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Harnessing nature's building blocks: potent and safe urease inhibition by simple amino acids

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ABSTRACT

Urease is a key metalloenzyme with broad industrial, agricultural, and medical relevance, and it is implicated in the virulence of numerous pathogenic microorganisms. Elevated urease activity supports the persistence of urease-producing microbes and contributes to nitrogen depletion in agricultural soils. Inhibitors of urease that are effective are therefore discovered and their discovery would be a plus. This research is looking at the possibility of amino acids being the safe, biocompatible, and effective urease inhibitors with prospective use in agriculture and medicine. The inhibition potentials of L-Valine, D-Phenylalanine, L-Methionine, L-Tyrosine, L-Arginine, and D-Threonine (Val, Phe, Met, Tyr, Arg, and Thr for short) against jack bean urease were evaluated systematically. The varying side-chain chemistries (aromatic, sulfur, hydroxyl, and ionic) of these compounds that could be involved in urease binding were the basis for selection. The study showed that Phe (IC_{50} = 0.53 μM) and Val (0.57 μM) were the strongest inhibitors, followed by Met (0.97 μM) and Arg (1.15 μM). A moderate level of inhibition was noted for Tyr (2.38 μM) and Thr (3.97 μM) was singled out as the least effective inhibitor. *In vitro* results have been validated by molecular docking and MM-GBSA calculations, which are in excellent agreement, as ligands with the highest predicted binding energies are also those with the lowest IC_{50} values. The conclusion reached is that natural amino acids can be considered non-toxic and environmentally friendly urease inhibitors for both agricultural soil management and the treatment of urease-associated infections.

1. Introduction

Urease (urea amidohydrolase; EC 3.5.1.5) is a metalloprotein enzyme that breaks down urea into ammonia and carbon dioxide and has two nickel ions at its active site [1]. It is commonly found in different forms of life such as plants, bacteria, and fungi and is of major importance in the environmental nitrogen cycle and the life cycle of pathogenic microorganisms [2–4]. In agricultural practices, a significant amount of the urea that is added to the soil is quickly transformed into ammonia and carbon dioxide by either plant or microbial ureases [5]. This reaction leads to the release of high amounts of ammonia into the environment, nitrogen losses, and consequently reduced agricultural productivity. Furthermore, the resulting ammonia increases greenhouse gas emissions, negatively impacting environmental sustainability [6]. Urease activity is considered a virulence factor from the medical point of view of numerous pathogens like *Helicobacter pylori*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Cryptococcus neoformans* [7–9]. The mentioned microorganisms decompose urea and, in turn, produce ammonia that not only neutralizes but also damages host tissues [10–12]. Thus, urease, as an enzyme, becomes a key biochemical area of focus in agricultural as well as clinical applications.

Inhibitors developed to reduce urease activity are used in agriculture to reduce nitrogen loss and in medicine to control infections [13]. The literature reports many synthetic urease inhibitors, such as hydroxamic acids, phosphoramidates, imidazoles, thioureas, and various heterocyclic compounds [14–17]. However, most of these compounds have limitations such as toxicity, low biocompatibility, and environmental persistence [18,19]. This situation has necessitated the research of new inhibitors that are safer, biologically compatible, and environmentally friendly. At this point, natural amino acids emerge as an important alternative. Amino acids possess an environmentally friendly and non-toxic profile to humans, as they are low in toxicity, highly soluble,

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and have biocompatible structures [20,21]. Furthermore, the side chains of certain amino acids (*e.g.*, those containing sulfur or aromatic rings) have the ability to form coordinate bonds with metal ions, which could contribute to the enhancement of their binding ability to the active site of nickel-containing urease enzymes [22]. Nevertheless, only a small number of publications are available in the present day literature which assess compounds derived from amino acids as urease inhibitors directly. The majority of the research has been done on synthetic heterocyclic compounds derived from amino acid. This meritorious work has been carried out with a view to systematically explore and establish the inhibitory action of natural amino acids on the urease enzyme of Jack bean. The hypothesis of the study is that the amino acids might have a suppressive effect on enzyme activity *via* the impacting of metal-binding capacity due to their various side-chain chemistries (aromatic, sulfur-containing, hydroxyl, and basic groups). The research is seeking to prove the capability of natural amino acids to be used as safe and effective urease enzyme inhibitors by conducting both *in vitro* and molecular docking (*in silico*) analyses. In this particular context, the research is important in two principal domains: (i) offering an ecological solution for reducing urea losses in agricultural use and (ii) creating low-toxicity, biocompatible candidates of inhibitors for the control of urease-related infections in the medical sector. Therefore, an original and creative viewpoint is put forward that amino acids are not only metabolic building blocks but also potential future bioorganic inhibitors.

Previous studies have mostly been concentrated on the development of synthetic urease inhibitors such as hydroxamic acids and thiourea derivatives [23–25]. Above all, five-membered heterocyclic compounds like thiohydantoins and hydantoins created from amino acids have done very well with both plant and microbial ureases [25,26]. Nevertheless, not so many investigations were carried out about natural or amino acid-based inhibitors and their toxic free and biocompatible features. The gap in knowledge created by this situation accentuates the necessity to search for uncomplicated, naturally occurring compounds which have the same potency but are more environmentally friendly, *i.e.*, safe. Hence, the present research was planned to carry out a systematic evaluation of the inhibitory potential of different natural amino acids against Jack bean urease. The reason for this is their distinct characteristics: amino acids are natural, biocompatible, and very soluble molecules that possess marginal cytotoxicity [27,28]. Metal-binding ability, due to amino acids, allows them to interact with the metal ions present in the active site of the metalloenzyme which may hinder its catalytic role [29,30]. Because of such considerations, researchers have been encouraged to explore amino acids as safe and efficacious urease inhibitors *in vitro* and through molecular docking methods. The results indicate that amino acids are not only the metabolic building blocks but also the potential ones for biocompatible urease inhibitors.

2. Materials and methods

2.1. Chemicals and reagents

The research used analytically pure amino acids that were commercially available and the classic inhibitor thiourea, which were all obtained from Sigma-Aldrich (St. Louis, MO, USA) (Fig. 1). The selection of amino acids was done considering the differences in their structures and also the properties of their side chains. All the solutions, which included amino acids and thiourea, were prepared using distilled water.

2.2. In vitro urease inhibition

The urease inhibitory activities of amino acid derivatives were determined using a modified version of the indophenol method described in the literature by Weatherburn, based on phenol-hypochlorite color development [31,32]. The solutions were prepared immediately before the experiment; serial dilutions from 1 mM stock solutions yielded concentrations of 1.0, 3.125, 6.25, 11.25, 25, 50, 100, 300, 500, 700, and 1000 μM. The reaction medium was carried out in a buffer containing 100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA, and 0.01 M LiCl, adjusted to pH 8.2. Alkaline reagents consisting of a 1 % (*w/v*) phenol solution with a 0.005 % (w/v) sodium nitroprusside solution and a 0.5 % (w/v) NaOH solution with a 0.1 % (*v/v*) NaOCl solution were used for color development [33]. Each reaction mixture was prepared to contain 0.4 mL buffer, 0.2 mL enzyme solution (5 U/mL), and 0.1 mL test compound; in the control groups, solvent was added instead of the test compound. After incubating the mixtures at room temperature for 15 min, 0.65 mL of phenol reagent and 0.65 mL of alkaline reagent were added, and the tubes were left in the dark for 50 min. After the formation of the blue-violet color of the indophenol complex, the absorbance was measured at 625 nm using a spectrophotometer (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer).

All experiments were performed in triplicate. Inhibition percentages were calculated using control and sample absorbance values; IC_{50} values were determined *via* non-linear regression analysis from the obtained dose-response curves using GraphPad Prism (version 9.0) software (Fig. 2) [34].

2.3. Molecular docking

Molecular docking was performed using Schrödinger Molecular

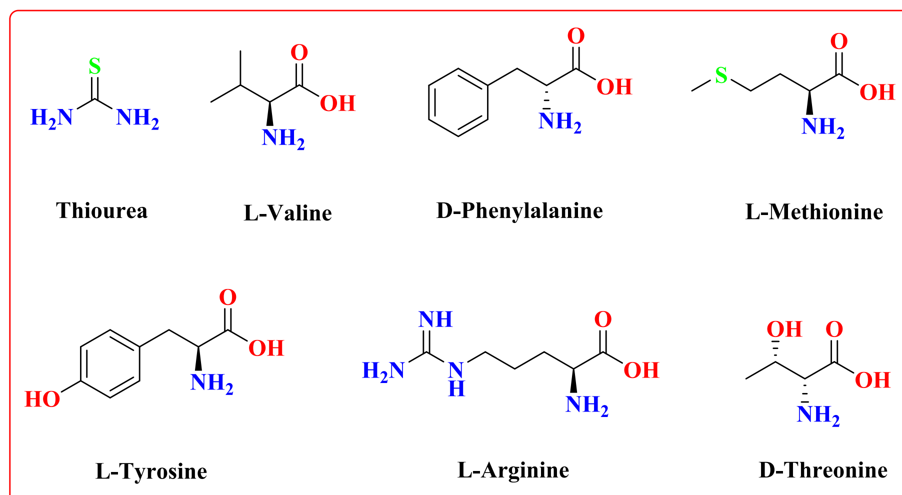

Fig. 1. Chemical structures of reference inhibitor (thiourea) and amino acids tested as urease inhibitors.

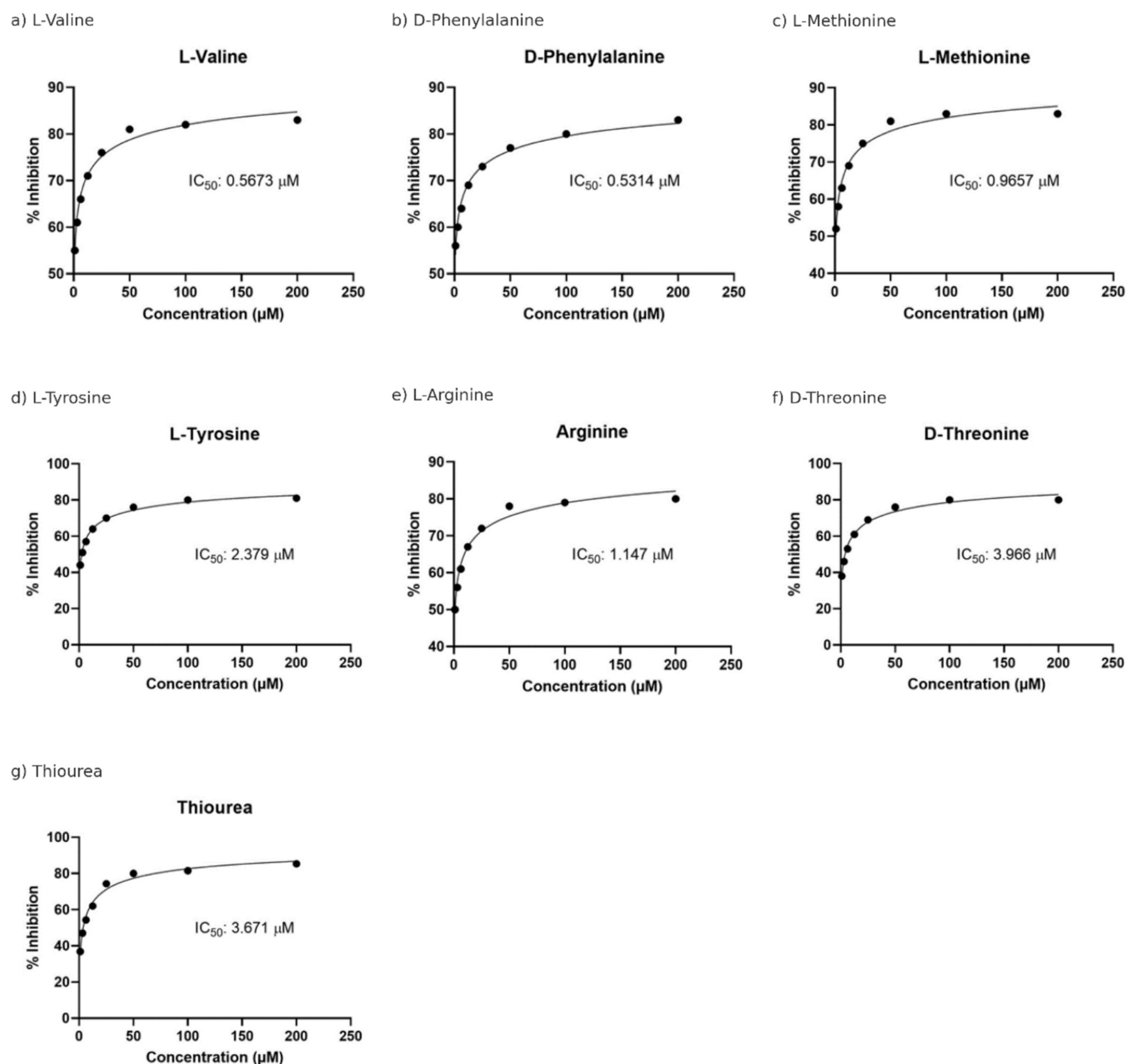

Fig. 2. Dose-response inhibition curves of amino acids and the standard inhibitor thiourea on the Jack bean urease enzyme. Each curve shows the mean inhibition (%) ± SD values obtained from triplicate experiments and has been fitted using non-linear regression based on a four-parameter logistic model. The IC_{50} values were calculated for (a) L-Valine, (b) D-Phenylalanine, (c) L-Methionine, (d) L-Tyrosine, (e) L-Arginine, (f) D-Threonine, and (g) Thiourea, respectively.

Modeling Software (2025–1) with the Maestro interface (v14.3) and Desmond. According to the established group protocols, preparation of proteins and ligands was done [35]. The three-dimensional structure of Jack bean urease (PDB ID: 3LA4) was obtained from the Protein Data Bank and then further refined with the protein Preparation Wizard of Schrödinger. The docking simulation employed Glide XP and Induced Fit Docking (IFD) to account for the receptor's flexibility [36,37]. For all ligands, twenty poses maximum were created and the best poses were picked based on the IFD scores. The binding free energies were determined by Prime MM-GBSA calculations employing the VSGB solvation model [37].

3. Results and discussions

3.1. Inhibition and IC_{50} values

The IC_{50} values determined in the present research reveal considerable variations in the capacity for urease inhibition among the amino acids that were examined (Table 1). The most potent inhibitors were identified as D-Phenylalanine (IC_{50} = 0.53 μM) and L-Valine (IC_{50} = 0.57 μM), demonstrating that these compounds possess a very high inhibition capacity at the submicromolar level. L-Methionine (0.97 μM) and L-Arginine (1.15 μM) are also among the potent inhibitors, and the activity of L-Methionine, which has a sulfur-containing side chain, is consistent with the potent inhibitory potential of methionine derivatives reported in the literature [24]. L-Tyrosine (2.38 μM), by virtue of presenting an aromatic side-chain, demonstrated intermediate inhibition

Table 1
IC_{50} values of amino acids and thiourea for urease.

Inhibitors	Urease (μM)	
	IC_{50}	R^2
L-valine	0.57 ± 0.05	0.9845
D-phenylalanine	0.53 ± 0.02	0.9870
L-methionine	0.97 ± 0.06	0.9785
L-tyrosine	2.38 ± 0.04	0.9903
L-arginine	1.15 ± 0.07	0.9814
D-threonine	3.97 ± 0.05	0.9857
Thiourea	3.67 ± 0.05	0.9856

with a higher IC_{50}. On the other hand, D-Threonine (3.97 μM), having a hydroxyl group, was the weakest inhibitor. Thiourea (3.67 μM), used as a standard inhibitor, showed less activity than almost all of the amino acids tested. In particular, D-Phenylalanine and L-Valine were observed to be approximately seven times more potent inhibitors than thiourea (Fig. 2). This demonstrates that amino acids, despite being natural building blocks, can also be potent inhibitors. Therefore, unlike synthetic derivatives, they have the potential to follow a different pathway. Additionally, due to their low toxicity and biological compatibility, amino acids are becoming more and more attractive as safer options compared to thiourea and other traditional inhibitors.

When the results are evaluated overall, it can be said that amino acids carrying aromatic and sulfur-containing side chains exhibit stronger inhibitory effects, while those with polar hydroxyl groups show weaker effects. These differences may be related to the interaction capacity of the side chains with nickel ions in the active site and the binding mode. Thus, the IC_{50} values obtained reveal a strong relationship between the structural properties of amino acids and their inhibition capacities.

3.2. Structure-activity relationships

The structure-activity relationship (SAR) analysis of the tested amino acids exposed unambiguous trends that connect side-chain characteristics with urease inhibition (Table 1, Fig. 1). The two compounds D-Phenylalanine and L-Valine were found to show the strongest inhibitory effect among the tested ones with the IC_{50} values of 0.53 μM and 0.57 μM, respectively. The exceptional result of D-Phenylalanine is due to its aromatic side chain, which allows π-π stacking and hydrophobic interactions in the urease active site, while the branched alkyl group of L-Valine facilitates the best positioning for metal coordination and compact binding. Amino acids that had longer or more flexible side chains including L-Methionine (IC_{50} = 0.97 μM) and L-Arginine (IC_{50} = 1.15 μM), showed moderate inhibition. The sulfur atom present in L-Methionine might have a weak interaction with the nickel ion, while the guanidinium group of L-Arginine provides electrostatic stabilization of the system but is not close enough to the hydrophobic area to cause stronger inhibition. On the other hand, L-Tyrosine and D-Threonine that have polar or less bulky substituents, were of much weaker activity because of their inability to establish hydrophobic or metal-mediated interactions.

The reported results of this study correspond closely to the findings available in the literature concerning amino acid derivatives. The derivatives of thiohydantoin as well as hydantoin have been shown to display very high inhibition by researchers. Moreover, the compounds of valine and methionine were characterized as having very good inhibition against urease [24,25]. This study contributes to the literature by demonstrating that amino acids themselves also possess inhibitory potential at submicromolar levels.

3.3. Molecular docking

Molecular docking is, without any doubt, the most important computer-assisted method in the modern drug discovery process, providing thorough understanding of the interaction and the binding strength of candidate small molecules with their target proteins. In order to increase the credibility of docking results, Induced Fit Docking (IFD) was used which enables the flexible movement of both ligand conformations and the protein binding site, thus simulating the actual physiological conditions more accurately. To this method an additional one, MM-GBSA which is a Molecular Mechanics-Generalized Born Surface Area method, was used for the computation of binding free energies in a quantitative way thus giving a thermodynamic viewpoint on the firmness and attractiveness of the ligand-protein complexes [38]. According to the IFD scores and MM-GBSA binding free energy calculations, the amino acids' binding affinities towards urease were very strong, and the reference inhibitor thiourea was easily surpassed (Table 2).

D-Phenylalanine had the most robust interaction with the target site compared to all the other samples, thus getting the IFD score of −12.351 kcal/mol and MM-GBSA ΔG Bind of −72.20 kcal/mol which corroborates its lowest IC_{50} value (0.53 μM) observed *in vitro*. The aromatic side chain of the compound makes possible the π-π and hydrophobic interactions with the residues that are neighboring the nickel ions located in the active site. L-Valine was, however, active to a high degree, though it scored only −11.26 kcal/mol on the docking scale which is still good. This indicates that the small branched alkyl side chain is very well positioned and coordinated with the metal. The amino acids L-Methionine and L-Arginine with their long and/or flexible side chains (−11.276 and − 11.473 kcal/mol, respectively) exhibited moderate activity both in the vitro experiment and through docking, their partial interaction with the metal being due to L-Methionine's sulfur-containing chain and L-Arginine's guanidinium group. In contrast, L-Tyrosine and D-Threonine displayed lower inhibitory effects, as their polar or less bulky side chains are less capable of establishing strong hydrophobic or metal-mediated contacts. The results indicate that amino acids combining appropriate size, hydrophobicity, and metal-binding potential are the most effective urease inhibitors, and the docking analyses closely correlate with the observed *in vitro* IC_{50} trends.

Ligand efficiency (LE) was calculated by dividing the MM-GBSA binding free energy by the number of heavy atoms in each ligand to normalize for molecular size differences. LE provides a measure of the binding energy contribution per non-hydrogen atom and serves as a size-independent indicator of binding efficiency. The stronger binding per atom is indicated by more negative LE values, which have the effect of completely removing any potential bias that may arise from the larger number of atoms interacting in the case of bulkier ligands [39–41]. In this study, thiourea got the best LE value (−9.70) among all the substances because it is a very small molecule consisting of only four heavy atoms. D-Threonine (−6.93) and D-Phenylalanine (−6.02) were the amino acids that gave the highest ligand efficiencies, meaning these compounds not only have strong but also relatively small-size-efficient bindings. L-Arginine, L-Methionine, and L-Valine were in the moderate (∼ − 5) category, while L-Tyrosine was the least efficient with LE (−3.28), which implies that the interaction was not very favorable on a per-atom basis.

To provide evidence that the selected amino acids are located in the active site of the enzyme and to compare their interactions with those of the reference inhibitor thiourea, the binding pose and interactions of thiourea within the active site were also analyzed. As shown in Fig. 3, thiourea formed only two metal coordination interactions with Ni798 and Ni799 ions with bond lengths 2.19 Å and 2.42 Å. Besides this, no electronic or hydrophobic interactions were observed.

In light of the *in vitro* urease inhibition results, D-Phenylalanine and L-Valine, which are the two most active amino acids, were chosen to study their interactions with the atoms in the active site of urease. The interaction analysis of D-Phenylalanine with urease, both in 2D and 3D, shows several major contacts that most probably play a role in its strong inhibitory activity (Fig. 4). The coordination of carbonyl oxygen with Ni798 results in a metal coordination and a salt bridge interaction, while

Table 2
IFD scores and MM-GBSA ΔG binding free energies of the inhibitors against urease.

Inhibitors	Urease (PDB ID: 3LA4)		
	IFD score (kcal/mol)	MM-GBSA ΔG bind. (kcal/mol)	Ligand efficiency (LE)*
D-phenylalanine	−12.351	−72.20	−6.02
L-arginine	−11.473	−62.62	−5.22
L-methionine	−11.276	−50.98	−5.66
D-threonine	−11.270	−55.47	−6.93
L-tyrosine	−11.653	−42.58	−3.28
L-valine	−11.255	−44.31	−5.54
Thiourea	−5.542	−38.80	−9.70

* LE represents the average binding energy contribution per heavy atom and was calculated according to the formula MM-GBSA ΔG/Number of heavy atoms (excluding H).

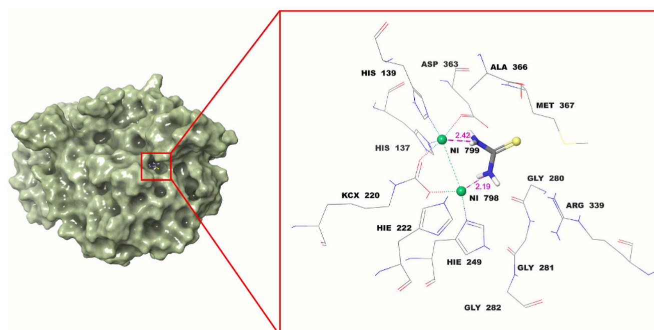

Fig. 3. Molecular docking 3D ligand-protein interactions of Thiourea-Urease complex.

carboxylate oxygen is involved in similar interactions with both Ni798 and Ni799, thus fortifying the stabilization in the active site [42,43]. The amino group interacts through two hydrogen bonds with Gly280 and Asp363. In addition, Asp363 forms a salt bridge, which emphasizes the position of the residues in the process of positioning the inhibitor and making its binding more secure. At the same time, the phenyl ring of D-Phenylalanine is involved in the formation of a π-π stacking interaction with His323, which is very important for the hydrophobic packing within the active site (Fig. 4a) [44]. D-Phenylalanine's high inhibitory potency can thus be attributed to the strong mechanistic interactions with nickel centers, urease residues that are catalytically and structurally important, and key active site residues.

Fig. 5 illustrates in detail the interactions of L-valine with the residues in the active site of urease.

Similar to *D*-phenylalanine, L-valine formed metal coordination and salt bridge interactions with Ni798 and Ni799 ions. Likewise, its amino group participated in a salt bridge interaction with Asp363. However, unlike *D*-phenylalanine, L-valine did not form hydrogen bonds with Gly280 or Asp363. Moreover, the π-π stacking interaction with His323 observed for *D*-phenylalanine was not detected in the case of L-valine. The similarities and differences between the interactions of these two amino acids clearly explain the variations observed in their *in vitro* and *in silico* activities.

As a result, molecular docking and *in vitro* assays consistently demonstrated that D-Phenylalanine and L-Valine are the most potent urease inhibitors among the tested amino acids. The highly significant binding affinities of the compounds are due to the perfect coordination of the metal with Ni and furthermore to the active site interactions like hydrogen bonding and π-π stacking that up the stability of the ligands. In contrast, thiourea, the standard inhibitor, showed only minimal coordination with the metal, which was a clear sign of the superior capacity for the selected amino acids to inhibit the enzyme. So, the main point is that the balanced hydrophobicity, size, and metal-binding capability are the factors that determine how effective the urease can be inhibited.

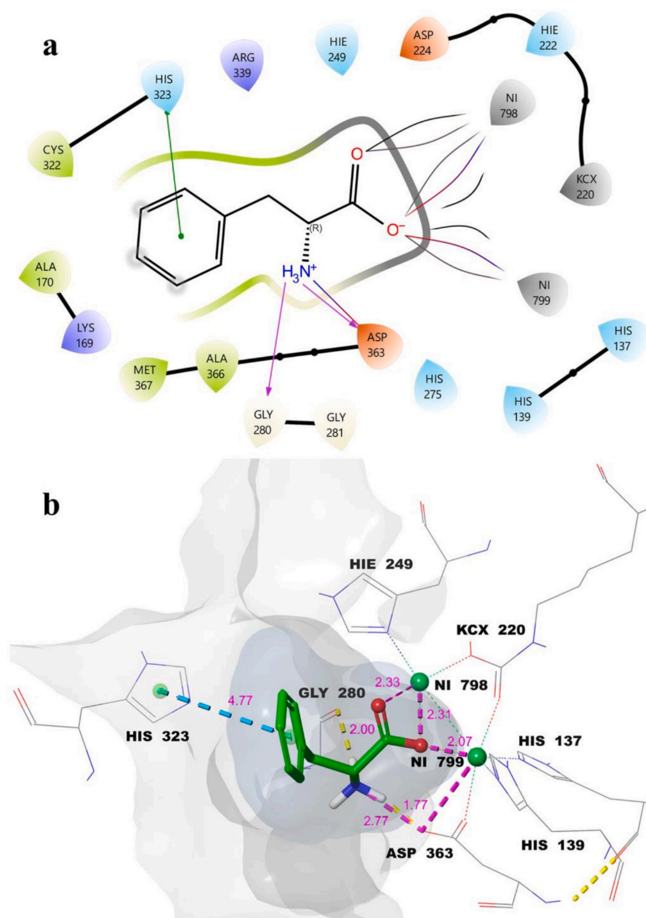

Fig. 4. Molecular docking 2D (a) and 3D (b) ligand-protein interactions of D-Phenylalanine-Urease complex.

4. Conclusions

In the present investigation, the inhibitory power of different natural amino acids on Jack bean urease was comprehensively assessed. The *in vitro* IC_{50} values indicated that D-Phenylalanine and L-Valine were submicromolar inhibitors, L-Methionine and L-Arginine were potent inhibitors, L-Tyrosine was a moderate inhibitor, and D-Threonine was a weak inhibitor, with the majority of the amino acids showing greater inhibition than the reference compound thiourea. The complementary molecular docking studies reinforced these discoveries by demonstrating that the amino acids form stabilizing interactions with the active site residues and the nickel ions that are responsible for the catalytic process and these interactions include hydrogen bonding, salt bridges, metal coordination, and π-π stacking, thus, giving the mechanistic

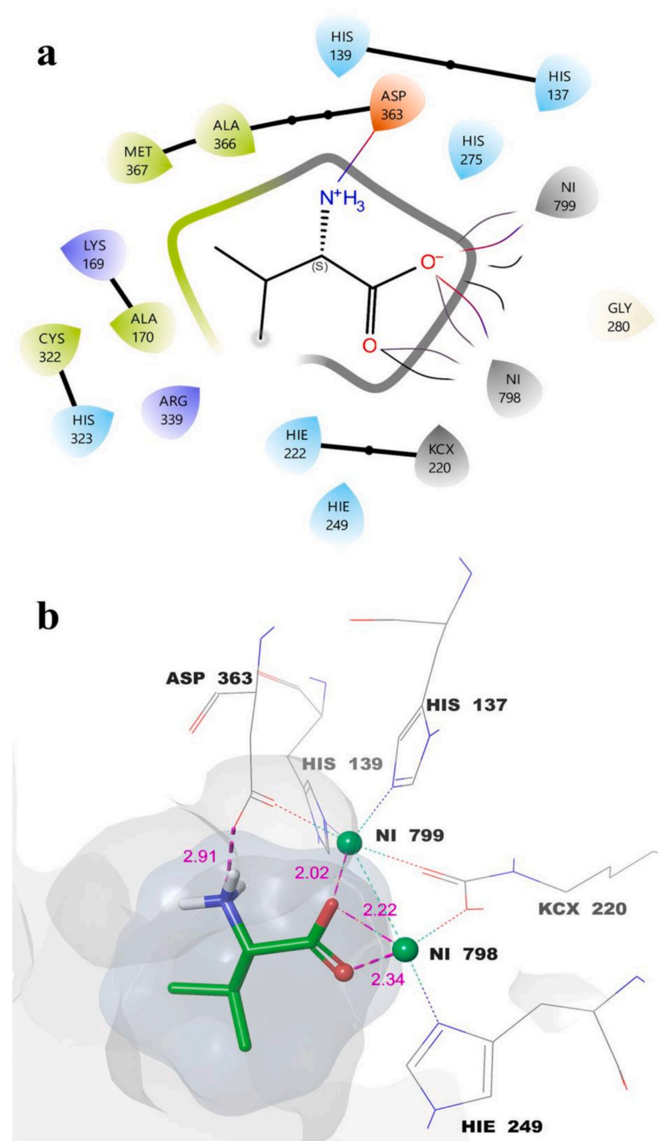

Fig. 5. Molecular docking 2D (a) and 3D (b) ligand-protein interactions of L-Valine-Urease complex.

explanation for the potency that has been observed. The findings emphasize amino acids as non-toxic and potent urease inhibitors that could find uses in agriculture and medicine. Further research with more amino acids and their derivatives together with enzyme kinetics and molecular modeling will not only elucidate the mechanisms of inhibition but also open up new avenues for producing biocompatible urease inhibitors.

One of the drawbacks of this research is that the inhibitory action of the free amino acids was assessed against Jack bean urease isolated under controlled *in vitro* conditions. In real soils or in microbiological environments, free amino acids might quickly get adsorbed, consumed, or even locked away by other enzymes, thus, lowering their accessibility and selectivity towards urease. This restriction can be solved by presenting a number of strategies for future development: *(i)* the construction and examination of di- and tri-peptides or peptidomimetics, which might have greater binding strength and longer stability because of their increased molecular weight and lower diffusivity; these modifications could make the soil areas experiencing the decay of the inhibitor less than before; *(ii)* the usage of D-amino acids or N-modified/non-canonical amino acids, as these are very resistant to microbial degradation and enzymatic hydrolysis, therefore possibly giving rise to increased environmental persistence and improving bioavailability to urease; and *(iii)* the adoption of formulation-based methods like slow-release matrices, polymeric or nanoparticle encapsulation, or co-formulation with urea that can bring down volatilization losses and enable controlled delivery to the enzyme's microenvironment. In general, the amino acid-based urease inhibitors will most likely gain tremendous stability, selectivity, and thereby practical use in agriculture and environmental areas thanks to these strategies.

Major findings were obtained from the research regarding natural amino acids as urease inhibitors, however, it was conducted solely on the basis of *in vitro* enzyme assays and *in silico* molecular docking studies. The lack of *in vivo* testing and thorough enzyme kinetics studies prevent the findings to be applied to more complex biological systems. Besides, the study was done exclusively on free amino acids and did not look into their derivatives or formulations which could have had an impact on the stability, selectivity, and bioavailability. More in-depth kinetic studies, *in vivo* trials, and testing the longer derivatives should be part of the future work to validate and generalize the potential of these findings.

CRediT authorship contribution statement

Fulya Bağ: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Orhan Uluçay:** Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Feyzi Sinan Tokalı:** Writing – original draft, Methodology, Investigation. **Halil Şenol:** Visualization, Software, Methodology, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The article is based on the MSc thesis of Fulya Bağ. The thesis study has been expanded with the contributions of Dr. Feyzi Sinan Tokali and Dr. Halil Şenol through molecular modeling. In the compilation of this article, the authors have been assisted by programs to improve spelling and readability. These include Grammarly, AI (ChatGPT), DeepL, Google Translate, and Quillbot. The authors reviewed and edited the content using these tools. The authors take full responsibility for the contents of this manuscript.

No external funding was received for the study.

Data availability

No data was used for the research described in the article.

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