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ACE inhibitors hypothesis generation for selective design, synthesis and biological evaluation of 3-mercapto-2-methyl-propanoyl-pyrrolidine-3-imine derivatives as antihypertensive agents

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ABSTRACT

A series of new 3-mercapto-2-methyl-propanoyl-pyrrolidine derivatives (**V**, **VIa–e**) were designed. A new validated ACE inhibitors pharmacophore model (hypothesis) was generated for the first time in this research from the biologically active (frozen) conformation of Lisinopril–Human ACE complex that was downloaded from PDB, using stepwise technique of CATALYST modules. The molecular modeling compare–fit study of the designed molecules (**V**, **VIa–e**), with such ACE inhibitors hypothesis was fulfilled,and several compounds showed significant high simulation fit values. The compounds with high fit values were synthesized and biologically evaluated in vivo as hypotensive agents. It appears that the in vivo hypotensive activity of compounds **V**, **VIa**, **VIb**, and **VIe** was consistent with their molecular modeling results, and compound **VIe** showed the highest activity in comparison to Captopril.

1. Introduction

Renin is a proteolytic enzyme produced mainly in the juxta-glomerular apparatus of the kidney, which acts on the circulating R-globulin angiotensinogen produced by the liver resulting in the formation of angiotensin I (Ang I), a decapeptide that has very little biological activity. Angiotensin-converting enzyme (ACE) then converts Ang I into the octapeptide Ang II,[1,2] a potent vasoconstrictor that plays an integral role in the pathophysiology of hypertension,[3–5] through sympathetic Ang II receptor activity.[6] This directed many researchers toward designing drugs to inhibit the effects of ACE.[7]

ACE inhibitors have gained wide acceptance clinically and are commonly prescribed for the treatment of hypertension, congestive heart failure,[8,9] protection against hypertension-related organ damage,[10,11] The first breakthrough in the area of ACEIs was Captopril®,[12] which was launched in 1981 for hypertension management. Furthermore new ACEI leads; **1**,[13] **2**,[13] **3**,[14] **4**,[15] **5**,[15] and Lisinopril[16] (Fig. 1) were also developed to have more activity as antihypertensive agent than Captopril. Our current investigation is based on developing new Me-Too ACEIs bearing pyrrolidine ring system which possess potential antihypertensive activity.

The design of new 3-mercapto-2-methyl-propanoyl-pyrrolidine derivatives, as ACE inhibitors, was based on structural modification of the lead compound; Captopril, aiming to get optimum activity. These modifications were performed through the following strategies: considering the SAR of the lead compounds that have ACE inhibitor activity, prompted us to retain the sulfhydryl-side chain linked to pyrrolidine ring system. This is because, it was reported that SH function of the ACE inhibitor could strikingly bind to Zn ion (present in the skeleton of the ACE).[13,16] In addition, the *p*-methoxybenzyl moiety as well as substituted imino functions was introduced onto the pyrrolidine ring system, in order to get the essential lipophilic pharmacophores in the designed molecules (**V** and **VIa–e**).[13] Meanwhile, such lipophilic features could improve the pharmacokinetic properties and potencies of these molecules.[13] Furthermore, the design of the proposed molecules (**V** and **VIa–e**) was also, based upon molecular modeling studies between a newly generated ACEIs pharmacophore model (hypothesis) and these test set proposed molecules (**V** and **VIa–e**). In this research, the ACEIs hypothesis was generated for the first time from the docked bioactive conformer of Lisinopril molecule that was complexed with the Human ACE. Such complex was downloaded from the website of PDB and the docked conformer of Lisinopril was selected and used without any conformational changes (as a frozen bioactive conformation), to generate the ACEIs hypothesis using CATALYST modules adopting stepwise technique. Also, such hypo-

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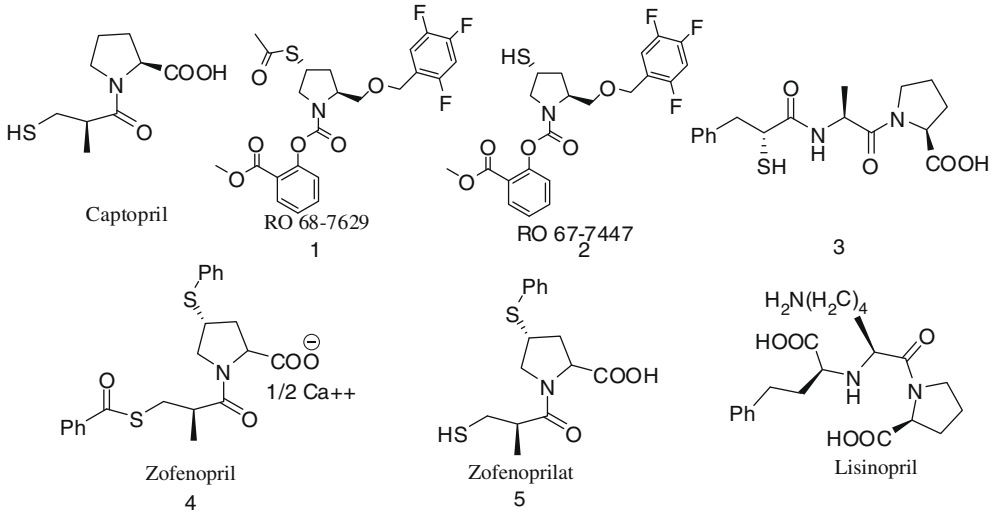
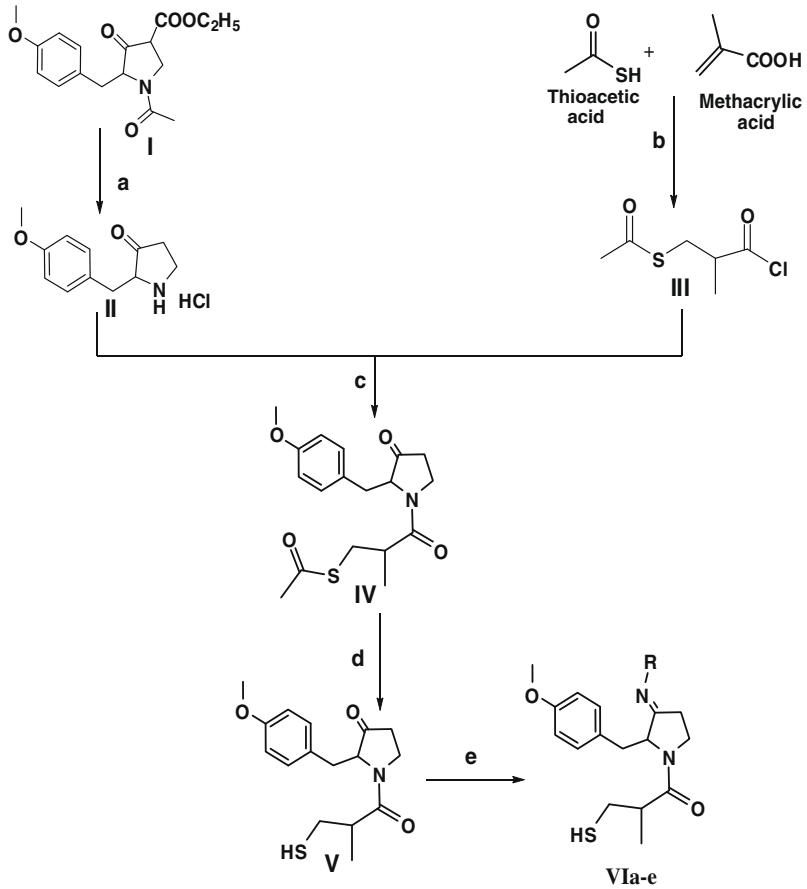


Figure 1. The structures of the lead ACE inhibitors.

thesis was perfectly validated and used for virtual studies to predict the biological activity of database molecules as ACEIs. The compare–fit studies between generated hypothesis and **V**, **VIa–e** were performed in order to prioritize their activities. The result of virtual study revealed that the molecules (**V** and **VIa–e**) have high fitting values and they could be considered active hit molecules as ACEIs.

Accordingly, the designed molecules (**V** and **VIa–e**) were synthesized following Scheme 1 and evaluated for their hypotensive activity on normotensive anesthetized cats. The study revealed that the hypotensive activities of **V** and **VIa–e** were similar to or higher than those of Captopril and matched with the virtual screening.



Scheme 1. Compounds: **VIa**, R = OH; **VIb**, R = CH_2CH_2OH; **VIc**, R = NH–CS–NH_2; **VId**, R = CH_2–C_6H_5; **VIe**, R = $CH(CH_3)COOC_2H_5$. Reagents and conditions: (a) 5 N HCl, reflux 5 h; (b) (i) aqueous KOH 7%; (ii) $SOCl_2$, reflux 1 h; (c) anhyd K_2CO_3, acetonitrile; (d) 5.5 N NH_4OH, MeOH; (e) NH_2–R, anhyd CH_3COONa, EtOH, reflux 6 h.

2. Results and discussion

2.1. Molecular modeling. Stepwise generation of the ACEIs hypothesis

The molecular modeling studies for constructing the hypothesis for the ACEIs, derived from the frozen conformation of Lisinopril docked at the human ACE were performed using both Cerius2 and CATALYST modules. The crystal structure of human ACE in complex with the Lisinopril (1086)[17] was obtained from the World Wide Web based protein data bank (PDB). The PDB file in a simplified view contains a listing of the amino acid sequence, then a listing of connectivity data that contain information about how the amino acid sequence (the primary structure) is arranged in three dimensional space (Fig. 2A). Using the protein tools deck in Cerius2 Modules, all the non-protein residues present in the structure can be identified and selected as independent models. Thus, Lisinopril in its presumed biologically active conformation was selected together with adjacent binding site (about 10 Å distance) in a new independent file of PDB format (Fig. 2B), then the frozen bioactive conformer of Lisinopril was isolated from the remaining protein structures. Such conformer included atom types in its basic architecture by only single bonds. Thus the structure needed modifications in order to correct the atom types and bond orders, using Cerius2 3D-sketcher, then the corrected bioactive conformation of Lisinopril was saved (without changing its conformation, i.e; frozen conformation) (Fig. 2C and D).

The bioactive frozen conformer of Lisinopril was transferred to Catalyst workstation and the reported crucial five features,[13,18,19] required for effective ACE inhibitory activity, were added manually, depending on the reported data, to generate the hypothesis in stepwise manner (Fig. 3A and B). The added crucial features[18] are; two HB-Acceptor features (A1 and A2), one negative ionizable feature (N), and two hydrophobic features (H1 and H2).

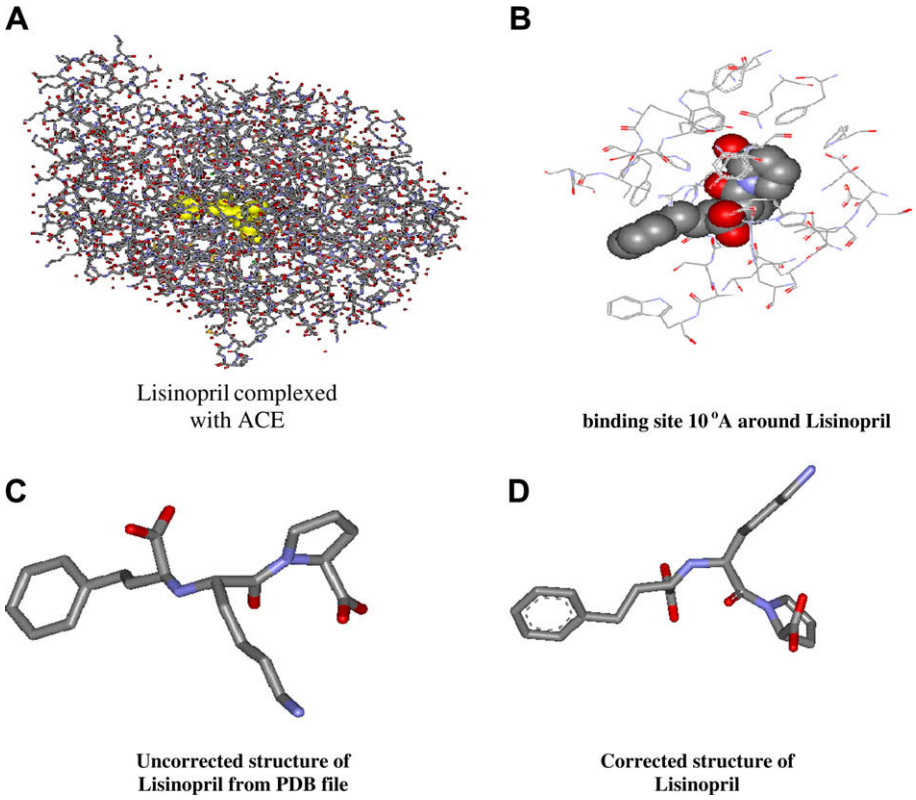


Figure 2. Isolation steps for bioactive conformation of Lisinopril.

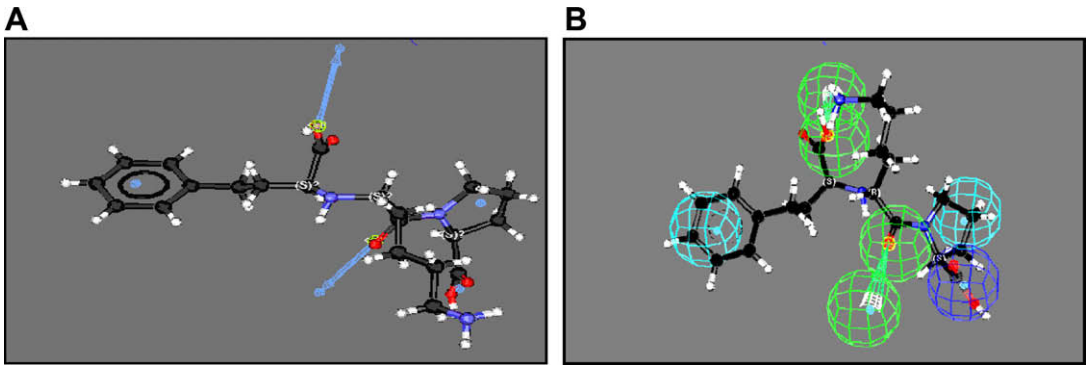


Figure 3. (A and B) selected features for ACEIs.

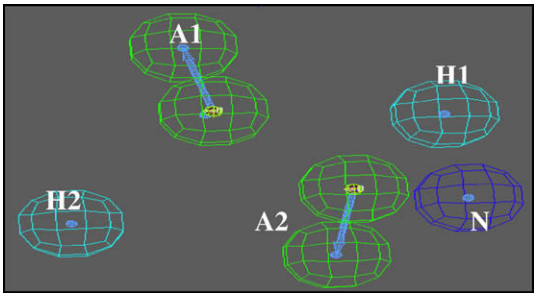


Figure 4. Generation of ACE inhibitors hypothesis.

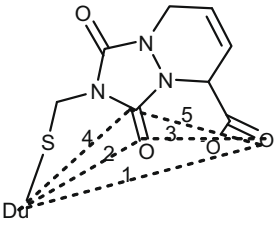


Figure 5. Constraint distances between the active site of a reported hypothetical bioactive lead.

The structure of Lisinopril was then deleted, and constraint dimension (distances and angels between all the features) were automatically determined and the corresponding hypothesis was then generated using the stepwise technique of CATALYST modules.[20] Such generated hypothesis, reported herein, in this investigation, for the first time, showed all the features reported for a potent ACE inhibitor[18] (Fig. 4).

Dammkoehler et al.[18] reported hypothetical constraint distances between the most essential groups in a biologically active molecule as outlined in Figure 5.

Meanwhile, the same authors did not mention the constraint angles between these essential groups. But, in this research we reported the range of the constraint distances and the angles between the essential features existed in the generated hypothesis, as shown in Figures 6, 7 and Table 1.

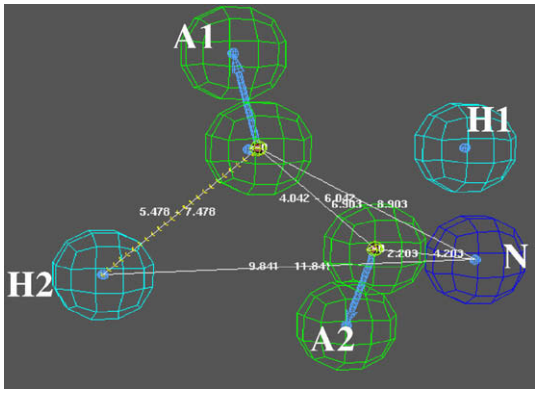


Figure 6. Constraint distances of ACEIs hypothesis.

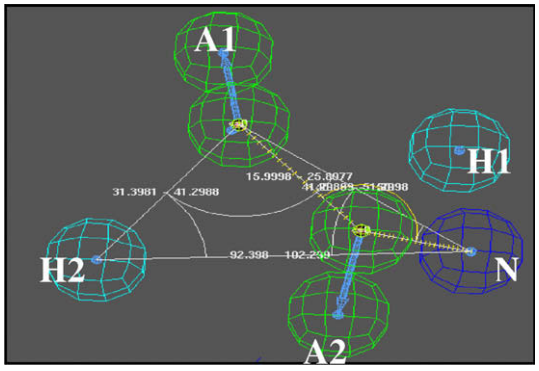


Figure 7. Constraint angles of ACEIs hypothesis.

2.1.1. Validation of the generated Catalyst hypothesis of ACE inhibitors

(a) Mapping of Lisinopril and the other lead compounds such as Captopril to the generated hypothesis showed complete mapping of all the features with high fit values (4.9 and 3.9, respectively) and with low conformational energies (3.7and 2.7) (Fig. 8 and Table 2).

Table 1
Constraint dimensions of the generated ACEIs hypothesis in comparison to Richard's ACEI hypothetical bioactive molecule

Dimensions	Richard's ACEI hypothetical bioactive lead	Our catalyst ACEI hypothesis (values are recorded in ranges)
Constraint distances between different features (Å)	1, 8.543; 2, 4.938; 3, 3.808; 4, 5.278; 5, 4.095	A1–N, 6.903–8.903; A1–A2, 4.042–6.042; A2–N, 2.203–4.203; A1–H2, 5.478–7.478; H2–N, 9.841–11.841
Constraint angles between different features (°)	Not reported	A1–N–A2, 31.39–41.298; N–A1–H2, 92.372–102.34; A1–H2–N, 41.29–51.291; A1–A2–N, 140.90–150.90; A1–N–H2, 31.398–41.298

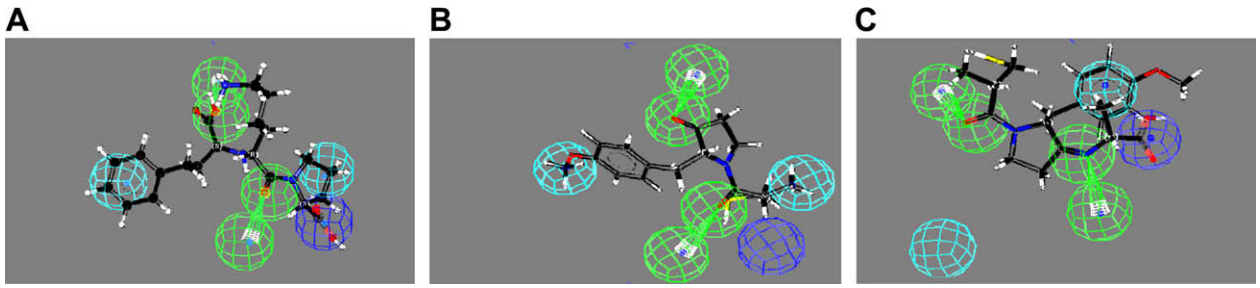


Figure 8. Mapping of (A) Lisinopril (B) compound **V** (C) hydrolyzed ester of compound **VIe** with the generated ACE inhibitors hypothesis.

Table 2
Mapping of the best fit conformers of lead and test set compounds (**V** and **VIa–e**) to the generated ACEIs hypothesis

Compd no.	No. of conformers	Conf. energy (kcal mol^{-1})	Fit value (out of five)
Lisinopril	210	3.7	4.9
Captopril	131	2.7	3.9
V	125	6.4	3.95
VIa	85	5.8	3.33
VIb	126	5.6	3.47
VIc	175	13	2.9
VId	149	12.3	3.1
De-esterified **VIe**	220	9	3.87

Table 3
Mapping of Catalyst database molecules with ACE inhibitors hypothesis

Database	Total numbers of molecules	Number of the retrieved (mapped) hits	% of retrieved (mapped) hits
NCI 2000	238,819	291	0.122
Maybridge 2001	55,273	9	0.016
MiniMaybridge	2000	0	0
Total	296,092	300	0.101

(b) The simulated fitting values of such hypothesis with the hypotensive test set compounds (**V** and **VIe**) were consistent with the experimental results, as it will be mentioned later, in the biological evaluation results (Table 2).

(c) Additional validation of our ACEIs hypothesis was achieved by databases search study with the molecular structures of the provided Catalyst databases (Maybridge 2001, MiniMaybridge 2001 and NCI 2000). The results showed that 0.101% of the databases molecules was retrieved among 296,092 molecules (Table 3). The low percentage of the retrieved molecules of the databases proved the high selectivity of such hypothesis.[21]

In addition, it was found that the ACEI molecules; **3**, **4**, and **5** (Fig. 1), that exist among the used databases molecules, were among the recognized mapped molecules in this search.

The above finding indicates the validity of the generated ACE inhibitors hypothesis (Fig. 4).

2.1.2. Molecular modeling simulation study of the generated ACE inhibitors hypothesis with the test set molecules; 3-mercapto-2-methylpropanoyl-pyrrolidine derivatives (V and VIa–e)

Examination of the mapping of the test set compounds (**V** and **VIa–e**) to the generated ACE inhibitors hypothesis for predicting their activity was performed using compare/fit (Best Fit) process. Different mappings for all conformers of each compound of the test set to the generated hypothesis were visualized. Such simulation study revealed that pyrrolidine compounds **V**, **VIa**, **VIb**, and the de-esterified **VIe** have high fitting values in comparison to the lead compounds (Fig. 8 and Table 2).

2.1.3. Conclusions of simulation studies

The above molecular modeling simulation studies indicated that the pyrrolidine derivatives (**V** and **VIa–e**) have potential ACE inhibitor activity and thereby they are considered as active antihypertensive hits. It seems that the high fit values of **V**, **VIa**, **VIb**, and **VIe** with the ACEIs hypothesis, are attributed to the hydrophobic interaction of the *p*-methoxybenzyl group with the hydrophobic feature (H2). Accordingly, we concluded that compounds **V**, **VIa**, **VIb**, and **VIe** could be considered as promising active hits as ACE inhibitors. Hence such active hits were synthesized (Scheme 1) and evaluated for their hypotensive activity in normotensive adult cats.

2.2. Synthesis

The intermediates (**I**,[22] **III**[23]) were synthesized according to reported methods. Compound **I** was deethoxycarboxylated and N-deacetylated in one step through refluxing in 5 N HCl to afford **II**. Acylation of **II** with equivalent amount of **III**, afforded the 2-(4-methoxybenzyl)-1-acetylthio-2-methylpropanoyl) pyrrolidine-3-one (**IV**) Hydrolysis of thioester function of (**IV**) using 5.5 N methanolic ammonia gave the target compound; 2-(4-methoxybenzyl)-1-(3′-mercapto-2′-methyl propanoyl) pyrrolidine-3-one (**V**). A new series of 3′-mercapto-2′-methyl-propanoyl-pyrrolidine-3-imines (**VIa–e**) were prepared by condensation of the 2-(4-methoxybenzyl)-1-(3′-mercapto-2′-methyl propanoyl) pyrrolidine-3-one (**V**) with the respective primary amines (viz; hydroxylamine HCl, 2-ethanolamine, thiosemicarbazide, benzylamine, and 2-aminopropionic acid ethyl ester) in the presence of anhydrous sodium acetate and absolute ethanol (Scheme 1).

2.3. Biological screening. Assessment of the hypotensive activity in normotensive cats

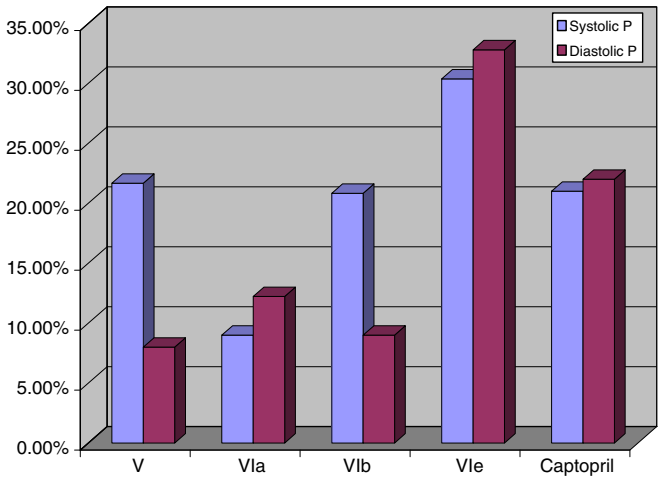
Preliminary pharmacological testing of selected compounds (**V**, **VIa**, **VIb**, and **VIe**), which have high compare/fit scoring value, was carried out in anesthetized normotensive adult cats. All tested compounds demonstrated a hypotensive activity on adult normotensive cats (Table 4 and Graph 1).

Compounds **V** and **VIe** showed higher hypotensive activity in comparison to Captopril at doses, 0.0014 and 0.0012 mmol/kg, respectively. Whereas compounds **VIa** and **VIb** showed moderate hypotensive activity in comparison to the same reference compound at doses 0.0031 and 0.00285 mmol/kg, respectively. The hypotensive effects of the different congeners of this group were arranged in the following decreasing order: **VIe > V > VIb > VIa**. This indicated that the required SAR for these series of compounds should involve 3-one and 3-(1-carbethoxy)ethylimino moieties to exert higher hypotensive activity than Captopril, while

Table 4
Mean percentage reduction in the systolic and diastolic blood pressure of normotensive adult cats in comparison with their fitting affinities with ACEIs hypothesis

Compd no.	Fit value (out of five)	Conf. energy (kcal mol^{-1})	Dose (mmol/kg)	Systolic BP (mmHg) mean ± SE	Mean change %	Diastolic BP (mmHg) mean ± SE	Mean change %
Control[!]	—		—	134.1 ± 2.01	—	125 ± 1.35	—
Captopril	3.9	2.7	0.00246	105.9 ± 1.4	−20.91%	97.6 ± 1.56	−21.89%
V	3.95	6.4	0.00246	104.9 ± 23.65	−21.67%	115 ± 0	−8%
VIa	3.33	5.8	0.00246	121.9 ± 0.89	−9%	109.7 ± 0.75	−12.22%
VIb	3.47	5.6	0.00246	106 ± 2.76	−20.83%	113.7 ± 2.32	−9%
VIe[a]	3.87	9	0.00246	93.3 ± 1.6	−30.37%	84 ± 0.89	−32.8%

[!] Statistically significant difference from the control group at $p < 0.05$.
[a] Fit value is measured using the hydrolyzed ester form.



Graph 1. Percentage reduction in systolic and diastolic BP compared to the reference compound (Captopril).

incorporation of 3-oxime or 3(2-hydroxyethylimine moieties will lead to lower hypotensive activity.

2.4. Correlation between molecular modeling simulation studies and the in vivo hypotensive activity

The in vivo hypotensive activity of the designed compounds (**V** and **VIa–e**) was found to matching with the fit values. However, compound **VIe** having the in vivo hypotensive activity was found to be the most active molecule, in spite of **V** having higher fit value (3.95) than the fit value of **VIe** (3.87). A similar disparity was explained in one of our previous reported researches,[21] where it was found that the existence of an electron-rich edge on the molecule may interfere in the interaction forces between the compound and its binding site. Accordingly, we decided to determine the atomic charge distribution for compounds **VIe** and **V**, using Cerious2 software, version 4.7 Accelrys, in order to predict the effect of charge distribution of these compounds on the binding affinity with their binding sites. The result is given in (Figs. 9 and 10). We could find that compound **V** has low electron-rich edge around the pyrrolidin-3-one ring system, which may weakly interact with the complementary positive ionizable feature of the receptor side, and hence it has lower hypotensive activity. The opposite is true in compound **VIe**, where it showed high electron-rich edge around the pyrrolidine-3-alkyl carboxylate moiety, and hence it could easily combine with the positive ionizable feature of the binding site; that is, it will have higher in vivo hypotensive activity than **V**.

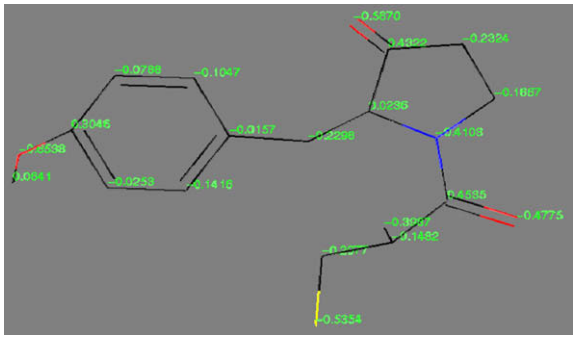


Figure 9. Atomic charge distribution on compound **V**.

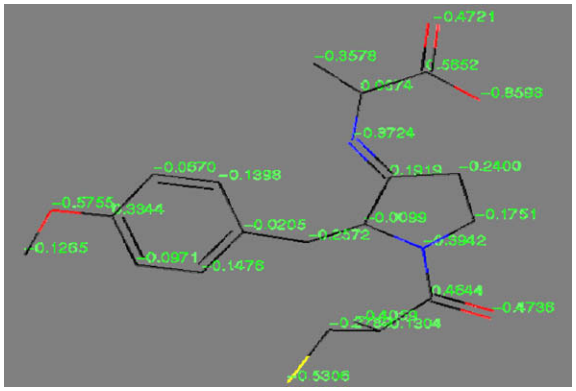


Figure 10. Atomic charge distribution on the de-esterified; **VIe**.

3. Experimental

All reagents were supplied by Aldrich, Merck, and Acros Organics, and were used with no further purification. TLC was performed on Silica Gel 60 F_{254} plates (Merck). Melting points were determined on Stuart Scientific apparatus and are uncorrected. FT-IR spectra were recorded on a Perkin–Elmer FT spectrophotometer. ^{1}H NMR spectra were measured in δ scale on a Perkin–Elmer 300 MHz spectrometer. EIMS spectra were recorded on Finnigan Mat SSQ 7000 (70 eV) mass spectrometer. Elemental analyses were performed at the Microanalytical Center, Cairo University, Egypt. Preparation of 1-acetyl-4-ethoxycarbonyl-2-(4-methoxybenzyl)pyrrolidin-3-one (**I**) and 3-acetylthio-2-methyl-propionic acid chloride (**III**) was performed adopting reported procedures.[22] A solution of **I** (5 g, 0.084 mol) and 5 N hydrochloric acid (25 mL) was refluxed for 5 h and evaporated under vacuum and the residue was crystallized from isopropanol to give 4 g (82%) of **II**, mp: 225–227 °C. IR 3340 and 1719 cm^{-1}. ^{1}H NMR ($CDCl_3$) 2.8–3.2 (m, 6H, $C_6H_4CH_2$, N–CH_2CH_2C=O), 3.75 (s, 3H, OCH_3), 4.4 (s, 1H, NC*H*C=O) and 6.7–7 (dd, J = 8.4, 8.6 Hz, 4H, Ar-H).

3.1. 1-(3-Acetylthio-2-methylpropanoyl)-2-(4-methoxybenzyl) pyrrolidin-3-one (IV)

A mixture of **II** (3.27 g, 17.5 mmol), 3-acetylthio-2-methyl-propionyl chloride (**III**) (8.75 g, 19.3 mmol), and potassium carbonate (4.83 g, 35 mmol) in acetonitrile (100 mL) was stirred at 25 °C for 24 h. The reaction mixture was filtered and the filtrate was evaporated under vacuum. The residue was diluted with water and the resulting mixture was then extracted with ethyl acetate. The combined organic phases were washed with water, dried over anhydrous magnesium sulfate, and evaporated under vacuum. The appropriate products as crude oil residue were purified by column chromatography using *n*-hexane/chloroform (3:1) to produce the titled compound **IV** as pale yellow oil (4.9 g, 92%). IR ν (neat, cm^{-1}): 3010, 2293, 1756, 1689 and 1642. ^{1}H NMR (DMSO-d_6) δ ppm 1.7 (d, J = 7.1 Hz, 3H, –CH–CH_3), 2.1–3.6 (m, 12H, CH_3C=O, S–CH_2–CH–, N–CH_2–CH_2, –CH_2-ph), 3.9 (s, 3H, CH_3O–), 4.22 (t, J = 7.49 Hz, 1H, CH–N) and 6.66–7.2 (dd, J = 8.34, 8.61 Hz, 4H, Ar-H); MS (EI$^+$): m/z: 349.12 [M·]$^+$; Microanalysis calcd C, H, N for $C_{18}H_{23}NO_4S$: 61.87, 6.63, 4.01, found 61.69, 6.54, 3.92.

3.2. 1-(3-Mercapto-2-methylpropanoyl)-2-(4-methoxybenzyl) pyrrolidin-3-one (V)

Compound **VI** (0.85 g) was dissolved in methanolic ammonium hydroxide (7.8 mL). The solution was stirred for 2 h at room temperature.[24] The reaction mixture then concentrated under vacuum

and the residue then washed with water and extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and evaporated under vacuum to give the oily residue which was purified by column chromatography using *n*-hexane/chloroform (3:1) to produce 0.6 g (92%) of the titled compound **V** as pale yellow oil. IR ν (neat, cm^{-1}): 3020, 22933–2836, 1732 and 165. ^1H NMR (DMSO-d_6) δ ppm 1.7 (d, J = 7.45 Hz, 3H, CH_3–CH–CO), 2.3–3.4 (m, 9H, S–CH_2–CH–, N–CH_2–CH_2, –CH_2-ph), 3.7 (s, 3H, CH_3O–), 4.2 (t, J = 7.51, 1H, ph-CH_2–CH–N), 6.7–7.2 (dd, J = 8.74, 8.8 Hz, 4H, Ar-H); MS (EI^+): m/z: 307.1 $[M^{\cdot}]^+$; Microanalysis calcd C, H, N for $C_{18}H_{23}NO_4S$: 61.87, 6.63, 4.01, found 61.67, 6.45, 4.18.

3.3. 2-(4-Methoxybenzyl)-1-(3-mercapto-2-methylpropanoyl)-3-substituted imino pyrrolidine (VIa–e) (cf.[22])

A mixture of **V** (1.5, 0.012 mmol), primary amine (viz; hydroxylamine HCl, 2-ethanolamine, thiosemicarbazide, benzylamine, and 2-aminopropionic acid ethyl ester) (0.02 mmol), and anhydrous sodium acetate (1.4 g, 0.03 mmol) in absolute ethanol (25 mL) was refluxed under stirring for 6 h. After cooling, the solvent was evaporated under vacuum and the residual solid was treated with ice cold water and filtered. The precipitate was crystallized from ethanol to produce the titled compounds (**VIa–e**).

3.4. 1-(3-(Hydroxyimino)-2-(4-methoxybenzyl)pyrrolidin-1-yl)-3-mercapto-2-methyl propan-1-one (VIa)

Yield 75%, mp 185–186 °C; IR ν (neat, cm^{-1}): 3520 (br, OH), 1664 (C=O, amid); ^1H NMR (DMSO-d_6) δ ppm 1.3 (d, J = 7.4 Hz, 3H, CH_3–CH–CO); 2.1–3.4 (m, 9H, N–CH_2–CH_2–C=N, S CH_2CH–CO, ph-CH_2–); 3.75 (s, 3H, CH_3O–); 4.2 (t, J = 7.49 Hz, 1H, CH–N); 4.9 (br, 1H, SH); 5.7 (br, 1H, OH) and 6.6–7.2 (dd, J = 8.61, 8.66 Hz, 4H, Ar-H); MS (EI^+): m/z: 322.2 $[M^{\cdot}]^+$. Microanalysis calcd C, H, N for $C_{16}H_{22}N_2O_3S$: 59.60, 6.88, 8.69, found 59.55, 6.77, 8.51.

3.5. 1-(3-(2-Hydroxyethylimino)-2-(4-methoxybenzyl)pyrrolidin-1-yl)-3-mercapto-2-methylpropan-1-one (VIb)

Yield 77%, mp 174–175 °C; IR ν (neat, cm^{-1}): 3560–3200 (br, OH), 1660 (C=O, amid); ^1H NMR (DMSO-d_6) δ ppm 1.25 (d, J = 7.1 Hz, 3H, CH_3–CH–CO); 1.8–3.4 (m, 13H, N–CH_2–CH_2–C=N, S CH_2CH–CO, ph-CH_2–, N=CH_2–CH_2–); 3.75 (s, 3H, CH_3O–); 4.4 (t, J = 7.29 Hz, 1H, CH–N); 4.7–5.4 (br, 2H, OH and SH) and 6.8–7.2 (dd, J = 8.71, 8.41 Hz, 4H, Ar-H); MS (EI^+): m/z: 350.1 $[M^{\cdot}]^+$. Microanalysis calcd C, H, N for $C_{16}H_{22}N_2O_3S$: 61.69, 7.48, 7.9, found 61.37, 7.28, 8.01.

3.6. 2-(1-(3-Mercapto-2-methylpropanoyl)-2-(4-methoxybenzyl) pyrrolidin-3-ylidene) hydrazinecarbothioamide (VIc)

Yield 80%, mp 155–156 °C; IR ν (neat, cm^{-1}): 3320–3150 (br, NH), 1683 (C=O, amid); ^1H NMR (DMSO-d_6) δ ppm 1.3 (d, J = 81 Hz, 3H, CH_3–CH–CO); 2.3–3.5 (m, 9H, N–CH_2–CH_2–C=N, S CH_2CH–CO, ph-CH_2–); 3.9 (s, 3H, CH_3O–); 4.2 (t, J = 7.9 Hz, 1H, CH–N); 5 (br, 1H, SH) and 6.7–7.3 (dd, J = 8.9, 8,71 Hz, 4H, Ar-H), 8.1–8.5 (br, 3H, N$HCSNH_2$); MS (EI^+): m/z: 380.1 $[M^{\cdot}]^+$. Microanalysis calcd C, H, N for $C_{17}H_{24}N_4O_2S_2$: 53.66, 6.36, 14.72, found 53.69, 4.92, 15.00.

3.7. 1-(3-(Benzylimino)-2-(4-methoxybenzyl)pyrrolidin-1-yl)-3-mercapto-2-methylpropan-1-one (VId)

Yield 65%, bright yellow oil; IR ν (neat, cm^{-1}): 1675 (C=O, amid); ^1H NMR (DMSO-d_6) δ ppm 1.2 (d, J = 8 Hz, 3H, CH_3–CH–CO); 2.3–3.4 (m, 11H, N–CH_2–CH_2–C=N, S CH_2CH–CO, ph-CH_2–, N–CH_2ph); 3.8 (s, 3H, CH_3O–); 4.3 (t, J = 7.19 Hz, 1H, CH–N); 5 (br, 1H, SH); and 6.6–8.3 (m, 9H, Ar-H); MS (EI^+): m/z: 396.1 $[M^{\cdot}]^+$. Microanalysis calcd C, H, N for $C_{23}H_{28}N_2O_2S$: 69.66, 7.12, 7.06, found 69.37, 7.28, 6.89.

3.8. Ethyl-2-(1-(3-mercapto-2-methylpropanoyl)-2-(4-methoxybenzyl)pyrrolidin-3-ylidene amino)propanoate (VIe)

Yield 75%, mp 135–136 °C; IR ν (neat, cm^{-1}): 1731 (C=O, ester), 1665 (C=O, amid); ^1H NMR (DMSO-d_6) δ ppm 1.2 (d, J = 8 Hz, 3H, CH_3–CH–CO); 1.35 (t, J = 7.3 Hz, 3H, CH_3–CH_2–O); 1.9–3.5 (m, 9H, N–CH_2–CH_2–C=N, S CH_2CH–CO, ph-CH_2–); 3.75 (s, 3H, CH_3O–); 4.2 (q, J = 6.3 Hz, 2H, CH_3CH_2–O); 4.4 (t, J = 7 Hz, 1H, CH–N) and 6.9–7.2 (dd, J = 8.41, 8.62 Hz, 4H, Ar-H); MS (EI^+): m/z: 406.2 $[M^{\cdot}]^+$. Microanalysis calcd C, H, N for $C_{21}H_{30}N_2O_4S$: 62.04, 7.44, 6.89, found 61.85, 7.23, 7.11.

3.9. Molecular modeling experiments

All molecular modeling studies were performed using a Silicon Graphics desktop (SGI) Fuel workstation under an IRIX 6.8 operating system, at the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. The bioactive conformer of Lisinopril was isolated from the remaining protein structures using Cerius2 module (Fig. 2). The generation of the pharmacophore model for ACE inhibitors hypothesis was accomplished using Accelrys CATALYST, version 4.8. Molecules were built within CATALYST modules and conformational models for each compound were generated automatically using the poling algorithm. This emphasizes representative coverage over a 20 kcal/mol energy range above the estimated global energy minimum and the best quality generation technique was chosen. The bioactive conformer of Lisinopril was used for stepwise hypothesis generation using the stepwise technique. In this study, hydrogen bond Acceptors, hydrophobic features, and negative ionizable points were used as the chemical features, which were reported to be crucial for the ACE inhibitors activity.[16,18]

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