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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel phthalamide derivatives as antihypertensive agents: rapid and clean synthesis, in silico and in vivo evaluation

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Abstract

Hypertension is a prevalent progressive disorder and a key risk factor for cardiovascular disease, stroke, and kidney disease. Angiotensin-I-converting enzyme (ACE) inhibitors are the first-line drugs for treating hypertension, but they have many side effects. ACE is a zinc dipeptidyl carboxypeptidase that cleaves the decapeptide angiotensin-I to form the vasopressor angiotensin-II. Since the latter molecule is the main bioactive product of the renin–angiotensin system, its inhibition is a key strategy for hypertension therapy. The aim of this study was to conduct an in silico evaluation of a series of new phthalamides as ACE inhibitors, examine the acute toxicity (in mice) of three of these molecules, and test the hypertensive effect of the most promising compound in a spontaneous hypertensive rat (SHR) model. The new phthalamide derivatives were synthesized with a fast, cheap, high-yield green (solventless) procedure. Three molecules (DD-01, DD-13, and DD-14S) from the current series of phthalamides were selected as the most promising ACE inhibitors based on in silico analysis of their physicochemical properties, Gibbs free energy and ADME profile. After synthesis, these three molecules showed low toxicity ($LD_{50} > 1600$ mg/kg) in the acute toxicity test (Lorke's method). Finally, DD-01 significantly decreased systolic, diastolic, and mean arterial pressure in the SHR model, being ~7-fold more potent than captopril (the reference drug). Three novel phthalamide derivatives were synthesized in good yields with a fast and efficient green procedure. They all displayed low toxicity. The one tested in the SHR model proved to be efficient for reducing blood pressure.

Keywords Hypertension · Spontaneously hypertensive rats · Green chemistry · ADME profile · Molecular docking

Introduction

Hypertension, a chronic medical condition that affects >1.3 billion people worldwide (Bloch 2016; Abdel-Rahman et al. 2017), represents the main risk factor for the development of cardiovascular disease, stroke, and kidney disease. These three disorders account for 17 million deaths annually, approximately 31% of all disease-related deaths worldwide (WHO 2013; Abdel-Rahman et al. 2017; Ben Salah et al. 2018).

One in four people suffers from hypertension in Mexico, according to ENSANUT 2016 (Campos-nonato and Hernández-barrera 2018). The values used for diagnosing hypertension have changed over time. Since a reduced range of values for defining this disorder yields better results in patient therapy and prognosis, new guidelines for diagnosis, management, and treatment were published in 2017 by the American Heart Association and the American College of Cardiology (AHA/ACC) (Lloyd-Jones et al. 2017; Magvanjav et al. 2018).

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The physiopathology of hypertension is still poorly understood. The known factors that contribute to this multifactorial disease are high salt (Na^+) intake, obesity, insulin resistance, and improper regulation of the sympathetic nervous system and the renin–angiotensin system (RAS) (Luo et al. 2017; Grootaert et al. 2017; Vildmyren et al. 2018). One of the most important mechanisms in blood pressure regulation is the RAS, responsible for controlling hemodynamic stability, fluid volume, and sodium–potassium balance (Te Riet et al. 2015; Fu et al. 2016).

Renin is synthesized in the kidneys in its inactive form and released into blood circulation in response to low levels of intratubular sodium, hypotension in the afferent arterioles of renal glomerulus, and sympathetic activation. In the bloodstream, proteolytic and nonproteolytic mechanisms activate pro-renin (Jan Danser et al. 2007), which hydrolyzes angiotensinogen to form angiotensin-I (Ang I). The latter decapeptide is cleaved by the Ang I-converting enzyme (ACE, a zinc dipeptidyl carboxypeptidase) to generate a potent vasoconstrictor, angiotensin-II (Ang II) (EC3.4.15.1) (Lv et al. 2018), the main bioactive product of the RAS.

In addition to catalyzing the conversion of Ang I into Ang II, ACE inhibits the degradation of the vasodilator bradykinin (Paiva et al. 2017; Fienberg et al. 2018). The two distinct isoforms of ACE are the somatic and testicular form. The somatic isoform, the most important, is mainly expressed on the endothelial surface. It is particularly abundant in the lungs, intestine, choroid plexus, and placenta, as well as on the brush border membranes in the kidney (Sparks et al. 2015).

In cases of hypertension, the inhibition of ACE is known to lower blood pressure and protect organs. Consequently, ACE inhibitors (ACEis) have been considered the first-line drugs for hypertension therapy. Recently, many researchers have been developing new ACEis based on peptides derived from plants, animals, and eggs (Li et al. 2014; Jenis et al. 2017; Tai et al. 2018) because they can control blood pressure and decrease cardiac and pulmonary fibrosis without producing the secondary effects of other treatments (Fienberg et al. 2018).

Ang II receptor blockers are also employed to control blood pressure, but it has been demonstrated that ACEis have additional benefits due to their capacity to increase the concentration of bradykinin and improve endothelial function (Shen et al. 2017). The main adverse effects of ACEis, including a dry cough, hyperkalemia, fatigue, dizziness, headaches, and loss of taste (Parish and Miller 1992; Nawaz et al. 2017; Yu et al. 2018), could probably be avoided by the design of new molecules like those proposed in the current contribution.

Phthalamides are one possible source of new ACEis. Phthalic anhydride derivatives are an important moiety in the development of new acetylcholinesterase inhibitors to treat a wide variety of neurodegenerative diseases such as Alzheimer's (Aliabadi et al. 2013; Si et al. 2016; Andrade-Jorge et al. 2018). Some phthalamides act as selective inhibitors of COX-2 with high affinity, giving better results than diclofenac (a nonsteroidal anti-inflammatory drug) (Alanazi et al. 2015), while others have been administered as anticonvulsant agents. Moreover, $\alpha_{1A/1D}$-AR subselective antagonists have been designed (Xu et al. 2015).

The aim of the present study was to design, characterize (in silico), synthesize, and evaluate (in vivo) a series of phthalamides as ACEis. The one molecule from this series that was tested in a spontaneous hypertensive rat (SHR) model was more potent than some of the current ACEis such as captopril.

Materials and methods

Theoretical calculations

The in silico calculations for the parameters of the absorption, distribution, metabolism, and excretion (ADME) profile, physicochemical properties and toxicity were carried out on the online server Molinspiration (Molinspiration Cheminformatics 2016), OSIRIS property explorer, and StarDrop software for all ligands in this job.

Docking

All molecules were modeled on GaussView 5.0.9 software and the protonation state was considered at physiological conditions (pH 7.4) for all ligands. The conformational analysis was performed on Gaussian 09 (Frisch et al. 2009) with a semi-empirical method (PM3). Docking conditions were programmed with AutoDock tools 1.5.4 and Raccoon (Forli et al. 2016) by utilizing a hybrid Lamarckian Genetic Algorithm (Morris et al. 1998), an initial population of 100 randomly placed individuals, Kollmann partial charges for all protein atoms, and Gasteiger charges for ligands. Ligands were prepared by adding all rotating bonds, torsional degrees of freedom, atomic partial charges, and nonpolar H-bonds with AutoDock tools 1.5.4. (Morris et al. 2009). The crystal structure of human ACE was downloaded from the Protein Data Bank (PDB code: 1O86) (Natesh et al. 2003). The grid box was set at $80 \times 60 \times 70$ with 0.375 Å spacing and the following coordinates: $X = 37.531$, $Y = 33.432$, and $Z = 44.336$. With AutoDock4 software in a Linux operative system (Fedora 22), the lowest energy conformations were obtained for each ligand bound to the enzyme, expressed as Gibbs free energy (ΔG), and the dissociation constant (Kd) and the $-\log_{(10)}$ dissociation constant (pKd) were ascertained for the complex showing the highest affinity. The number of

interactions, the binding distance, and type of binding were determined with the Visual Molecular Dynamics program (VMD v.1.8.6) (Humphrey et al. 1996).

Synthesis and characterization

The reagents and solvents were used as received from the commercial supplier (Sigma-Aldrich, St. Louis, MO, USA). All reactions were carried out in an oven-dried flask at the melting point of the starting material, agitating the mixtures with a stirring bar for a few minutes under solventless conditions (green chemistry). The solution was purified by utilizing the appropriate non-toxic solvent and then concentrated by means of a standard rotary evaporator. Melting points were measured on a Stuart® SMP40 automatic melting point apparatus and are uncorrected. Infrared spectra (IR) were obtained on a 100 FT-IR spectrometer (Perkin-Elmer) with a universal ATR accessory. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer with tetramethylsilane as an internal reference. The parameters reported are chemical shifts in ppm (δ), the integration area, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constants (Hz). Electrospray ionization (ESI) high-resolution mass spectrometry was performed on a Bruker micrOTOf-Q-II instrument.

General procedure for the synthesis of *N,N′*-substituted benzene-1,2-dicarboxamides (DD-01 is representative)

After placing 202.88 mg (1 mmol) of phthalic anhydride and 328.65 mg (2 mmol) of phenylethylamine in a 50-mL round-bottom flask, the mixture was agitated with a stirring bar and heated from 100 to 150 °C for 10–15 min to achieve gentle melting. The reaction was cooled at room temperature and monitored by thin layer chromatography. Subsequently, ethyl acetate (40 mL) was added and the mixture was sonicated until a white powder formed. The precipitate was filtered and washed three times with water (NaOH 0.1 M, pH 13).

In vivo evaluation

Ten-week-old male Wistar Kyoto (WKY) and spontaneously hypertensive rats (250–300 g) were obtained from the breeding colony of the Institute of Cell Physiology (UNAM). Rats were maintained in a pathogen-free environment under controlled conditions (22 ± 2 °C, 40–60% humidity, 12/12-h light/dark cycle), with food and water provided *ad libitum*. All procedures were approved by the Bioethics Committee of our institution and complied with the technical specifications of the Mexican Official Norm for the production, care and use of laboratory animals (NOM-062-ZOO-1999, Ministry of Agriculture).

All drugs were suspended in distilled water as the vehicle (VEH) and administered to rats intragastrically every 24 h for 32 days. The WKY rats formed the normotensive control group (WKY + VEH, $n = 4$). The SHR were randomly assigned to one of the following groups ($n = 4$): the hypertensive control group (SHR + VEH), reference group (SHR + CAPTOPRIL at 40 mg/kg), and experimental group (SHR + DD-01 at 10 mg/kg).

Blood pressure monitoring

Blood pressure (BP) was measured by a non-invasive indirect method by utilizing a tail-cuff device with a sensor and an inflatable latex ring (IITC Life Science Inc., Woodland Hills, CA, USA). During 1 week, rats were exposed to being inside a plastic restrainer at 37 °C before the evaluation of BP began. An average of seven BP readings was employed to establish systolic BP (SBP), diastolic BP (DBP), and mean arterial pressure (MAP). The measurements were recorded every week during the 4 weeks of the experiment.

Lethal dose 50 for mice

The lethal dose 50 (LD_{50}) was determined with Lorke's method. Briefly, CD1 male mice (20–25 g) were placed in three groups ($n = 3$), applying one dose (10, 100, or 1000 mg/kg) to each group of the test compounds (by intraperitoneal injection) to each animal per group. Observation of the animals for 24 h revealed that none had died. Therefore, three new groups were formed to administer higher doses (1200, 1400, and 1600 mg/kg), again finding no dead animals within 24 h (Lorke 1983; Chinedu et al. 2013).

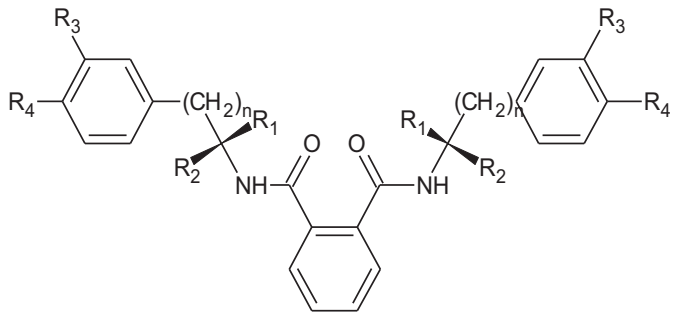
Statistical analysis

The data from the in vivo assays are expressed as the mean ± standard error of the mean (SEM). Each treatment group was compared with the corresponding control group. Repeated-measures analysis of variance (RM-ANOVA) and the Bonferroni post-test were carried out on GraphPad Prism statistical software, with significant differences considered at $p < 0.05$.

Results and discussion

Molecular docking and theoretical calculations

Twenty-three phthalamides derived from phthalic anhydride were proposed (Scheme 1) and their basic structures



Molecule	R_1	R_2	n	R_3	R_4
DD-01	H	H	1	H	H
DD-02	H	H	1	OH	OH
DD-03R	H	OH	1	OH	OH
DD-03S	OH	H	1	OH	OH
DD-04R	H	OH	1	H	H
DD-04S	OH	H	1	H	H
DD-05R	COOH	H	1	OH	OH
DD-05S	H	COOH	1	OH	OH
DD-06R	COOH	H	1	OCH_3	OH
DD-06S	H	COOH	1	OCH_3	OH
DD-07R	COOH	CH3	1	OH	OH
DD-07S	CH3	COOH	1	OH	OH
DD-08R	COOCH3	H	1	OH	OH
DD-08S	H	COOCH3	1	OH	OH
DD-09	CH3	CH3	0	OH	OH
DD-10R	H	OH	1	OCH3	OH
DD-10S	OH	H	1	OCH3	OH
DD-11	H	H	1	H	OH
DD-12A	H	H	1	OCH3	H
DD-12B	H	H	1	H	CH3
DD-13	H	H	1	OCH3	OCH3
DD-14R	H	CH3	0	H	H
DD-14S	CH3	H	0	H	H

Scheme 1 Proposed structure of the phthalamides

modeled with a semi-empirical method (PM3). They were then docked with ACE, obtaining ΔG, Kd, and pKd for the interactions between human ACE and the ligands, the latter comprising the test compounds and some reference drugs like benazepril, captopril, enalapril, and lisinopril (Table 1). Molecular docking results clearly show a high affinity of the test compounds for the enzyme, exceeding the capacity of some of the reference drugs. This is not enough for selecting the best candidate for synthesis and the in vivo test, in order to define the molecules with high probability to present in vivo effects, additional theoretical calculations were carried out with the online servers Molinspiration and OSIRIS property explorer to examine some physicochemical properties with Lipinski's rule of five (Lipinski et al. 1997), as well as the ADME profile and some toxicity parameters using StarDrop software, results are shown in Tables 2 and 3. The parameters estimated here are discussed briefly below in order to select the best candidates for the synthesis.

The octanol/water partition coefficient (LogP) is an important descriptor of molecular hydrophobicity and therefore serves as a parameter of absorption and

Table 1 Gibbs free energy (ΔG), the dissociation constant (Kd), and –log (10) dissociation constant (pK_d) for the interactions between the ligands and angiotensin-I-converting enzyme of human

Receptor			
Ligand	ΔG (kcal/mol)	Kd (μM)	pK_d
DD-01	−9.800	0.066	7.18
DD-02	−10.65	0.018	7.74
DD-03R	−9.660	0.082	7.08
DD-03S	−9.650	0.085	7.07
DD-04R	−9.290	0.156	6.81
DD-04S	−8.990	0.258	6.59
DD-05R	−8.080	1.200	5.92
DD-05S	−7.660	2.430	5.61
DD-06R	−7.760	2.050	5.69
DD-06S	−8.100	1.150	5.94
DD-07R	−8.010	1.350	5.87
DD-07S	−8.010	1.350	5.87
DD-08R	−8.550	0.543	6.26
DD-08S	−8.570	0.522	6.28
DD-09	−9.750	0.072	7.14
DD-10R	−9.900	0.056	7.25
DD-10S	−9.670	0.082	7.08
DD-11	−10.60	0.017	7.77
DD-12A	−10.88	0.011	7.97
DD-12B	−10.10	0.040	7.40
DD-13	−9.900	0.055	7.26
DD-14R	−9.170	0.191	6.72
DD-14S	−9.500	0.109	6.96
Benazepril	−10.67	0.015	7.82
Captopril	−7.120	6.010	5.22
Enalapril	−10.79	0.012	7.91
Lisinopril	−10.81	0.012	7.93

bioavailability. Although Lipinski suggested that the value must not be over 5.0, a negative number also represents low hydrophobicity and poor absorption, leading to the exclusion of some of the proposed molecules (DD-03R, DD-03S, DD-07R, and DD-07S, among others see Table 2). LogS (aqueous solubility) refers to solubility measured in mol/L, where values >−4 are desirable since it has been seen that >80% of all drugs on the market with these values present good absorption. Therefore, this parameter is a good descriptor of absorption. Some molecules had values >2.1 and were eliminated (DD-08R, DD-08S, DD-09, DD-10R, and DD-10S). Polar surface area (PSA) is a descriptor that has been found to correlate with the passive transport of molecules through membranes. Based on the recommendation that values should be <120, additional molecules were discarded (DD-02, DD-03R, DD-03S, and others). Furthermore, analysis was made of molecular weight (MW), the number of hydrogen bond acceptors (HBA) and the number of hydrogen bond donors (HBD). Lipinski pointed out that for good absorption, molecules must have a MW < 500, no >5 HBD and <10 HBA. The few molecules in accordance with these values were included in the next screening.

In drug development, the inclusion of the ADME profile is obligatory (see Table 3). The first parameter evaluated was human intestinal absorption (HIA), an indicator of the passive absorption from the intestine into the hepatic portal vein (without considering the effect of first pass metabolism). This parameter provided evidence of poor HIA for most of the test compounds (Table 3). Acceptable HIA values (absorption ≥ 30%) are desirable but insufficient to describe a good oral bioavailability. Greater plasma protein binding (PPB90) reduces the capacity of compounds to traverse cell membranes and therefore may affect drug efficiency. Only a few molecules exhibited strong binding (over 90%) to plasma proteins. Therefore, those molecules were discarded since the less bound a drug is, the more efficiently it can traverse cell membranes. Since metabolism is a crucial parameter of pharmacokinetic studies, two relevant isoforms of cytochrome P450 were herein tested for affinity to the ligands using the StarDrop software. Isoform 2C9 is responsible for the phase I metabolism of up to 20% of all drugs, mainly through oxidation (Van Booven et al. 2010). Isoform P450 2D6, located in the liver and brain (Wang et al. 2009), participates in the metabolism of up to 25% of the current clinically used drugs. Both of these isoforms are involved in the metabolism of aromatic rings, which are present in all the proposed molecules. All molecules were found to have similar values of affinity for isoform 2C9, with an average of 5.72. For isoform 2D6, most of the molecules displayed low affinity (see Table 3). P-glycoprotein, a protein belonging to the superfamily of ATP-binding cassette (ABC) transporters, is implicated in the absorption and disposal of drugs, acting as a pump to remove xenobiotics from cells (Lin and Yamazaki 2003). Accordingly, a determination was made as to whether the compounds in the series of phthalimides are a substrate of this protein, and if absorption is thus affected. Such was true for many of the test compounds. Finally, blood–brain barrier penetration (BBB log([brain]:[blood])) and blood–brain barrier penetration category (BBB category) are two relevant parameters in the development of central nervous system (CNS) drugs. However, ACEis do not need to cross the BBB to exert their action, and the present results indicated that they are unlikely to do so. In addition, 57 toxicity parameters were assessed by using the Derek Nexus module with StarDrop software (Table 4). The data reveal three negative properties for some ligands, including the following presumptive toxicity: skin sensitization, hepatotoxicity, and chromosome damage in vitro, such as DD-02, DD-03R/S,

Table 2 Physicochemical properties based on the Lipinski's rules of five

LIGAND	LogP < 5	LogS (−5 a 1)	PSA < 120	MW (g/mol) < 500	HBA < 10	HBD < 5	nrotb < 10
DD-01	3.634	1.08	58.2	372.5	4	2	10
DD-02	1.781	2.43	139.1	436.5	8	6	10
DD-03R	−0.2098	3.219	179.6	468.5	10	8	10
DD-03S	−0.2098	3.219	179.6	468.5	10	8	10
DD-04R	1.888	2.586	98.66	404.5	6	4	10
DD-04S	1.888	2.586	98.66	404.5	6	4	10
DD-05R	0.7275	2.553	213.7	524.5	12	8	12
DD-05S	0.7275	2.553	213.7	524.5	12	8	12
DD-06R	1.116	2.286	191.7	552.5	12	6	14
DD-06S	1.116	2.286	191.7	552.5	12	6	14
DD-07R	−1.311	2.521	220	556.6	12	10	12
DD-07S	−1.311	2.521	220	556.6	12	10	12
DD-08R	1.371	2.318	191.7	552.5	12	6	14
DD-08S	1.371	2.318	191.7	552.5	12	6	14
DD-09	2.215	2.142	139.1	464.5	8	6	8
DD-10R	0.3713	3.004	157.6	496.5	10	6	12
DD-10S	0.3713	3.004	157.6	496.5	10	6	12
DD-11	2.831	1.673	98.66	404.5	6	4	10
DD-12A	3.465	1.51	76.66	432.5	6	2	12
DD-12B	3.465	1.51	76.66	432.5	6	2	12
DD-13	3.018	2.023	95.12	492.6	8	2	14
DD-14R	3.369	0.9025	58.2	372.5	4	2	8
DD-14S	3.369	0.9025	58.2	372.5	4	2	8

LogP octanol/water partition coefficient, *LogS* aqueous solubility, *PSA* polar surface area, *MW* molecular weight, *HBA* number of hydrogen bond acceptors, *HBD* number of hydrogen bond donors, *nrotb* number of rotatable bonds

Table 3 ADME profile

LIGAND	HIA	PPB90	2C9 pKi	2D6 affinity	P-gp	BBB log([brain]:[blood])	BBB category
DD-01	+	High	5.447	Medium	Yes	−0.2858	–
DD-02	–	High	5.606	Low	Yes	−0.6993	–
DD-03R	–	Low	5.620	Low	No	−0.8803	–
DD-03S	–	Low	5.620	Low	No	−0.8803	–
DD-04R	+	Low	5.145	Low	Yes	−0.5687	–
DD-04S	+	Low	5.145	Low	Yes	−0.5687	–
DD-05R	–	Low	5.715	Low	Yes	−1.2720	–
DD-05S	–	Low	5.715	Low	Yes	−1.2720	–
DD-06R	–	Low	5.915	Low	Yes	−1.3810	–
DD-06S	–	Low	5.915	Low	Yes	−1.3810	–
DD-07R	–	Low	5.793	Low	Yes	−1.5030	–
DD-07S	–	Low	5.793	Low	Yes	−1.5030	–
DD-08R	+	Low	5.737	Low	Yes	−0.9135	–
DD-08S	+	Low	5.737	Low	Yes	−0.9135	–
DD-09	–	High	5.904	High	Yes	−0.7201	–
DD-10R	–	Low	5.985	Low	Yes	−0.9973	–
DD-10S	–	Low	5.985	Low	Yes	−0.9973	–
DD-11	+	High	5.65	Medium	Yes	−0.6184	–
DD-12A	+	High	5.896	Medium	No	−0.4444	–
DD-12B	+	High	5.852	Medium	No	−0.4444	–
DD-13	–	low	6.118	Low	Yes	−0.4697	–
DD-14R	+	High	5.675	Medium	No	−0.2397	–
DD-14S	+	High	5.675	Medium	No	−0.2397	–

HIA: "+" indicates absorption ≥ 30% and "−" absorption < 30%

PPB90: "Low" denotes < 90% and "high" ≥ 90% of the compound bound to plasma protein

2D6 affinity: "Low" refers to a pKi < 5, "medium" a pKi in the range 5–6, "high" in the range 6–7, and "very high" > 7

BBB category: "+" means a ratio ≥ −0.5 and "−" a ratio < −0.5

HIA human intestinal absorption, *PPB90* plasma protein binding (90% threshold), *2C9 pKi* cytochrome P450 CYP2C9 affinity, *2D6* cytochrome P450 CYP2D6 affinity, *P-gp* P-glycoprotein substrate, *BBB log([brain]:[blood])* blood–brain barrier penetration, *BBB category* blood–brain barrier penetration category

Table 4 Properties of toxicity evaluated by using the Derek Nexus module with StarDrop software

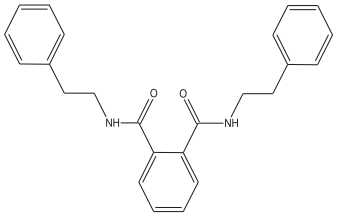
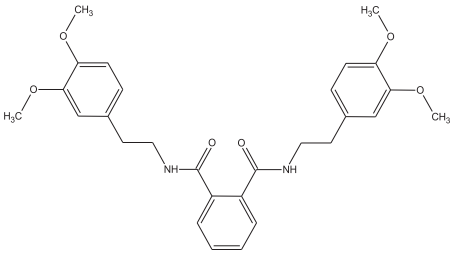
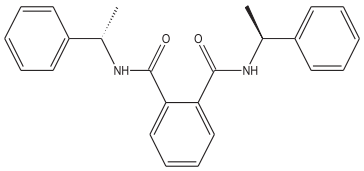
Carcinogenicity, irritation of the skin, phototoxicity, kidney function-related toxicity, photocarcinogenicity, lachrymation, cholinesterase inhibition, nephrotoxicity, chromosome damage in vitro, anaphylaxis, neurotoxicity, ocular toxicity, chromosome damage in vivo, blood in urine, adrenal gland toxicity, pulmonary toxicity, photo-induced chromosome damage in vitro, cerebral edema, bladder disorders, splenotoxicity, mutagenicity in vitro, chloracne, bladder urothelial hyperplasia, thyroid toxicity, mutagenicity in vivo, cyanide-type effects, bone marrow toxicity, urolithiasis, photomutagenicity in vitro, high acute toxicity, cumulative effect on white cell count and immunology, developmental toxicity, nonspecific genotoxicity in vitro, methaemoglobinaemia, bradycardia, teratogenicity, nonspecific genotoxicity in vivo, mitochondrial dysfunction, cardiotoxicity, testicular toxicity, photo-induced nonspecific genotoxicity in vitro, uncoupler of oxidative phosphorylation, HERG channel inhibition in vitro, occupational asthma, photo-induced nonspecific genotoxicity in vivo, oestrogenicity, hepatotoxicity, respiratory sensitization, eye irritation, peroxisome proliferation, alpha-2-mu-globulin nephropathy, photoallergenicity, irritation of the gastrointestinal tract, phospholipidosis, kidney disorders, skin sensitization, irritation of the respiratory tract.

DD-05R/S, DD-06R/S, DD-12A, DD-12B, and others, except those molecules selected (DD-01, DD-13, and DD-14S).

As can be appreciated, theoretical analysis is a vital tool for drug discovery. Three molecules were chosen in accordance with the best corresponding scores obtained in the in silico study (docking, physicochemical properties, ADME profile, and toxicity parameters) (see Table 5), which indicated a high probability of producing the best effect in vivo. The next step was the examination of the binding mode of the three molecules chosen (Table 5) and also the references molecules with the ACE.

The main amino-acid residues involved in ligand-ACE recognition for the test compounds and some reference drugs were identified (Table 6). Some amino-acid residues are the same for the phthalamides and the reference drugs like Lys511, His353, Tyr523, His387, and the Zinc atom (Zn701), and also the kind of interaction are shown in Table 7, as well as the binding distances. Thee residues are very important for the inhibition of ACE (Natesh et al. 2003). The binding mode with ACE turned out to be very similar for the three selected test compounds and lisinopril (Fig. 1). Moreover, these test compounds and the reference drugs (benazepril, captopril, enalapril, and lisinopril) exhibited the same pattern in relation to the binding distances with the amino-acid residues of the enzyme (Figs. 2 and 3). The validation of the molecular docking was performed by comparison of docked and crystallographic lisinopril, obtaining the same pattern of interaction with the receptor for both molecules (Fig. 1d).

Table 5 Structure of the synthesized phthalamides and analyzed in the molecular approach with the angiotensin-I-converting enzyme

Nomenclature	Structure
DD-01	
DD-13	
DD-14S	

It can be concluded that the binding site for the three selected phthalamides is the catalytic active site of ACE. In addition, the principal interactions between these phthalamides and ACE are very similar to those described for the reference molecules. All the information above allowed us to select three molecules for the synthesis, since these molecules may act as good inhibitors, and estimate their lethal doses 50.

Chemical characterization

N,N′-bis(2-phenylethyl)phthalamide (DD-01)

A white solid was obtained in 90% yield; mp 167–168 °C; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 2.89 (4H, t, H-10,10'), 3.62 (4H, c, H-9,9'), 6.79 (2H, s, NH), 7.24 (6H, m, H-12,12',14,14',16,16'), 7.29 (4H, m, H-13,13',15,15'), 7.39 (2H, m, H-4,5), 7.46 (2H, m, H-3,6); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 134.6 (C-1,2), 128.4 (C-3,6), 130.1 (C-4,5), 169.1 (C-7,8), 41.3 (C-9,9'), 36.5 (C-10,10'), 138.6 (C-11,11'), 128.6 (C-12,12',16,16'), 128.7 (C-13,13',15,15'), 126.5 (C-14,14'). **IR (ATR, cm^{-1}) ύ:** 3254 (N-H), 3076 (C-H, Aromatic), 2929 (C-H, Aliphatic), 1626 (C=O), 1575 (C=C), 1442 (CH_2), 1313 (C-N). **MS (*m/z*):** 395.1713 (M +Na).

Table 6 Amino-acid residues involved in the interaction between angiotensin-I-converting enzyme of human and the ligands: phthalamides and reference molecules

Ligand	Residues
DD-01	Zn701, Lys511, His353, Tyr523, His387, Tyr520, His513, Ser513, Glu384, Ala354, Phe457, Glu162, Glu411, His383, Val380
DD-13	Zn701, Lys511, Tyr523, Tyr520, Phe457, His387, His383, His353, Ala354, Ser355, Glu384, Glu162
DD-14S	Zn701, Lys511, His353, Tyr523, Glu384, His383, Glu162, Phe457, Gln281, Trp279, Tyr520, His513
Benazepril	Zn701, Lys511, His353, Tyr523, Glu384, Ser355, His387, Ala354, His513, Tyr520, Glu162, Gln281
Captopril	Zn701, Lys511, Tyr523, Gly200, Glu384, Tyr520, His513, Gln281, Phe457
Enalapril	Zn701, Lys511, His353, Tyr523, Glu384, Ala356, His387, Glu411, Val518, His513, Tyr520, Phe512, Phe457, Gln281
Lisinopril	Zn701, Lys511, His353, Tyr523, His387, Glu384, Arg522, Glu411, His383, Ala354, Tyr520, His513, Phe512, Gln281

Table 7 Binding distances between amino-acid residues involved in the interaction and the selected ligands

Ligand	Interactions	
DD-01	4 H-bond:	5 π-π interactions:
		His387:C----C:DD-01 to 4.34 Å
	Tyr520:H----O:DD-01 to 1.92 Å	His383:C----C:DD-01 to 5.59 Å
	His353:H----O:DD-01 to 2.09 Å	His353:C----C:DD-01 to 3.94 Å
	His513:H----O:DD-01 to 2.37 Å	Phe457:C----C:DD-01 to 5.27 Å
	Lys511:H----O:DD-01 to 1.72 Å	Tyr523:C----C:DD-01 to 3.32 Å
	2 Hydrophobic interactions:	1 π-cation interactions:
	Val380:C----C:DD-01 to 5.44 Å	Zn701:Zn----C:DD-01 to 3.07 Å
	Ala354:C----C:DD-01 to 4.61 Å	
	2 π-anion interactions:	
	Glu411:O----C:DD-01 to 4.69 Å	
	Glu162:O----C:DD-01 to 3.07 Å	
DD-13	3 H-bond:	3 π-π interactions:
		His383:C----C:DD-13 to 5.67 Å
	Tyr520:H----O:DD-13 to 1.99 Å	Phe457:C----C:DD-13 to 4.97 Å
	His353:H----O:DD-13 to 2.47 Å	Tyr523:C----C:DD-13 to 3.51 Å
	Lys511:H----O:DD-13 to 1.84 Å	
	3 Hydrophobic interactions:	1 π-cation interactions:
	Ala354:C----C:DD-13 to 5.02 Å	Zn701:Zn----C:DD-13 to 3.38 Å
	His387:C----C:DD-13 to 3.75 Å	
	Ser355:C----C:DD-13 to 3.36 Å	
	2 π-anion interactions:	
	Glu384:O----C:DD-13 to 3.53 Å	
	Glu162:O----C:DD-13 to 4.61 Å	
DD-14S	4 H-bond:	4 π-π interactions:
		His383:C----C:DD-14S to 5.71 Å
	Tyr520:H----O:DD-14S to 2.16 Å	His353:C----C:DD-14S to 3.68 Å
	Gln281:H----O:DD-14S to 2.15 Å	Phe457:C----C:DD-14S to 4.93 Å
	His513:H----O:DD-14S to 2.05 Å	Tyr523:C----C:DD-14S to 3.35 Å
	Lys511:H----O:DD-14S to 1.81 Å	
	1 Hydrophobic interaction:	1 π-cation interactions:
	Trp279:C----C:DD-14S to 3.58 Å	Zn701:Zn----C:DD-14S to 2.33 Å
	2 π-anion interactions:	
	Glu384:O----C:DD-14S to 4.68 Å	
	Glu162:O----C:DD-14S to 2.88 Å	

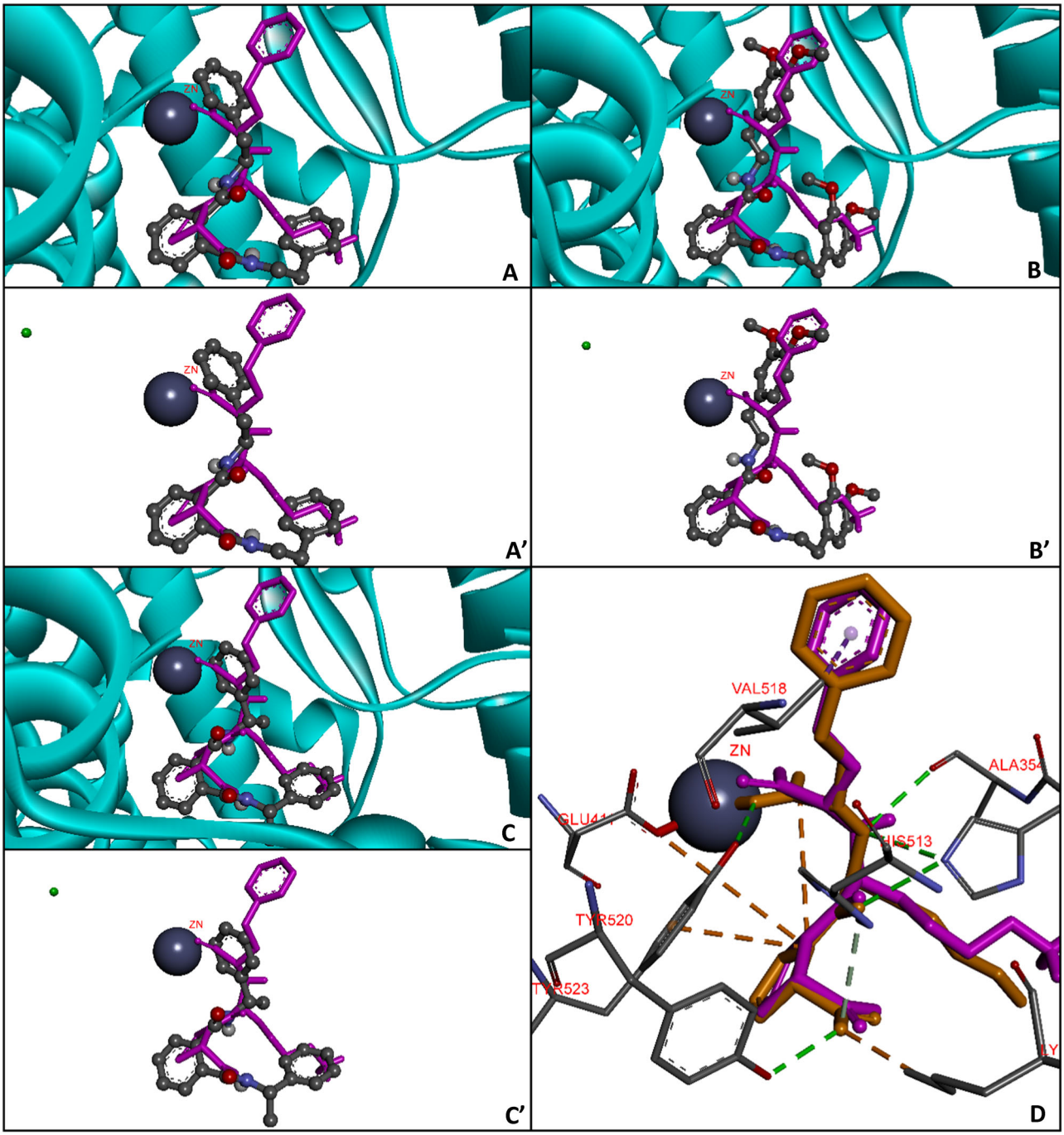


Fig. 1 Binding mode of the ligands in the catalytic active site of the human ACE established by molecular docking: **a** Binding site of DD-01 and lisinopril (fuchsia); **a'** the binding mode of DD-01 was similar to that of lisinopril (fuchsia); **b** binding site of DD-13 and lisinopril (fuchsia); **b'** a similar binding mode was found for DD-13 and lisinopril (fuchsia); **c** binding site of DD-14S and lisinopril (fuchsia); **c'** a similar binding mode was observed for DD-14S and lisinopril (fuchsia); **d** reproduction of the binding mode of lisinopril obtained by molecular docking (fuchsia) and by crystal structure (ocher; PDB code 1O86) (color figure online)

N,N'-bis[2-(3,4-dimethoxyphenyl)ethyl]phthalamide (DD-13)

The procedure gave a white solid in 92% yield; mp 147–148 °C; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 2.83 (4H, t, H-10,10'), 3.61 (4H, dd, H-9,9'), 3.83 (6H, s, H-17,17'), 3.84 (6H, s, H-18,18'), 5.28 (2H, s, NH), 6.77 (6H, m, H-12,12',15,15',16,16'), 7.41 (2H, m, H-3,6), 7.45 (2H, m, H-4,5); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 134.71 (C-1,2), 128.15 (C-3,6), 130.19 (C-4,5), 169.14 (C-7,8), 41.56 (C-9,9'), 35.11 (C-10,10'), 131.11 (C-11,11'), 111.30 (C-12,12'),

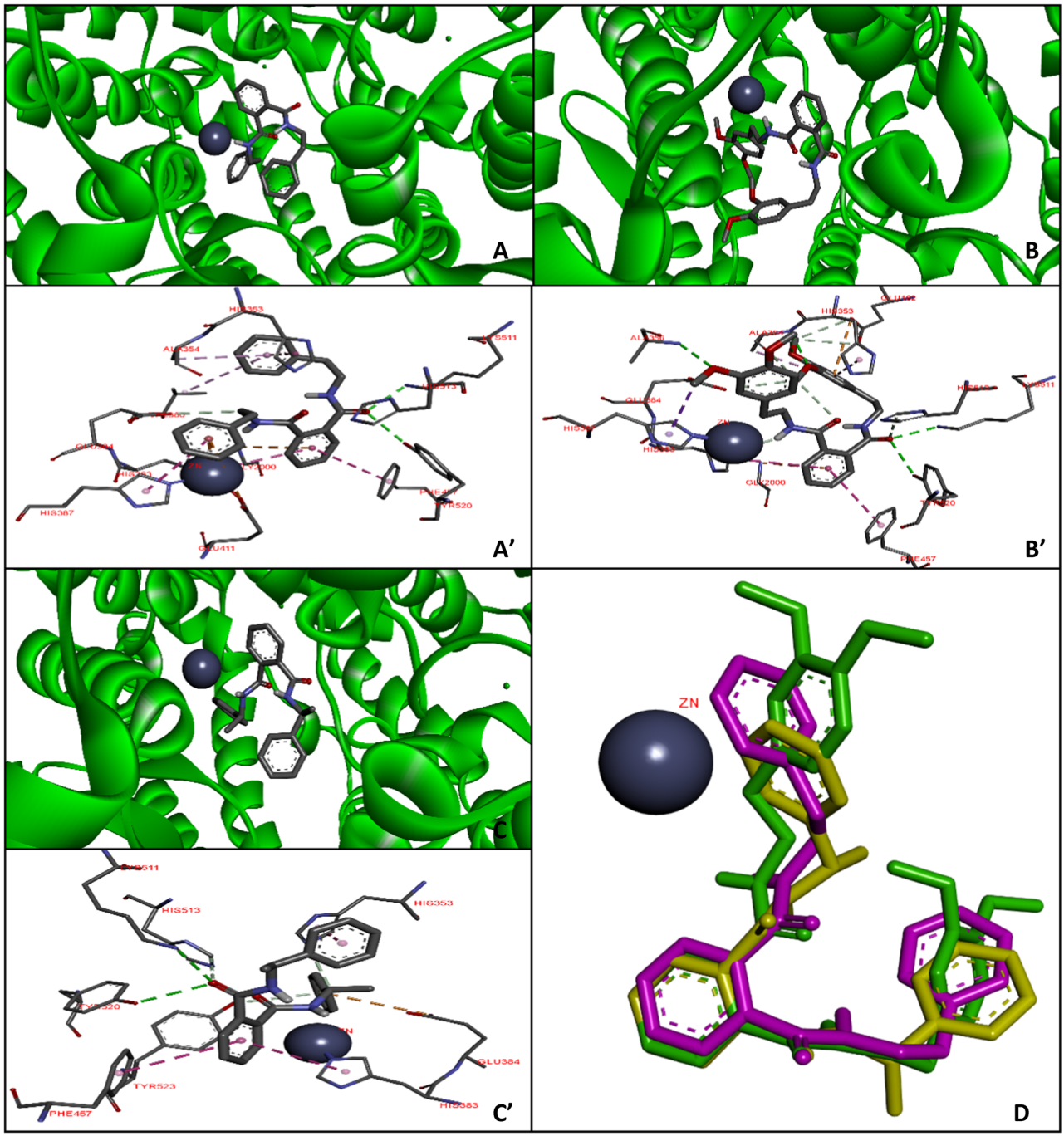


Fig. 2 Binding mode and main amino-acid residues in the catalytic active site of the human ACE when interacting with the ligands (observed by docking simulation): **a** Binding site of DD-01; **a'**) amino-acid residues interacting with DD-01; **b** binding site of DD-13; **b'** amino-acid residues interacting with DD-13; **c** binding site of DD-14S; **c'** amino-acid residues interacting with DD-14S; **d** binding mode of DD-01 (fuchsia), DD-13 (green), and DD-14S (yellow), each with great similarity to the others (color figure online)

148.94 (C-13,13'), 147.64 (C-14,14'), 111.87 (C-15,15'), 120.62 (C-16,16'), 55.84 (C-17,17'), 55.87 (C-18,18'). **IR (ATR, cm^{-1}) ύ:** 3238 (N-H), 3083 (C-H, Aromatic), 2928 (C-H, Aliphatic), 2836 (O-CH_3, Aliphatic), 1618 (C=O), 1590 (C=C), 1453 (CH_2), 1262 (C-N), 1239 (O-CH_3). **MS (m/z):** 515.2083 (M+Na).

N,N'-bis[(1S)-1-phenylethyl)phthalamide (DD-14S)

The procedure afforded a white solid in 89% yield; mp 218–219 °C; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.37 (6H, d, H-10,10'), 5.05 (1H, q, H-9,9'), 7.20 (2H, m, H-3,6), 7.27 (4H, m, H-13,13',15,15'), 7.35 (4H, m, H-12,12',16,16'),

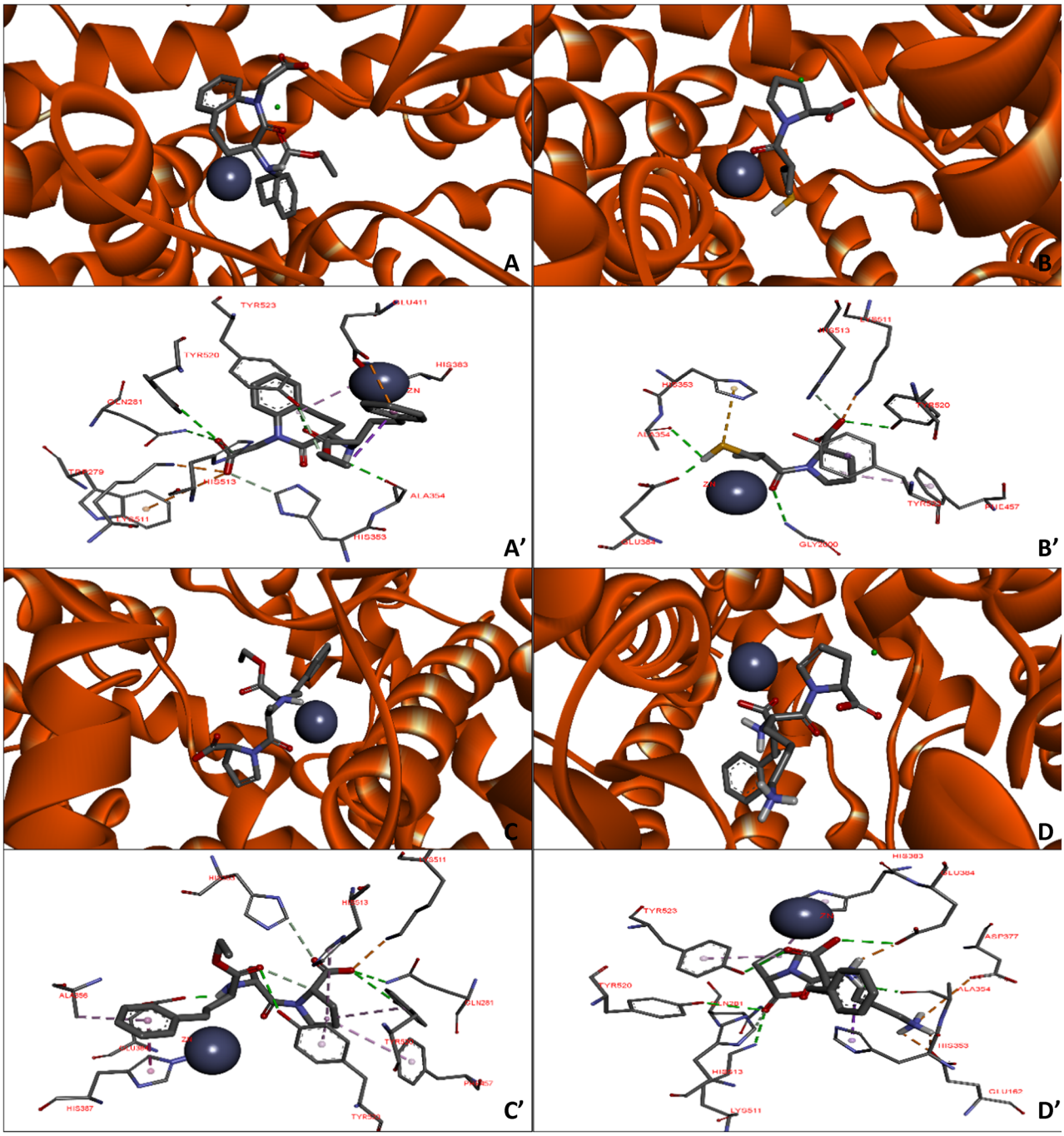
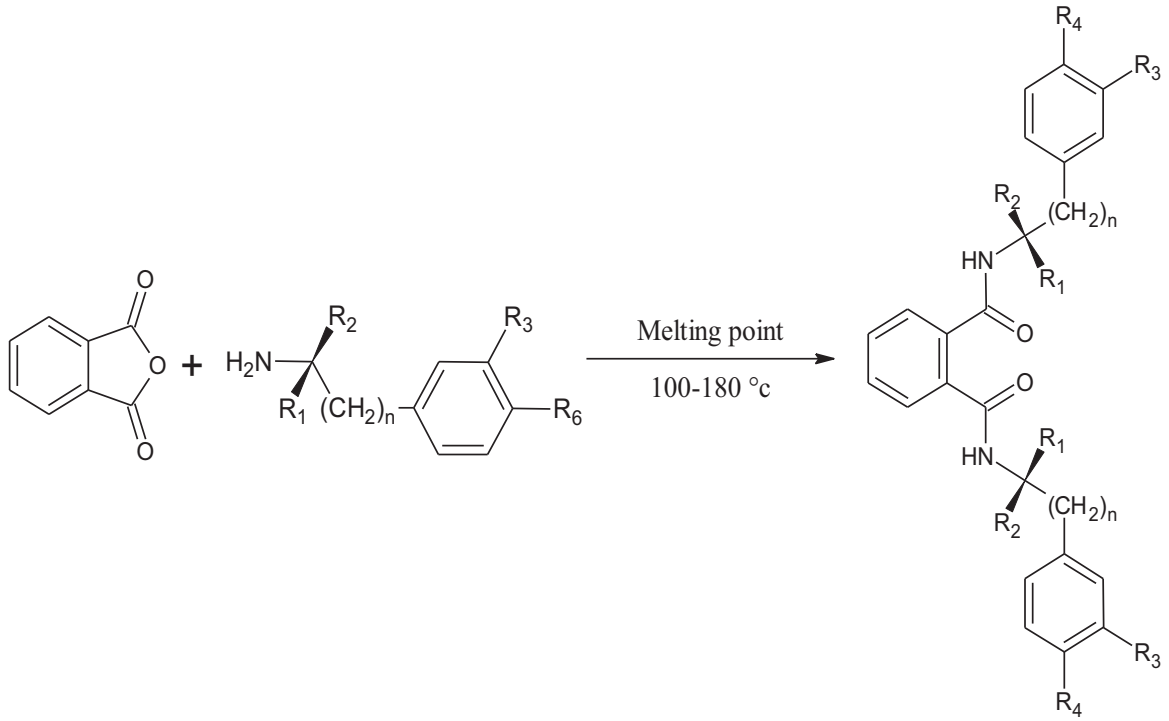


Fig. 3 Binding mode and main amino-acid residues in the catalytic active site of the human ACE when interacting with the reference drugs (observed by docking simulation): **a** Binding site of benazepril; **a'** amino-acid residues interacting with benazepril; **b** binding site of captopril; **b'** amino-acid residues interacting with captopril; **c** binding site of enalapril; **c'** amino-acid residues interacting with enalapril; **d** binding site of lisinopril; **d'** amino-acid residues interacting with lisinopril

7.45 (2H, m, H-14,14'), 7.46 (2H, m, H-4,5), 8.71 (2H, d, NH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 136.6 (C-1,2), 126.9 (C-3,6), 129.6 (C-4,5), 167.7 (C-7,8), 48.7 (C-9,9'), 22.9 (C-10,10'), 145.0 (C-11,11'), 126.4 (C-12,12',16,16'), 128.6 (C-13,13',15,15'), 128.1 (C-14,14'). **IR (ATR, cm^{-1}) ύ:** 3291 (N-H), 3029 (C-H, Aromatic), 2976 (C-H, Aliphatic), 1624 (C=O), 1542 (C=C), 1450 (CH_2), 1332 (C-N). **MS (*m/z*):** 395.1719 (M+Na).

Three phthalamide derivate of phenylethylamines were synthesized based on the green chemistry principles (Scheme 2). This strategy incorporates almost all reagents with good yield in very short times (10–15 min) without any



Scheme 2 The synthetic route under solventless conditions for the three molecules selected based on their favorable properties

solvent or catalytic reagent, with this technique we can be synthesizing up to 5 g each time, and also the purification is easy just basic water and ethyl acetate. Our new technique allows complying with at least nine principles of green chemistry using just the melting point of the reagents.

LD_{50} on mice

Acute toxicity of the three synthesized compounds was assessed by the LD_{50} on CD1 male mice by following Lorke's method. The LD_{50} for all compounds was >1600 mg/kg, which represents >160 times the dose used for the in vivo experiment. Hence, phthalic derivatives can be proposed for continued research on their potential as drugs with low toxicity and a potent antihypertensive effect.

In vivo evaluation

The SHR is the most common model for testing hypertension. Although the physiopathology of the increase in BP in these rats is still undefined, some evidence suggests that Ang II plays an essential role (Sueta et al. 2014). The RAS comprises systemic and local activity in several tissues such as the heart, lung, adrenal gland, kidney, blood vessels, and brain. The complex mechanisms of RAS activate several functions after ACE catalyzes Ang I to form Ang II. The interaction of Ang II with the AT_1 receptor triggers vasoconstrictor, trophic, proliferative and pro-thrombotic activity (Herichova and Szantoova 2013). However, excessive activity of Ang II has been implicated in hypertension and cardiovascular disease.

Ang II elevates BP through several pathways, the most important ones being vasoconstriction, sympathetic stimulation, and increased aldosterone biosynthesis and renal activity (Fyhrquist et al. 1995). Consequently, hypertension is commonly controlled by inhibiting the synthesis of this peptide. Indeed, the first-line drugs for the control of hypertension are ACEis and Ang II receptor blockers. ACEis decrease BP by preventing smooth muscle constriction in the vasculature and by reducing the release of aldosterone. Nevertheless, the recommendation has been made to use a different pathway that can enhance the activity of ACE/Ang (1–7)/MasR axis (Maia et al. 2004).

Based on the molecular docking results, the theoretical assessment of the physicochemical properties, and the determination of the ADME profile of the three molecules synthesized, the one with the best properties (DD-01) was selected. Thus, an in vivo evaluation of DD-01 and captopril (as the reference drug) was carried out with the SHR model. Compared with the normotensive control group (WKY + VEH), the hypertensive control group (SHR + VEH) exhibited higher SBP (Fig. 4), DBP (Fig. 5) and MAP (Fig. 6). DD-01 (10 mg/kg) significantly diminished all three parameters as of week 1 of treatment and for the subsequent 3 weeks. Captopril (40 mg/kg) presented the same pattern. The mol to mol comparison of both molecules

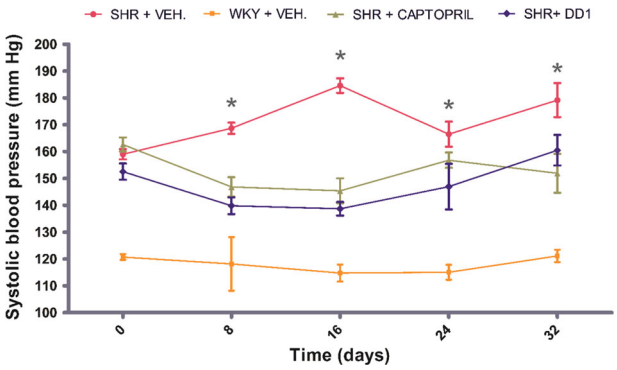


Fig. 4 Effect of DD-01 on the systolic blood pressure of male spontaneously hypertensive rats (SHRs). *Significant difference versus the SHR group treated with the vehicle (SHR + VEH). Data are expressed as the mean ± SEM ($n \geq 4$). Repeated-measures analysis of variance (RM-ANOVA) and the Bonferroni post-test were used for evaluating statistical significance ($p < 0.05$)

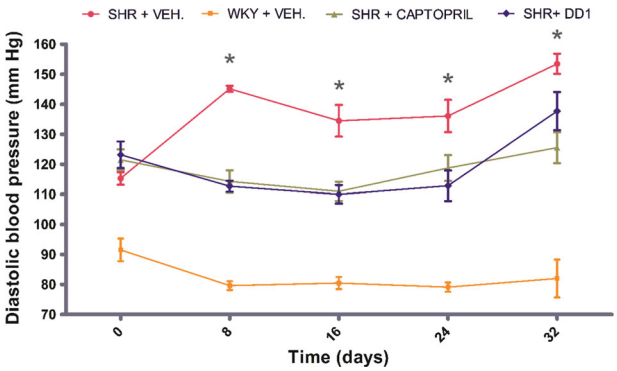


Fig. 5 Effect of DD-01 on the diastolic blood pressure of male spontaneously hypertensive rats (SHRs). *Significant difference versus the SHR group treated with the vehicle (SHR + VEH). Data are expressed as the mean ± SEM ($n \geq 4$). Repeated-measures analysis of variance (RM-ANOVA) and the Bonferroni post-test were used for evaluating statistical significance ($p < 0.05$)

revealed that DD-01 is 6.85-fold more potent than captopril. These two compounds produce an equivalent effect.

However, the normotensive values of the WKY + VEH group were not reached by either captopril or DD-01. These findings concur with those of other studies reporting a decrease of 40–50% in BP after ACEi monotherapy. ACEis administered in combination with diuretics or calcium antagonists reduced BP by 80–90% (Cheung et al. 2009). According to some researchers, the relatively low efficacy of ACEis in diminishing BP implies that chymase is the primary enzyme involved in forming Ang II from Ang I in humans (Dell'Italia and Husain 2002). Although the inhibition of chymase limits the synthesis of Ang II, it does not control BP in the short term, possibly because chymase inhibitors, unlike ACEis, only decrease Ang II locally and not systemically (Roszkowska-Chojecka et al. 2015).

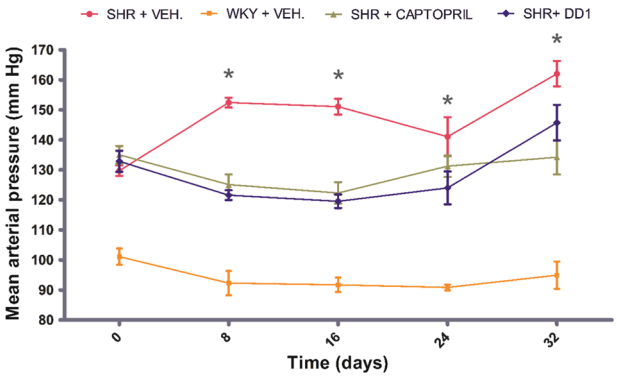


Fig. 6 Effect of DD-01 on the mean arterial pressure of male spontaneously hypertensive rats (SHRs). *Significant difference versus the SHR group treated with the vehicle (SHR + VEH). Data are expressed as the mean ± SEM ($n \geq 4$). Repeated-measures analysis of variance (RM-ANOVA) and the Bonferroni post-test were used for evaluating statistical significance ($p < 0.05$)

Conclusions

The design of new molecules with potential action on ACE is a worthwhile strategy to find alternative treatments for hypertension, especially if green chemistry is involved in their synthesis. Three molecules were herein selected by in silico analysis of their physicochemical properties, Gibbs free energy, and ADME profile. Their structures were confirmed by all spectrometry and spectroscopy methods. After finding low toxicity for these selected molecules, one was chosen for testing on the SHR model. It exhibited an effect similar to that of captopril but is ~7-fold more potent. Therefore, we propose further studies to explore the inhibitory activity of this and related phthalimide derivatives.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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