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In-silico design of new enalapril analogs (ACE inhibitors) using QSAR and molecular docking models

Jhon Alex Gonzalez Amaya, Daniella Zambrano Cabrera, Alejandra Mojica Matallana, Karen González Arevalo, James Guevara-Pulido *

Universidad El Bosque, Química Farmacéutica, INQA Research Group, Av Cra 9 No 131A-02, 110121, Bogotá, Colombia

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ABSTRACT

Angiotensin-converting-enzyme inhibitors (ACEI) are a group of drugs primarily used in the treatment of cardiovascular disease. In this study, MLR and PLS QSAR models were developed to evaluate the antihypertensive activity of new enalapril analogs, while binding affinities between each analog and ACE were determined with AutoDock. Consequently, analogs presenting para and meta trifluoromethyl substitutions, and a N,N-dialkyl aliphatic amide were the most promising analogs, exhibiting an IC_{50} of 0.009 nM and affinity energies of −8.9 kcal/mol, surpassing those of enalapril. Furthermore, all promising analogs were predicted to be less toxic than enalapril according to the software PreADMET.

1. Introduction

Hypertension is a long-term condition, often regarded as a significant risk factor for cardiovascular disease. The control and prevention of this condition have been considered critical to world health [1]. Currently, there are several types of pharmaceutical drugs available for the treatment of hypertension, such as renin inhibitors, angiotensin II receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs).

Angiotensin-converting enzyme (ACE) is a zinc-dependent dipeptidase that catalyses the conversion of angiotensin I to the vasopresor angiotensin II [2]. It possesses two catalytic domains, (N- and C- domains), with the pentapeptide C-domain being responsible for the enzyme's function [2]. ACE is produced by endothelial cells present in for example, the CNS, kidneys and lungs [3].

Angiotensin-converting enzyme inhibitors (ACEI) are a group of drugs used in the treatment of cardiac pathologies like hypertension, congestive heart failure, left ventricular dysfunction and myocardial infarction [4]. ACEI act as potent vasodilators by blocking the conversion of angiotensin I to angiotensin II, thereby lowering blood pressure by generating prolonged hypotensive responses [5].

In general, ACEI have been widely tolerated. However, common adverse effects include cough [6], eczematous reactions [7], hypotension, hyperkalemia [4], and small bowel angioedema [8]. The incidence of almost all of these effects could be reduced by designing ACEI with greater affinity to ACE's active site, lower IC_{50} values and lower toxicity.

In recent years, virtual screening has become an integral part of the drug discovery process. There are two types of virtual screening techniques: Structure-Based Virtual Screening (SBVS) and Ligand-Based Virtual Screening (LBVS). SBVS uses the 3D structure of a compound in order to predict its binding affinity to a receptor [9], whereas LBVS is used to predict pharmacological parameters such as the IC_{50} of a ligand, based on its structure-activity relationship using molecular desciptors, and not binding affinities.

One way to evaluate the binding affinity between a drug candidate and its receptor is by molecular docking, where simulating the ligand-receptor docking process allows for the calculation of scoring functions which in turn predict the binding affinity between drug candidate and receptor after they have been docked [9].

On the other hand, LBVS uses molecular descriptors of known active ligands, rather than the structure of the receptor, since it theorizes that ligands similar to active ligands will also show a similar binding activity to the receptor [9]. This type of virtual screening quantitatively relates structure to the biological activity of a family of compounds, known as QSAR which, based on the activity of several analogous ligands, allows the prediction of IC_{50} values for new compounds [10]. Currently, several QSAR models exist where the information available will be critical in selecting the best suited model for a study. For example, those based on MLR and PLS are used when there are few well-correlated input data, whereas when data is plentiful Machine Learning models (e.g ANNs, decision trees, SVMs) are chosen [9].

* Corresponding author.
E-mail address: coordinacionfacciencias@unbosque.edu.co (J. Guevara-Pulido).

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Considering that ACE plays a critical role in controlling blood pressure, inhibiting its function has been the target of hypertension control. San Juan & Cho [11], created a QSAR model showing the structural characteristics potential ACEI must have in order to inhibit the enzyme's C-domain, and therefore hinder its function. First, ACEI require a terminal carboxyl group to promote ionic interactions with the cationic site. Second, they must feature a hydrogen bond acceptor. Finally, they need an ionizable functional group capable of coordinating with the Zn^{2+} ion. Additionally, studies have stated that any additional ionizable groups or hydrophobic radicals increase the efficacy of the ACEI candidates [3]. Moreover, other QSAR models have, in turn, determined that the hydrophobicity and the polar interaction of ACEI play an important role in its inhibitory activity [12,13]. Also, recent studies using linear models have yielded promising results such as highlighting that positions C-1 and C-4 of possible ACEI oligopeptides are the most relevant at increasing their inhibitory activity [14].

The development of ACEI analogs through QSAR models by means of MLR and PLS regressions is one of modern medicine's priorities due to the possibility of discovering ACEI analogs with more favorable clinical profiles than those currently in use [3]. However, the toxicological profile of new ACEI must be considered seeing as a drug candidate can prove to be more potent and target-specific, yet more harmful than those ACEI currently used. Recently, predictive models have been developed to assess drug safety since they are inexpensive, eco-friendly and can be performed before a compound is synthesized [15].

2. Materials and methods

First, to carry out LBVS, molecular descriptors associated with ACEI activity and a suitable *training set* (commercial and non-commercial antihypertensives) were identified according to the literature. Those reference ACEI were built and optimized in Avogadro [16], through conformational analysis using the random rotor conformer search method and a MMFF94 force field.

Then, the VCCLAB [17], program was used to calculate values for those molecular descriptors where those constitutional and topological descriptors with a linear relationship with the experimental IC_{50} (via ORIGIN 8.5) were selected to develop a QSAR model that could predict IC_{50} values.

The correlation between the molecular descriptors and IC_{50} of the *training set* was explored using MLR and PLS QSAR models. Reference data to assess the quality of established models and their respective results were derived from the OCHEM database [18]. Both QSAR models yield equations that predict the IC_{50} of new ACEI analogs. Likewise, to carry out SBVS [9], binding affinities between molecules from the training set and ACE were determined by means of AutoDock Vina [19], with AutoDock4Zn force field [20].

Next, ACEI analogs were designed according to the structural requirements stated in the literature where enalapril derivatives were mainly studied. After their design, the ACEI analogs were characterized first through a conformational analysis using Avogadro [16] and ConfGen [21], second by the calculation of their molecular descriptors and IC_{50} with QSAR models, and third by the measurement of their binding affinities through AutoDock 4.2.6 [19]. The results were then compared with those of the *training set* where two analogs showed the most promise since they exhibited lower IC_{50} values and greater binding affinities to ACE.

Afterwards, more than 200 structural modifications were made to the two promising ACEI analogs and data was again obtained from the MLR, PLS models and from AutoDock. However, this step of the process also includes the use of PyMOL [22]. Finally, the toxicity profile of the five most promising analogs was predicted using PreADMET [15].

3. Results and discussion

The methodology sought to produce structural hybrids of a biologically active therapeutic with greater affinity and efficacy than the parent drug [23]. Ligand-based drug design includes QSAR modeling where regression, classification and machine learning models help determine the possible structure-activity relationships in order to predict the activity of new molecules due to physicochemical properties or theoretical molecular descriptors. This approach has become popular in past years since it seeks to reduce the cost and time related to drug discovery [24].

First, for the creation of the QSAR models used in this study, known antihypertensives like enalapril were modeled in Avogadro. Then, several molecular descriptors were evaluated with OCHEM [18] and VCCLAB [17]. A regression-based QSAR model relates the biological activity (dependent variable) to a set of molecular descriptors (independent variable) resulting in a mathematical equation correlating these two variables. In this study, MLR and PLS models were used to relate molecular descriptors to antihypertensive activity (IC_{50}). The molecular descriptors examined according to the literature were topological polar surface area, formal charge, octanol-water partition coefficient, isoelectric point, ionization potential, molar refractivity, van der Waals surface area, electronic density, dipole moment and pKa. Among the examined molecular descriptors only those with a linear relationship with the experimental IC_{50} values were selected to describe the sample and develop a QSAR model that could predict IC_{50} values. Based on the results, only partition coefficient, molar refractivity and dipole moment were chosen.

The MLR and PLS models must be examined in terms of the statistical quality of their results in order to assess their predictive capacity. A model with greater statistical quality, will yield more reliable and accurate predictions [25]. The statistical quality of a model can be evaluated using the coefficient of determination (R^2), Fisher's method (F) and standard deviation (s). However, the number of independent variables included in the models must also be considered, as there must be one variable per five or six compounds from the *training set* [26]. Table 1 shows molecular descriptor data and predicted IC_{50} for commercial and non-commercial ACEI used for the development of the MLR and PLS QSAR models via MATLAB. The commercial and non-commercial ACEI

Table 1
Molecular descriptor data, experimental and predicted IC_{50} of training and test sets.

Compound	Log P	AMR	DMo	IC_{50} (nM)Exp	IC_{50} (nM) MLR	IC_{50} (nM)PLS
Enalapril	−0.09	85.0	12.9	**1.2** [3]	**1.5**	**1.6**
Imidapril	−0.04	92.3	6.20	**1.7** [3]	**2.0**	**2.1**
Lisinopril	−1.23	96.7	5.82	**1.2** [3]	**1.2**	**1.2**
Moexipril	0.89	122	5.05	**2.6** [3]	**2.9**	**3.3**
Perindopril	−0.08	76.6	8.79	**1.5** [3]	**1.2**	**1.2**
Quinapril	0.81	109	7.60	**2.8** [3]	**2.0**	**2.1**
Ramipril	0.54	95.3	3.40	**2.0** [3]	**1.9**	**2.0**
Spirapril	0.90	109	11.1	**0.8** [3]	**0.9**	**0.9**
Trandolapril	0.77	98.3	7.09	**1.3** [3]	**1.2**	**1.3**
Benazepril	0.62	102	1.70	**1.7** [3]	**2.6**	**2.7**
Cilazapril	−0.48	92.5	6.42	**1.9** [3]	**1.9**	**1.9**
Temocapril	0.89	115	2.69	**3.6** [3]	**3.3**	**3.6**
CHEMBL100413	0.91	117	3.86	**1.7** [27]	**2.4**	**2.5**
CHEMBL100826	1.14	120	9.58	**2.3** [27]	**3.0**	**3.3**
CHEMBL101409	0.74	114	5.74	**11** [27]	**9.0**	**11.1**
CHEMBL317304	0.64	103	8.58	**6.7** [27]	**6.1**	**7.4**
CHEMBL431052	−0.38	86.6	12.4	**3.5** [27]	**2.1**	**2.3**
CHEMBL2111940	0.62	122	10.9	**4.8** [27]	**3.2**	**3.6**
CHEMBL2112767	−0.78	89.8	16.3	**17** [27]	**12.1**	**15.9**
CHEMBL2112768	0.62	122	19.8	**3** [27]	**2.8**	**3.2**
CHEMBL2112769	0.61	119	12.9	**7.7** [27]	**4.6**	**5.5**
CHEMBL2371228	−0.47	71.9	2.22	**1.9** [27]	**0.7**	**0.7**
CHEMBL2371229	−0.38	80.3	18.0	**230** [27]	**152**	**198**
CHEMBL3037879	−2.50	68.8	10.2	**700** [27]	**459**	**513**

Log P (P partition coefficient), AMR (Molar refractivity), DMo (Dipole moment).

are divided into two subsets of data: the *training set* (first 17 compounds, Table 1) and the *test set* (last 7 compounds, Table 1) which are used to estimate the precision of the model's performance when applied to new ACEI analogs [10,25].

The equations yielded by the MLR and PLS models respectively are presented as follows:

$$loglog\left(\frac{1}{IC_{50}}\right) = 10.567(\pm 0.328) + 0.248(\pm 0.045)(logP)^2 + 0.049(\pm 0.005)logP - 0.021(\pm 0.003)AMR + 0.074(\pm 0.008)DM_O \quad (1)$$

$r = 0.984;\ F = 1311.4;\ s = 0.115$

$R^2 = 0.968$

$$loglog\left(\frac{1}{IC_{50}}\right) = 10.77960 + 0.27168(logP)^2 + 0.05350logP - 0.02308AMR + 0.00584DM_O \quad (2)$$

$r = 0.978; F = 511.9;\ s = 0.741$

$R^2 = 0.956$

Based on the equations above, it can be observed that both models show high R^2 and F values ($R^2 > 0.96$) and low s-values, which indicate that they exhibit a high predictive capacity. However, between the two, the MLR model has the greatest statistical quality. Figs. 1 and 2 illustrate the relationship between experimental IC_{50} (from the literature) and predicted IC_{50} of both *training* and *test sets* (Table 1) derived from the MLR and PLS models. Both figures show an almost linear relationship among data indicating a suitable behavior for both subsets of data. In the interest of reducing variability, multiple rounds of cross-validation using different *training* and *test sets* partitions were rendered to estimate the predictive performance of the models.

Once the QSAR models were developed, 19 enalapril analogs were designed (Fig. 3) according to the structural requirements mentioned before, which include a terminal carboxyl group, an oxygen as the electron acceptor, an ionizable functional group that adapts to the enzyme's molecular geometry and an amide carbonyl group as hydrogen bond acceptor. During Phase I, 19 different molecules were created by modifying the R-group in enalapril (Fig. 3). These structural modifications were carried out considering that the structure of enalapril features two amino acids (ALA-PRO). The hypothesis in this study is based on modifying the alanine residue for another amino acid (Fig. 3). Therefore, the peptidic nature of the molecule would remain the same and only the

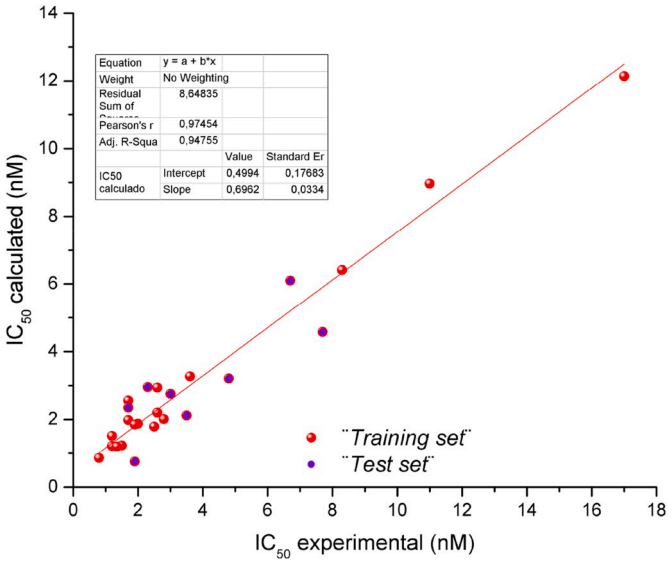


Fig. 1. Relationship between experimental IC_{50} (from the literature) and predicted IC_{50} of several commercial and non-commercial ACEI derived from the MLR model. Training and test set data points are used to estimate the predictive capacity of the model.

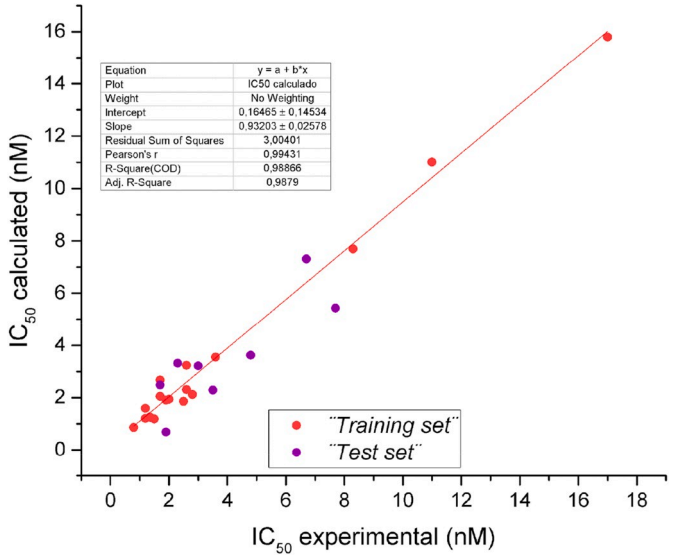


Fig. 2. Relationship between experimental IC_{50} (from the literature) and predicted IC_{50} of several commercial and non-commercial ACEI derived from the PLS model. Training and test set data points are used to estimate the predictive capacity of the model.

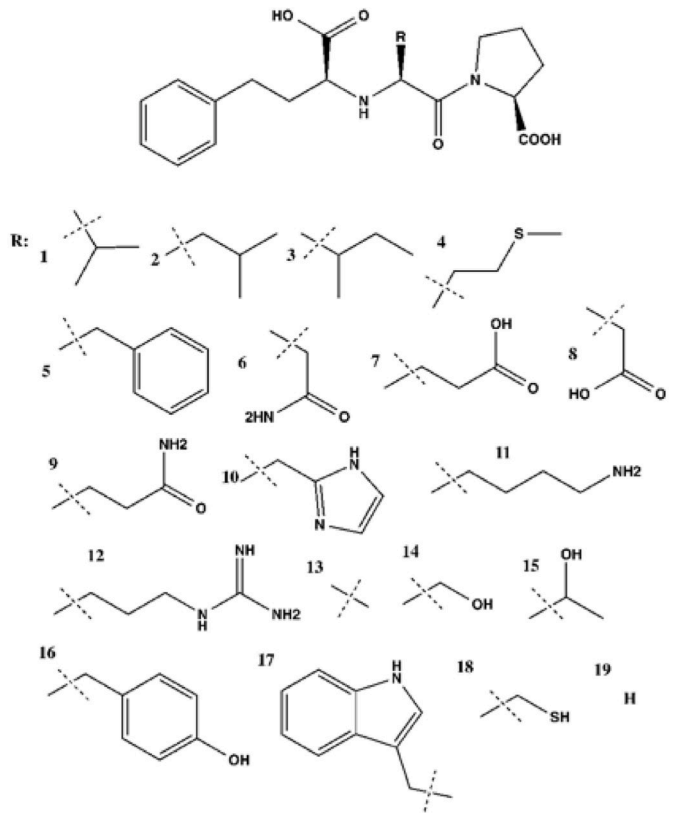


Fig. 3. Structure template of the enalapril analogs designed during Phase I where the R-group was replaced with different amino acid residues. The R-group in enalapril is a methyl radical.

influence of the ALA-residue on the biological activity of the molecule would be evaluated. Table 2 summarizes the structure of the two promising ACEI analogs from Phase I with their respective IC_{50} values derived from both QSAR models. As shown in Table 2, analogs 6 and 14 exhibit an IC_{50} equal to or lower than enalapril's ($IC_{50} = 1.2$) [3].

Moreover, molecular docking predicts the predominant binding modes of a ligand with a receptor protein [28]. This tool predicts the preferred orientation of one molecule to a second when bound together, and in turn predicts the binding affinity between those two molecules [29]. The receptor protein ACE was first downloaded from Protein Data Bank (PDB) [30] and was then optimized in AutoDock Vina [19]. The optimization algorithm is very efficient, with a success rate of 80%, because it takes into account several parameters of chemical interactions that allow for the evaluation of different scenarios, including the program's default one [31]. Afterwards, in order to determine the necessary parameters to carry out molecular docking (exhaustivity values, search area and number of cycles) the ACE-lisinopril complex was systematically evaluated. Ligand-binding sites were adjusted through multiple GRID arrangements and then estimated by means of the AutoDock Vina [20]. Later, the binding affinities (kcal/mol) between the enzyme and each ACEI analog were determined. Table 3 shows the binding affinities of the two most promising ACEI analogs from Phase I and two commercial ACEI (enalapril and captopril), where analogs 6 and 14 require less binding energy than both commercial ACEI.

Then, in order to have a greater notion of the affinity between the enzyme and the ACEI analogs from Phase I, their ligand-enzyme interaction distance was evaluated through PyMOL [22,30]. Thus, it was necessary to first evaluate the interaction between the enzyme and known antihypertensives like enalapril and captopril. Table 3 also shows the predicted ligand-Zn interaction (Å) of the two most promising ACEI analogs from Phase I and known antihypertensives (enalapril and captopril). Based on Table 3, it can be observed that all two analogs have similar interaction lengths to those of the known ACEI. However, it must be noted that the ligands experience other types of interactions with certain amino acid residues present in the enzyme such as Tyr523, Ala354, Hys353, Hys513, Lys511 and Tyr520 [3].

Afterwards, during in Phase II, structural modifications were made upon analogs 6 and 14 in an effort to improve their binding affinity and IC_{50}. In total, 220 new molecules were designed all of which maintained the structural template of enalapril. With each new design, molecular descriptors, IC_{50}, binding energies and ligand interaction lengths were calculated. Changes made to the two analogs took into account Lipinski's Rule of Five which states that drug-likeness is exhibited when a molecule abides by the following criteria: molecular mass <500Da, LogP$\leq$ 5, no more than five hydrogen bond donors and no more than ten hydrogen bond acceptors.

First, the R_1 group of analogs 6 and 14 (Fig. 4) was substituted with alkyl groups, successively substituting from a methyl to a decyl group, in order to decrease their polarity and evaluate hydrophobic analog-enzyme interactions. Next, R_2 was consecutively substituted from a propyl to decyl group. Then, changes to both R groups were done at the same time; thus resulting in the evaluation of 80 systematic structural changes. It was observed that the IC_{50} decreased in both analogs as the size of the alkyl substituent increased. The alkylation of both functional groups was carried out considering that current toxicity classification states that compounds containing large quantities of hydrocarbons are less toxic (Class 1). Next, changes meant to increase the polarity of the analogs (Fig. 5) were also explored by the consecutive substitution of R_2 with hydroxyl, amine and halide groups, combining each substitution with the ones made to R_1 in the previous step. The addition of hydroxyl groups resulted in the decrease of the IC_{50}, whereas addition of aromatic substituents proved unpropitious. Then, the substitution of the phenyl group with alkyl groups was examined, where a decrease in binding affinity made the substitution unfavorable. However, *para*-alkylation of the phenyl group significantly decreased the ligand's inhibition potency. On the other hand, halogenation of phenyl ring was carried out to evaluate if the inductive effect increased ligand-enzyme hydrogen bonding. Addition of methyl halides yielded the best results with an IC_{50} of 0.009 and an increase in binding affinity (Figs. 6 and 7 and Table 4). Moreover, all new designs obtained a partition coefficient less than five, therefore indicating that they shouldn't exhibit bioavailability problems. Fig. 7 shows the two new analogs with the most promising antihypertensive activity from Phase II.

Lastly, promising analogs from Phase I and Phase II underwent *in silico* drug safety assessments through PreADMET [15]. In this study, toxicity was evaluated by simulating growth inhibition, aquatic species reproduction, rodent carcinogenicity and bacterial mutagenicity assays [15]. Table 4 shows toxicity results for all four promising analogs and enalapril. Assessment of ecotoxicity demonstrated that analogs from Phase II (20 and 21, Fig. 7) are much less ecotoxic than enalapril, while analogs from Phase I (6, 14, Table 2) don't exhibit a significant toxicological difference to enalapril. Furthermore, the mutagenicity assessment (Ames_test) showed that only analog 6 proved to be mutagenic. Finally, all four analogs and enalapril presented negative for carcinogenicity in mice, while only analogs 14 and 21 were found negative for carcinogenicity in rats. Based on these results, it can be inferred that the trifluoromethyl para-substitution on the aromatic ring of analog 21 would yield less toxic compounds.

Likewise, results presented in Table 4 show that analogs 20 and 21 display greater affinity with the enzyme, lower IC_{50} and more acceptable toxicity results compared to enalapril and captopril. Consequently, it can be concluded that both analogs exhibit promising antihypertensive activity according to the parameters evaluated *in silico*, and should therefore be synthesized and evaluated in vitro and in vivo in order to study their viability as future antihypertensive drugs.

Table 2
Structure of the two promising ACEI analogs from Phase I with their respective IC_{50} values derived from the MLR and PLS models.

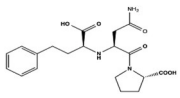
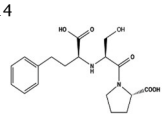
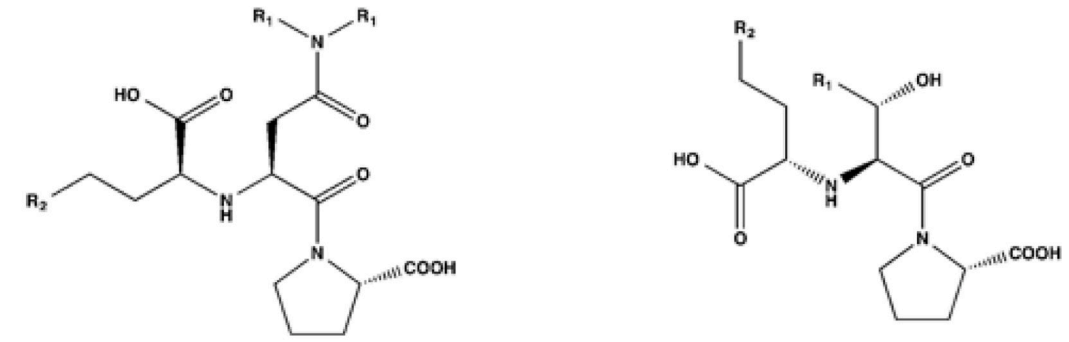
Structure	Predicted IC_{50} with MLR (nM)	Predicted IC_{50} with PLS (nM)
6 	1.3	1.3
14 	1.1	1.1

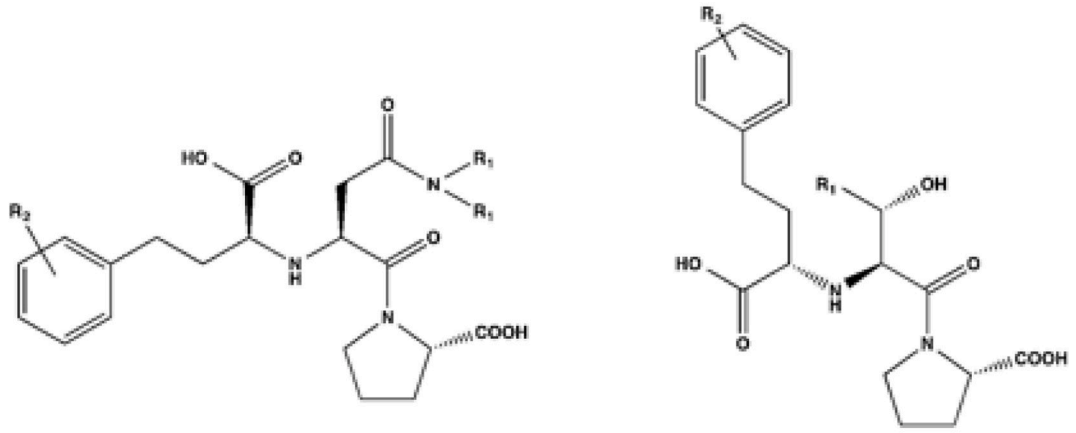
Table 3
Predicted ligand-Zn interaction (Å) of the two most promising ACEI analogs from Phase I and two commercial ACEI (enalapril and captopril). Binding energies between ligand and enzyme by means of molecular docking are also featured.

Interaction	Crystal structure (Å)	Predicted interactions through PyMOL (Å)			
		Captopril	Enalapril	6	14
1	8.485–8.673	8.3	8.5	8.0	8.0
2	5.718–5.981	5.6	5.1	5.5	4.9
3	3.532–3.628	3.5	3.6	3.5	3.6
4	4.876–5.181	4.8	4.5	5.1	5.1
5	3.989–4.047	4.0	4.2	4.3	3.9
Molecular Docking	**Binding energy (kcal/mol)**	−5.99	−6.38	−6.53	−5.90
	Total intermolecular energy (kcal/mol)	−7.48	−7.50	−10.11	−9.48



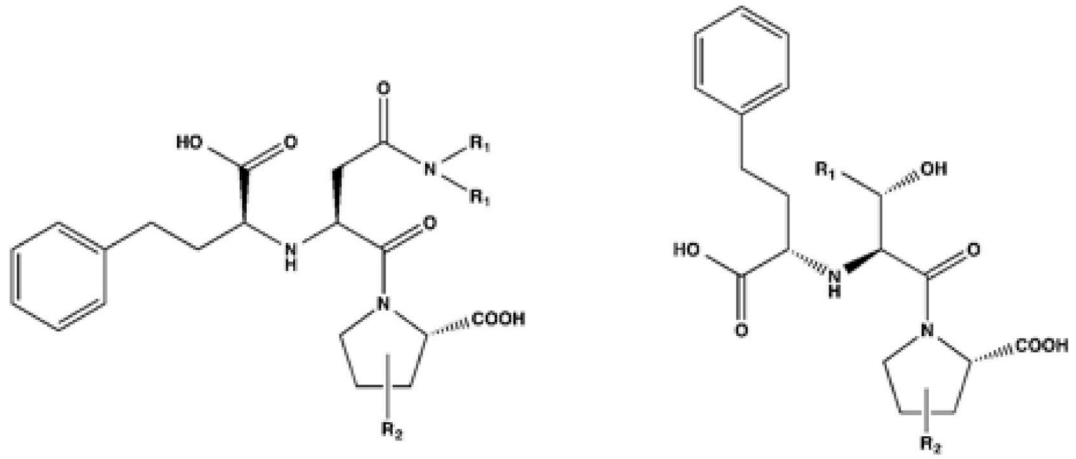
R_1: Methy to Decyl
R_2: Propyl to Decyl

Fig. 4. First structural modifications made upon analogs 6 and 14.



R_1: Methyl to Decyl
R_2: Br, Cl, I, F, CF_3, Methyl, NH_2, OH

Fig. 5. Additional modifications made upon analogs 6 and 14, to evaluate the influence of substituting the aromatic ring.



R_1: Methyl to Decyl
R_2: Br, Cl, I, F, CF_3, Methyl

Fig. 6. Another set of modifications made upon analogs 6 and 14 to evaluate the influence of substituting the proline ring.

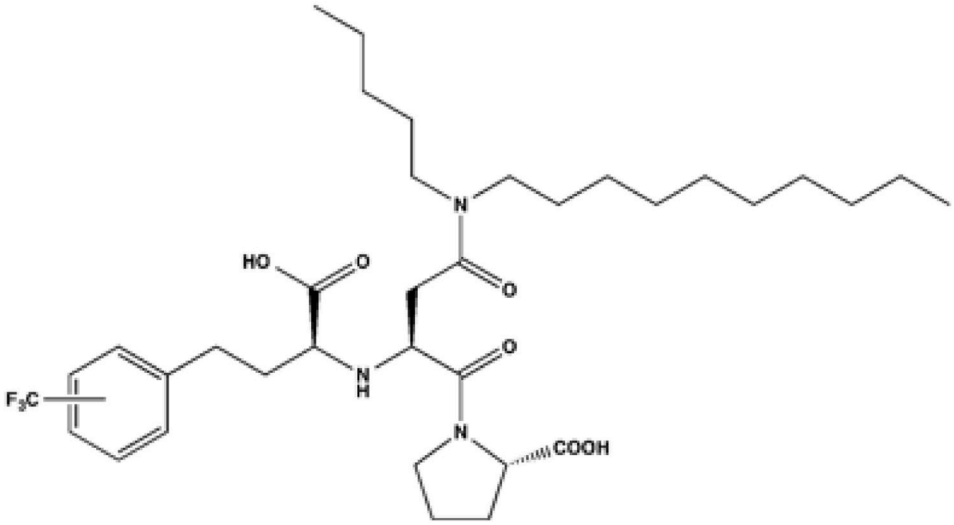


Fig. 7. Structure of the two most promising enalapril analogs designed during Phase 2. Analog 20 features meta substitutions while analog 21 features para substitutions.

Table 4
Predicted IC_{50} by means of MLR QSAR model, ligand-enzyme intermolecular energy through molecular docking and PreADMET toxicity assessments for enalapril and for the most promising ACEI from Phase I and Phase II.

		Enalapril	6	14	20	21
MLR QSAR Model	**Predicted IC_{50} nM (MLR)**	1.2 (1.2)[a]	1.3	1.1	0.009	0.009
Molecular Docking	**Intermolecular Energy (kcal/mol)**	−7.50	−10.11	−9.48	−8.90	−9.30
Toxicity PreADME	**Algae_at**	0.0263535	0.0547759	0.0443204	0.0002601	0.0005878
	Carcino_Rat	Positive	Positive	Negative	Positive	Negative
	Carcino_Mouse	Negative	Negative	Negative	Negative	Negative
	Daphnia_at	0.132963	0.521971	0.411032	0.000911	0.0023671
	Ames_test	Non-mutagenic	Non-mutagenic	Non-mutagenic	Non-mutagenic	Non-mutagenic

[a] Experimental IC_{50} [3].

4. Conclusion

MLR and PLS QSAR models were developed to predict antihypertensive activity of enalapril analogs. Both models demonstrated statistical quality by yielding r^2-values greater than 0.97, relatively high F-values and low s-values. These models, along with molecular docking, were used to predict the binding affinity and antihypertensive activity of more than 200 new ACEI analogs. Those presenting para and meta trifluoromethyl aromatic substitutions, and a N,N-dialkyl aliphatic amide turned out to be the best candidates. The most promising ACEI analogs were 20 and 21 since they presented an IC_{50} of 0.009 nM and binding affinities of −8.90 and −9.30 kcal/mol respectively, yielding better results than those of enalapril (IC_{50} 1.2 nM and binding energy of −7.50 kcal/mol). As for the toxicity assessments, both analogs were less ecotoxic than enalapril and both presented as non-mutagenic in an *in silico* drug safety study. However, only 21 proved non-carcinogenic on both rat and mouse cell lines, while 20 only presented non-carcinogenic on mouse cell lines. Therefore, it can be widely concluded that an *in silico* methodology may contribute to the design and discovery of new antihypertensives with greater biological activity than those currently used.

Ethical statement

N/A

Declaration of competing interest

None declared

Acknowledgements

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Appendix A. Supplementary data

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