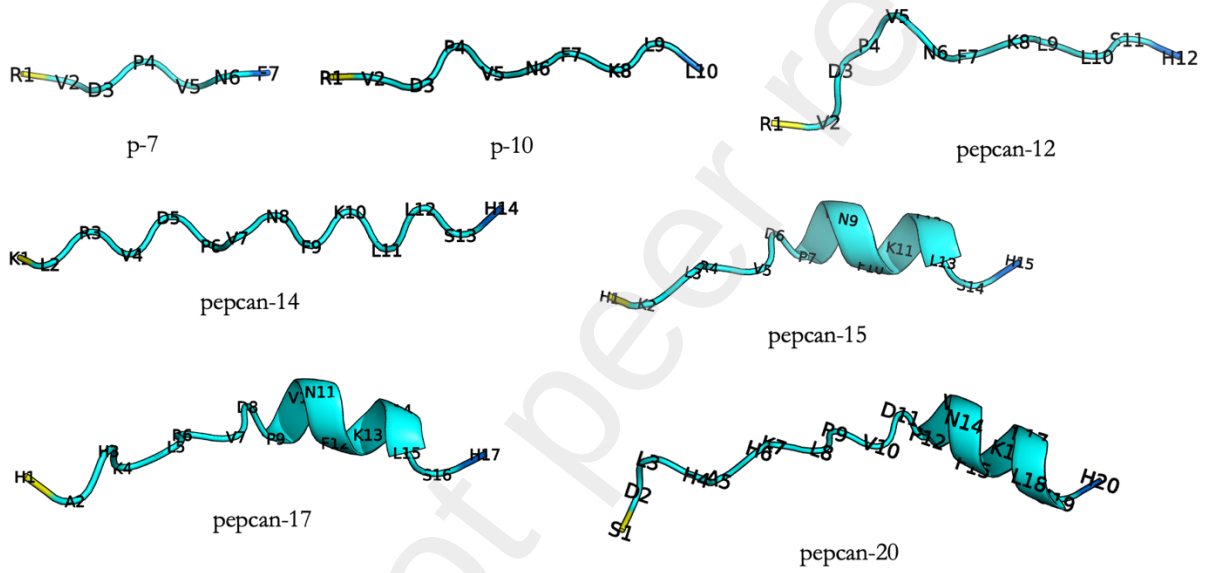
Computable physicochemical properties from the amino acid sequence such as net charge and hydrophobicity were computed using the Peptides package of R software [29]. Structural models of the seven peptides were used in order to compute: i) the accessible area of a molecule using the program Naccess [30] and ii) the context-dependent lipophilicity of the peptides using the ProtL scale which is a pH-dependent and structure-based lipophilicity scale based on the IEFPCM/MST continuum solvation method where the lipophilicity of each amino acid is calculated taking into account its specific structural features and the pH of the medium [31].

Quantitative structure-activity relationship (QSAR) of peptides and their experimental inhibitor constants to CB1 receptors

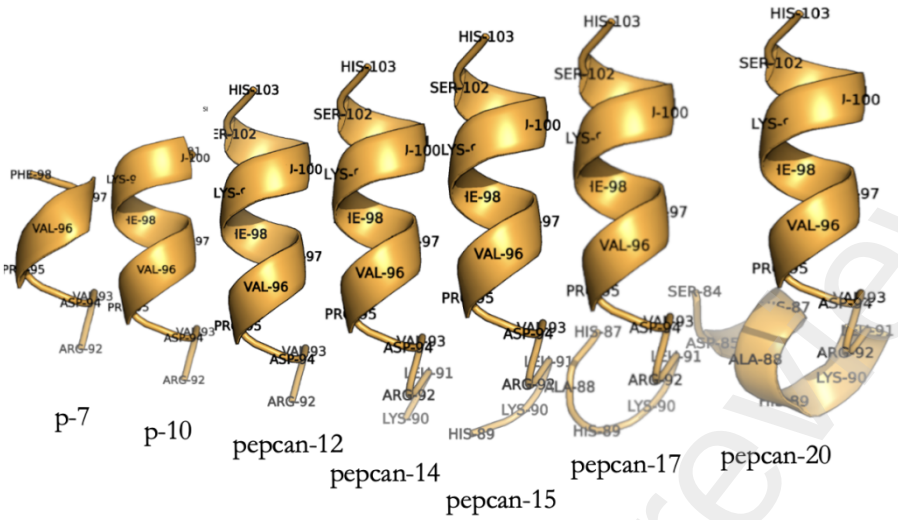
QSAR modeling was performed using the computable physicochemical properties from the amino acid sequence mentioned above. On the other hand, 3D-QSAR studies were achieved using both the accessible area and the context-dependent lipophilicity of the peptides. The endpoint in the QSAR studies was the experimental pepcan-induced pepcan-F4 displacement from CB_1 receptors expressed as $\log K_i$ [14].

Results and Discussion

The peptide models obtained of the ColabFold Server (cyan cartoon models) as well as those extracted of human haemoglobin (orange cartoon models) for the seven peptides used in this study - five peptides derived of hemopressin peptides (RVD-Hp or pepcan-12, pepcan-14, pepcan-15, pepcan-17, and pepcan-20) and two peptides corresponding to C-terminally truncated versions of the RVD-Hp peptide (p-7 and p-10) – are show in Figure 2.



(a)



(b)

Figure 2. Cartoon representation of the tridimensional model structures of seven peptides obtained from (a) ColabFold Server (N-terminal is marked in yellow and C-terminal in blue) and (b) helix G of human haemoglobin in the deoxy form (PDB code: 2DN2).

The predicted models show well-established turn regions, which is very useful for the objective of this work because it has been found that regular turn structures in the central portion of Hp (PVNFKFLSH) and PVNFKF are critical for an effective interaction with the receptor CB1 [32]. Let us note that pepcan-15, pepcan-17 and pepcan-20 the α-helical domains are present, which goes in line with what was found in low polarity environments for pepcan peptides where they can adopt α-helical structures that are similar to the native conformation of the N-terminal region of the helix G of hemoglobin [17] (see Figure 2b).

Both the sequences and the peptide structural models are necessary to perform quantitative structure-activity relationship (QSAR) using one- and three-dimensional descriptors, respectively. Therefore, for our purposes here, two different approaches were applied to correlate with the experimental inhibitor constants of the peptides towards CB1 human receptors (see Table 1). The first one uses non-hydrophobic descriptors such as net charge and solvent accessible surface area of peptides (see Table S1) using the

seven peptides but also discarding the pepcan-20 in view of the fact that it has been reported that this kind of longer pepcans - more than 17 amino acids – are not able to interact with CB1 receptors and are considered to be precursors to the shorter length pepcan peptides [14], [33].

Table 2 shows the Pearson correlation coefficient (*r*) of the net charge of peptides and the solvent-accessible surface area using both the models extracted from the human hemoglobin structural models and ColabFold structural models. The net charge represents a discrete numeric variable and although in general, it indicates that the peptides with the lowest net charge are the least active, it does not show a linear trend (see Fig. S1). On the contrary, as can be seen in figure 3, a significant improvement is observed upon the exclusion of pepcan-20 in the SASA computations where both structural models yield similar results. Interestingly, the solvent-accessible surface area shows a linear trend and makes it clear that the longer peptide such as pepcan-20 is an anomalous point, which agrees with what has been reported from experimental observations of the inactivity of this peptide towards CB1 human receptors [14], [33]. On the other hand, those short peptides with C-terminally truncated (p-7 and p-10) do not seem to have enough contact area to establish a good protein-peptide interaction with the CB1 receptor, which is an indispensable factor for biomolecular binding events [23], [24], in comparison with the other pepcans (pepcan-12, pepcan-14, pepcan-14, pepcan-15, and pepcan-17).

Table 2. Pearson correlation coefficient (*r*) of the solvent-accessible surface area (SASA) using both the models extracted from the human hemoglobin and ColabFold the net charge of peptides with the experimental inhibitor constants - expressed as logK_i - to CB_1 human receptors.

Property	**Type**	**Pearson correlation coefficient (*r*)**	
		All peptides	**Six peptides (no pepcan-20)**
Net charge	Sequence-based property	-0.81	-0.85
SASA (Å^2) human haemoglobin structural models	Context-dependent properties	-0.40	-0.89
SASA (Å^2) ColabFold structural models		-0.27	-0.84

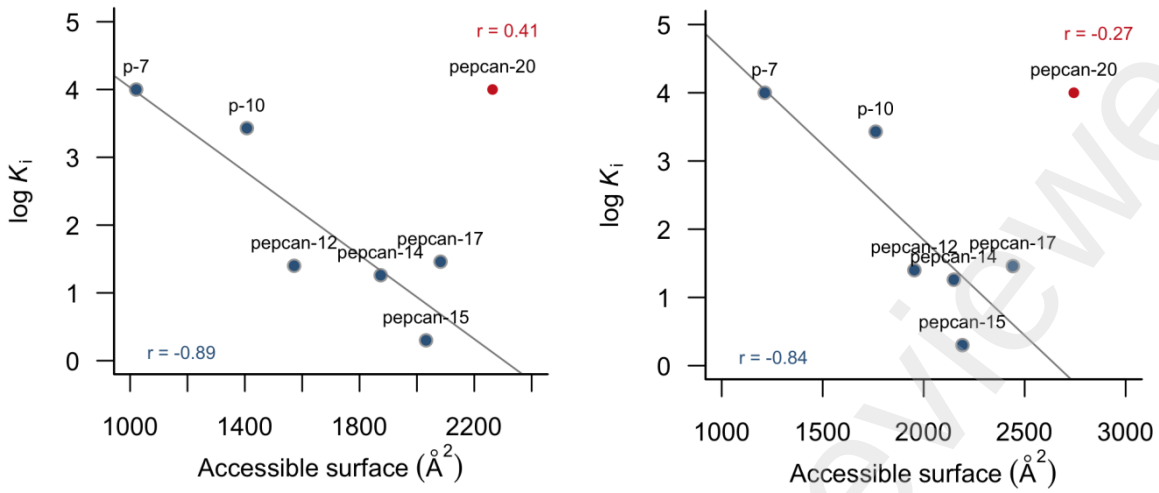


Figure 3. Relationship between experimental inhibitor constants - expressed as logKi – versus the solvent-accessible surface area for the hemopressin peptides using both the models extracted from the human hemoglobin models (left) and ColabFold structural models (rigth). Pearson correlation coefficients were determined with (top right, red) and without (bottom left, blue) pepcan-20.

The second approach is based on the use of several hydrophobicity scales of amino acids in order to compute the lipophilicity of each peptide and thus, compare the suitability of each scale in correlating the experimental inhibitor constants for the endocannabinoid peptides in this work. The values of the lipophilicity of the seven peptides at physiological pH are shown in the Supporting Information (see TableS2_Pepcans_All_properties.xls) and the Pearson correlation coefficient (*r*) considering or not pepcan-20 in Table 3. Thirty-eight hydrophobicity scales just use the amino acid sequence - all scales reported in Table 3 except for ProtL scale – and as expected, for the vast majority of correlations, a substantial improvement is observed when considering the pepcan-20 as an outlier.

Table 3. Pearson correlation coefficient (*r*) of the lipophilicity of the seven peptides using the hydrophobicity scales implemented in the Peptides package of R software [29] and the ProtL scale [31] with the experimental inhibitor constants - expressed as $\log K_i$ - to CB_1 human receptors.

Hydroph. Scale	Pearson correlation coefficient (*r*)		Hydroph. Scale	Pearson correlation coefficient (*r*)	
	All peptides	Six peptides (no pepcan-20)		All peptides	Six peptides (no pepcan-20)
Aboderin	0.12	0.45	Kidera	0.47	0.65
Abraham-Leo	0.53	0.82	Kuhn	0.32	0.15
Argos	0.11	0.65	Kyte-Doolittle	0.25	0.51
BlackMould	0.07	0.43	Levitt	0.29	0.72
BullBreese	0.09	0.14	Manavalan	0.14	0.60
Casari	0.05	0.15	Miyazawa	0.08	0.40
Chothia	***0.56***	***0.87***	Parker	0.34	0.19
Cid	0.20	0.17	Ponnuswamy	0.30	0.81
Cowan3.4	***0.51***	***0.86***	Prabhakaran	0.35	0.61
Cowan7.5	0.06	0.47	Rao	0.26	0.48
Eisenberg	0.29	0.29	Rose	0.39	0.60
Engelman	0.36	0.61	Roseman	-0.32	-0.15
Fasman	0.23	0.55	Sweet	0.01	0.40
Fauchere	0.02	0.23	Tanford	0.57	0.74
Goldsack	0.11	0.61	***Welling***	***0.51***	***0.87***
Guy	0.07	0.11	Wilson	0.37	0.83
Hopp-Woods	0.36	0.32	Wolfenden	0.01	0.01
Janin	0.74	0.72	Zimmerman	0.15	0.19
Jones	0.11	0.65	***ProtL[b]***	***0.66***	***0.89***
Juretic	0.18	0.39	***ProtL[c]***	***0.30***	***0.96***

[a] Using the models extracted from the human hemoglobin (PDB code: 2DN2)
[b] Using the models generated using ColabFold.

Figure 4 shows the relationship between experimental inhibitor constants with the hydrophobicity of the seven endocannabinoid peptides using the sequence-dependent scales that showed the best correlation, these scales were those reported by Cowan[34] ,Chothia[35] , and Welling[36]. Even though the importance of lipophilicity scales has been demonstrated for a better understanding of the interactions between biomolecules[24], [37]–[39], it must be careful where they are used keeping in mind the experimental conditions. Thus, it can be noted that the scale of Cowan (see Fig. 4) seems

to be efficient considering a linear trend, however, the hydrophobicity values of amino acids on this scale rely on measurements based on retention time in HPLC at pH 3.5 [34], conditions very different from the experimental determination of K_i -protein environment at pH 7.4. On the other hand, the Welling and Chothia scales present acceptable correlations and may be more appropriate because are derived from antigenic regions in proteins[36] and the proportion of exposed and buried residues in proteins[35], respectively. The main disadvantage that these scales show is their low resolution, since they have very narrow hydrophobicity ranges and, since they depend solely on the sequence, they do not capture details that depend on the local context of the peptides, that is, their bioactive conformation.

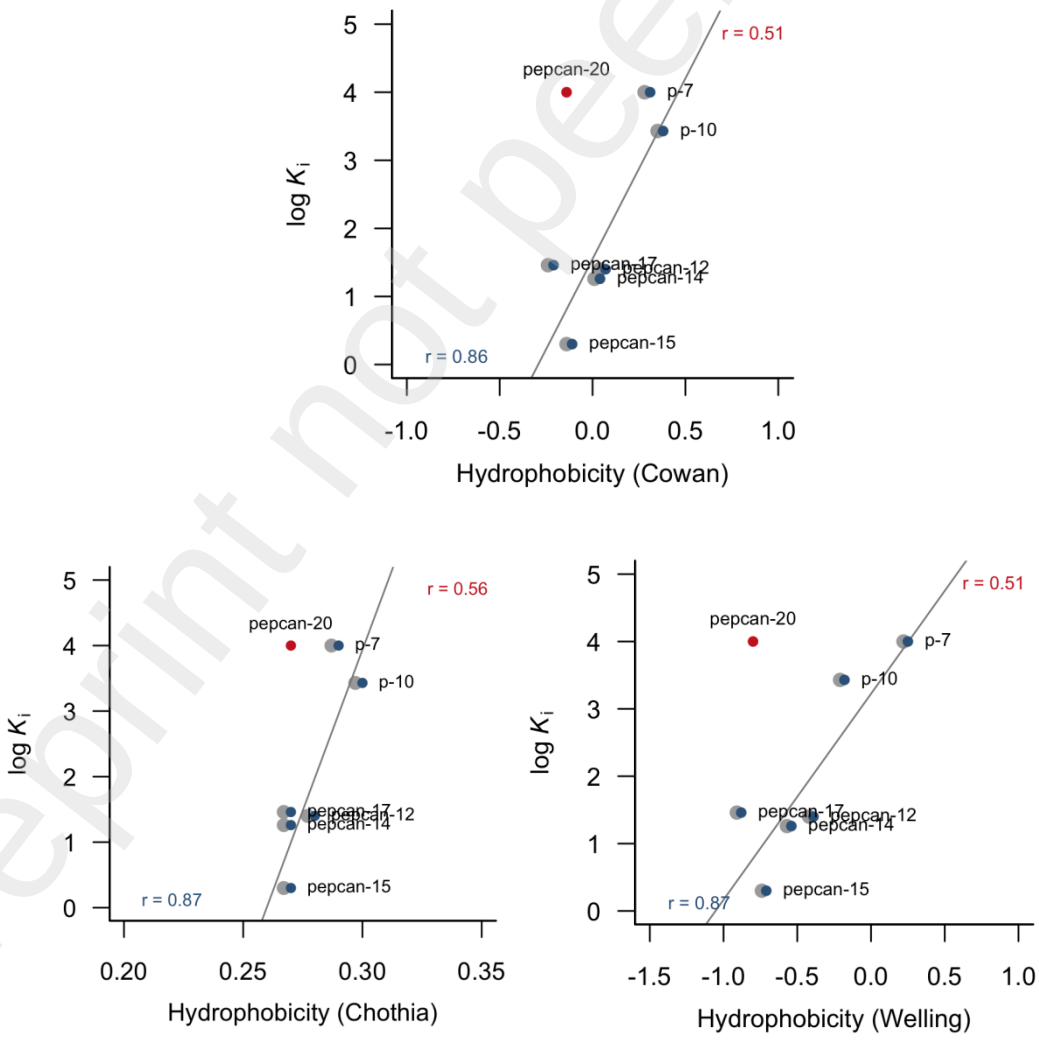


Figure 4. Relationship between experimental inhibitor constants - expressed as logKi – versus the hydrophobicity of the seven peptides using the scales reported by Cowan[34] (top), Chothia[35] (bottom left), and Welling[36] (bottom right). Pearson correlation coefficients were determined with (top right, red) and without (bottom left, blue) pepcan-20.

One of the initial aims of this study was to evaluate the importance of using a proper description of lipophilicity on peptides in order to improve the results in QSAR studies. To this end, we also compute the lipophilicity of the structures of seven peptides using a state-of-the-art lipophilicity scale, called ProtL scale (see Table 3), which is a pH-dependent and structure-based lipophilicity scale based on the IEFPCM/MST continuum solvation method where the lipophilicity of each amino acid is calculated taking into account its specific structural features and the pH of the medium [31] (see Figure 5), thus, the models from the ColabFold Server and those extracted of the helix G of human haemoglobin in the deoxy form (PDB code: 2DN2) were used.

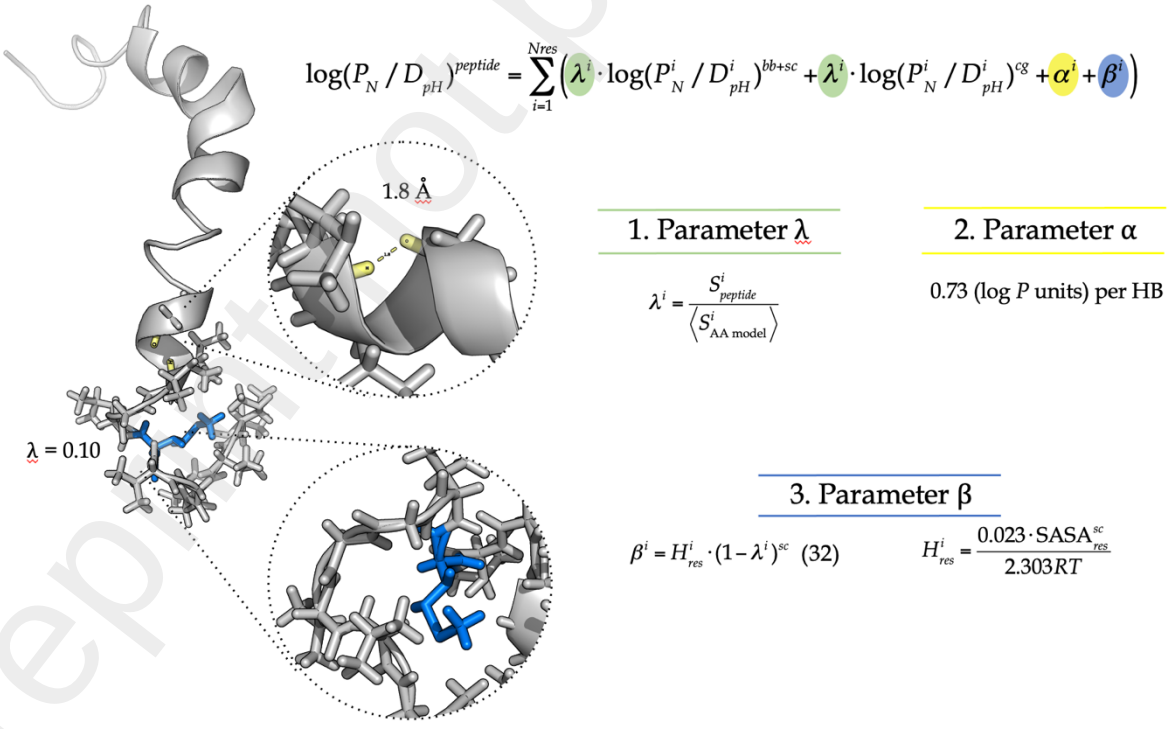


Figure 5. Representation of the *ProtL* lipophilicity scale. The lipophilicity of a peptide is calculated from each amino acid as a cumulative partition or distribution coefficient ($\log P_N/D_{pH}$) but considering local-context-dependent correction factors. The parameter λ stands for the fraction of solvent-exposed surface area (SASA) of the amino acid (backcnone=*bb*+side chain=*sc*) or capping group (*cg*) according to the local structural environment of in a peptide/protein. The parameter α introduces a correction to the hydrophobic contribution when the backbone participates in a hydrogen bond (HB). This contribution can be estimated to amount, on average, to 0.73 (log *P* units) per HB[40], the occurrence of this kind of HBs in a given 3D structural model was determined with the DSSP program[41]. Finally, the β factor accounts for a correction due to the burial of the side chain of hydrophobic residues (Ala, Leu, Ile, Val, Pro, Phe, Trp, Met and Tyr) from water to a lipophilic environment. This contribution has been estimated to be 0.023 kcal $mol^{-1}Å^{-2}$ according to the studies reported by Moon and Fleming[42] for the transfer of nonpolar side chains from water into a lipid bilayer and H_{res} stands for the hydrophobic contribution (in log*P* units) of a specific apolar residue.

The Pearson correlation between $\log K_i$ and the lipophilicity of the seven peptides using the *ProtL* scale is shown in Figure 6. Similarly, a significant improvement is observed upon the exclusion of pepcan-20. It is worth noting that the ProtL scale outperforms the results obtained with the other experimental scales reported in Table 3. First, the Person correlation amount to 0.96 for the ColabFold structural models of peptides, an improvement of more than 10% over the scales that use only the sequence. Then, the range of lipophilicity is much wider allowing us to distinguish in better way the differences between the peptides studied. This is not unexpected keeping in mind that the lipophilicites in *ProtL* scale are derived from a more complex algorithm that considers both local environment effects and pH effects giving higher sensitivity to this scale.

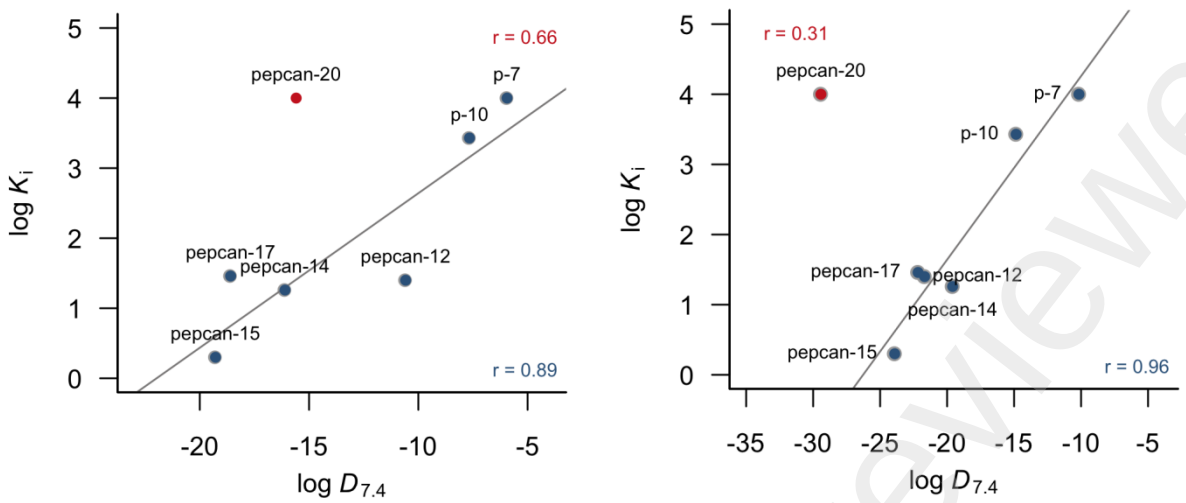


Figure 6. Relationship between experimental inhibitor constants - expressed as logKi – versus the lipophilicity of the seven hemopressin peptides using both the models extracted from the human hemoglobin models (left) and ColabFold structural models (rigth) with the *ProtL* scale. Pearson correlation coefficients were determined with (top right, red) and without (bottom left, blue) pepcan-20.

Finally, taking into consideration that the *ProtL* scale describes excellent trends, especially with ColaFold models, and has been reported in the literature that the first six amino acids (PVNFKF) of hemopressin peptides are required for CB1 binding and the deletion of C-terminal three residues did not affect receptor recognition[17], it led us to analyse which residues have the greatest impact on the description of the experimental inhibition constants. Figure 7 shows that the more hydrophobic the N97 residue is in the local context of the conformations of the analyzed peptides, the greater affinity it will have toward human CB1 receptors. This agrees with what was indicated in the experimental observations mentioned above and with in silico results where the smallest peptide NFKF displayed the better docking score to bind CB1 than small molecules antagonists (AM6538, cannabidiol, and rimonabant) [43] .

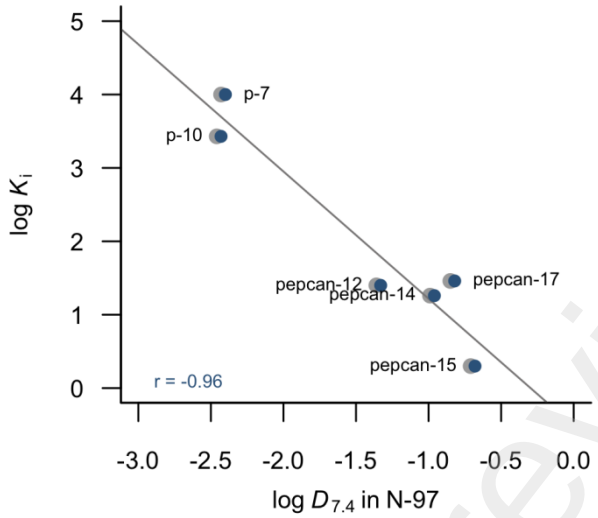


Figure 7. Relationship between experimental inhibitor constants - expressed as logK_i – versus the lipophilicity with the *ProtL* scale for the residue N-97 (α 1-globin chain numbering) present in six hemopressin peptides using the ColabFold structural models.

Conclusions

Overall, the results point out the use of state-of-the-art lipophilicity scales for describing the lipophilicity of peptides which take into account the local-context dependency of the conformations of peptides but also the pH of the environment outperforms the results obtained with other experimental scales which only consider sequence-based information. In addition, the ProtL scale proved to be useful for QSAR studies on pepcan peptides and could give details that the N97 amino acid is crucial in the binding of peptides to human CB1 receptors. Despite the fact that there are reviews in search of which is the best lipophilicity scale of amino acids, we considered that is more valuable to know when and where to use a lipophilicity scale, as they all have useful information on particle conditions and environments. Finally, the solvent-accessible surface area of pepcan peptides showed an important trend with the inhibition constants, indicating that large peptides such as pepcan-20 are inactive at CB1 receptors, which agrees with what was observed experimentally.

Acknowledgments

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