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SYNTHESIS, ANTIMICROBIAL ACTIVITY, UREASE INHIBITION AND MOLECULAR DOCKING STUDIES OF NEW PROLINE LINKED THIOUREA DERIVATIVES

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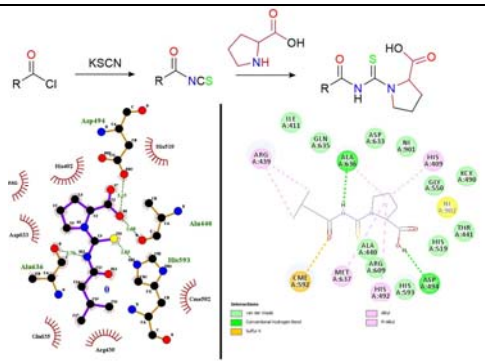
A series of new thiourea based carboxylic acids (**I_a-I_e**) were synthesized and characterized by elemental analysis, FTIR and NMR (^{1}H and ^{13}C) spectroscopy. They were preliminary bioassayed for their antibacterial, anifungal and urease inhibition activities. Molecular docking simulations were carried out to determine the probable binding mode of the synthesized compounds. The bioassay results showed that some of titled compounds exhibited encouraging results.



INTRODUCTION

Thiourea derivatives contain unique molecular structures with diverse pharmacological activities such as anti-inflammatory,[1–2] antidiabetic, antioxidant,[3] antiulcer,[4] antimycobacterials,[5] antimicrobial[3] and anticancer.[6] The significance of thiourea derivatives is attributed to the nature of both thiocarbonyl (C = S) and carbonyl (C = O) groups in the molecular structure,[1,7] which have an interesting influence on pharmacological behavior. Additionally, such interesting biological behaviors demonstrated by thiourea derivatives may also be attributed to their π-electron clouds as well as lone pairs present on oxygen, sulfur and nitrogen in their molecular configuration.[1, 8–9] Moreover thiourea derivatives can be also used as intermediates to construct other biologically active molecules with a thiourea backbone.[10] Thiourea derivatives act as selective analytical reagents particularly for the determination of a specific metal in the presence of many other metal ions.[11–12] Thiourea derivatives serve as corrosion inhibitors, and with conjugated sulfur, oxygen or nitrogen donor sites, thiourea derivatives function as good chelating ligands.[12] Metal complexes of these thiourea derivatives are utilized in supramolecular chemistry, homogenous catalysis, magnetic materials and chemical vapor deposition.[11, 13]

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Keeping in view the above aspects and in the continuation of our previous research work,[14–15] we report herein the synthesis, structural characterization and biological activities of some substituted thiourea derivatives **(I_a-I_e)**.

RESULTS AND DISCUSSION

The compounds (**I_a-I_e**) were synthesized by the reaction of respective acid chloride and potassium thiocyanate with the proline in stoichiometric amounts. They were fully characterized by FTIR and multinuclear NMR (^{1}H and ^{13}C) spectroscopies. The results of elemental analysis data are consistent with their proposed compositions. The spectroscopic data have been shown in the Experimental Section.

1. FTIR spectra

The pertinent IR absorption bands for **(I_a-I_e)** have been identified by comparing the data with literature values.[10, 14] Bands in the range 3387-3467 cm^{-1} and 3227-3261 cm^{-1} are attributed to stretching frequencies of OH and NH groups. Bands, due to C=O $_{carboxylic}$ stretching occurred in range 1712-1733 cm^{-1} and C=O $_{amide}$ in range 1667-1698 cm^{-1}.

2. NMR spectra

The chemical shifts were identified by their multiplicity patterns, intensity and compared with reported values. From the integration curve, total number of protons were calculated, which match exactly with expected molecular structures of newly synthesized compounds **(I_a-I_e)**. The ^{1}H NMR spectra for **(I_a-I_e)** show two distinct singlets in range 11.00-11.61 and 9.36-9.87 ppm which are attributed to carboxylic protons and amide protons respectively. Moreover, protons of pyrrolidine were found to resonates in the expected range 1.73-4.44 ppm.[10]

The ^{13}C NMR analysis further supports the structure of the target compounds **(I_a-I_e)**. All distinct carbon atoms present in the compounds were explicitly resolved by ^{13}C NMR. On the basis of alkyl signal intensities, alkyl carbon resonances are easily designated. By comparison of calculated chemical shifts from incremental method and experimental chemical shifts, the alkyl carbon resonances were designated [1]. As suggested from the previously published data, pyrrolidinic group shows signals in anticipated values (in range 26.8-59.8 ppm).[10,16–17] Whereas carboxylic carbon resonates in range 173-178.9 ppm and C=S resonate in range 180.6-188.5 ppm.

In accordance with the elemental analysis, FTIR and NMR spectral data, we suggest that the structures of **(I_a-I_e)**, resembles the general formula as suggested by Odame *et al*.[10]

3. Biological Studies

3.1. Antimicrobial activity

The synthesized compounds **(I_a-I_e)** were preliminary bio-assayed against four fungal strains (*A. Flavus, Trichoderma spp., A. Fumigatus,* and *A. Niger*) and four bacterial strains (*P. Aeruginosa, S. Aureus, E coli,* and *B. Subtlis*) for their antifungal and antibacterial activity (data is given in Table 1). The synthesized compounds **(I_a-I_e)** represented good to significant antibacterial activity (especially **I_a and I_c)** and has the ability of inhibiting the growth of tested bacterial strains to various levels. This significant antibacterial activity of the **(I_a and I_c)** can be attributed to its antibacterial effects or from its bacteriostatic effects. In case of antifungal data, **(I_b and I_d)** exhibited good performance in inhibiting the growth of fungal strains. Antifungal activities of **(I_b and I_d)** are comparable to the commercially available drug (Clotrimazole).

3.2. Antiurease activity

Antiurease properties of the compounds **(I_a-I_e)** were screened by urease inhibitory assay (Table 2). The screening of the antiurease activities of the synthesized compounds **(I_a-I_e)** revealed that compounds **(I_d** and **I_e)** inhibit more than 50% of urease inhibition activity. The compounds were further analyzed for the calculation of IC_{50} as compared to the standard drug thiourea. These were ascertained to be effective inhibitors of the urease and have shown significant activity comparable to the standard (thiourea). The potential inhibitory activity of the target compounds may be ascribed to their structural similarity to that of the thiourea (a natural substrate of urease). Subsequently, the bioactivity of the molecules cannot be decided by the impact of a lone variable or parameter. Moreover, in most of the cases, the existence of several groups in a molecule does not permit to exactly elucidate the potency and type of its bioactivity.[18]

Table 1

Antimicrobial data for **(I_a-I_e)**

	S. Aureus **(ZI^1)**	***B. subtlis*** **(ZI^1)**	***E. coli*** **(ZI)**	***P. aeruginosa*** **(ZI^1)**	***A. fumigatus*** **(ZI^1)**	***Trichoderma spp.*** **(ZI^1)**	***A Niger*** **(ZI^1)**	***A Flavus*** **(ZI^1)**
I_a	15	29	22	17	09	08	11	13
I_b	08	09	13	07	13	14	18	15
I_c	19	06	22	15	10	13	19	16
I_d	12	10	16	11	17	15	18	16
I_e	12	20	18	14	18	12	09	10
SD*	22	33	28	18				
SD**					24	17	21	19

ZI^1: Zone of inhibition (in mm)
SD*= Ciprofloxacin
SD**= Clotrimazole

Table 2

Urease inhibition data for **(I_a-I_e)**

Sample Code	Concentration (mM)	Inhibition (%)	IC_{50} (μmol.)
I_a	0.5	42.3	-
I_b	0.5	78.3	49.8
Ic	0.5	51.7	-
I_d	0.5	89.4	29.8
I_e		81.1	33.19
Thiourea	0.5	97.60	23.64

4. Molecular docking analysis

Molecular docking was implemented by following the previously published protocol [19]. The docking study was performed to clarify the binding mode of the synthesized compounds **(I_a-I_e)** in the active site of jack bean urease (**PDB code: 4H9M**). For this purpose, **(I_a-I_e)** were docked onto the active site of the enzyme using Autodock Vina. The Autodock tool was used to anticipate the best fitted conformational orientation of the synthesized compounds against jack bean urease. The generated docked complexes were evaluated in accordance with minimum energy value (kcal/mol) as well as bonding interaction configuration (van der Waal, hydrogen, π-alkyl and alkyl). Docking results are in good agreement with our experimental findings. The minimum energy value (-5.9 kcal/mol) was found for **(I_d)**, which was comparatively higher than acetohydroxamic acid (-3.8 kcal/mol, during its re-docking), which is a well-known urease inhibitor.

Docking analysis showed that **(I_d)** interacted with the active binding region of jack bean urease (as shown in Figures 2-3). The target compound was placed near the Ni^{+2} ions in the binding pocket of jack bean urease. In this position, **(I_d)** interacted with ASP A:494 and ALA A:636 through hydrogen bond. The π-alkyl interactions were also observed between the **(I_d)** and HIS (A:409 and A:492). The alkyl interactions were seen in the hydrophobic pocket containing side chain MET A:637 as well as ARG A:439. Two and three dimensional interaction patterns are shown in Figures 1-2. The relative SAR evaluation and the binding energy demonstrated that the essentialness of **(I_d)** may consider as powerful inhibitor by attacking jack bean urease.

EXPERIMENTAL

All chemical reagents were purchased from Merck (Germany) and were used as obtained from the supplier. Flash 2000 (elemental analyzer) was used to perform elemental analyses. Melting point was measured on the Gallenkamp (UK) instrument. Bio-Rad Excalibur FTIR Model FTS 3000 MX, was used to record Infrared spectra as KBr discs 4000–400 cm^{-1}. On a Bruker 300 spectrophotometer working on 300 and 75.45 MHz at ambient temperature, NMR spectra (^1H and ^{13}C) in solution were recorded. TMS was used as the internal reference.

1. Synthesis

Thiourea Based Carboxylic Acids **(I_a-I_e)**

The methodology used to synthesize the target compounds **(I_a-I_e)** was the same as reported earlier[14] with slight variation (Scheme 1). Solution of a substituted acid chloride (5 mmol) in acetone (20 mL) was added dropwise to a suspension of KSCN (5 mmol) in acetone (20 mL). The reaction mixture was refluxed for an hour, resulting in the formation of intermediate isothiocyanate. A solution of proline (5 mmol) in acetone (20 mL) was added and the resulting mixture was refluxed for additional 5 hours and afterwards the reaction mixture

was poured into the ice containing beaker. The solid product obtained was filtered and recrystallized with methanol. The reaction scheme may be depicted as follows.

***Synthesis of 1-[(2,2-dimethylpropanamido) methanethioyl]pyrrolidine-2-carboxylic acid* (I_a)**

Pivaloyl chloride (0.62 mL, 5 mmol), KSCN (0.49 g, 5 mmol), and proline (0.58 g, 5 mmol) were used. Yield: 78 %. M.p.: 117 °C. Anal. Calcd for $C_{11}H_{18}N_2O_3S$ (258.34): C, 51.14; H, 7.02; N, 10.84; S, 12.41. Found: C, 51.11; H, 6.96; N, 10.80; S, 12.46%. IR (KBr pellets, ν cm^{-1}): 3404 (O-H), 3240 (N-H), 2969 (C-H $_{alp}$), 1728 (C=O $_{carboxylic}$), 1689 (C=O $_{amide}$), 1367 (C-N), 772 (C=S). ^{1}H NMR (300M Hz, $CDCl_3$): δ 11.00 (1H, OH), 9.74 (1H, NH), 4.44 (1H, N-CH), 4.03 (m, 2 H), 2.18 (m, 2 H), 1.77 (m, 2H), 1.27 (9H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.6 (C=S), 173.5 (C=OOH), 172.1 (C=O), 27.4-58.4 (C $_{Pyrrolidine}$).

***Synthesis of 1-[(2-methylpropanamido) methanethioyl]pyrrolidine-2-carboxylic acid* (I_b)**

Isobutyryl chloride (0.52 mL, 5 mmol), KSCN (0.49 g, 5 mmol), and proline (0.58 g, 5 mmol) were used. Yield: 67 %. M.p.: 124 °C. Anal. Calcd for $C_{10}H_{16}N_2O_3S$ (244.31): C, 49.16; H, 6.60; N, 11.47; S, 13.12. Found: C, 49.12; H, 6.64; N, 11.46; S, 13.13%. IR (KBr pellets, ν cm^{-1}): 3387 (O-H), 3227 (N-H), 2983 (C-H $_{alp}$), 1719 (C=O $_{carboxylic}$), 1667 (C=O $_{amide}$), 1362 (C-N), 777 (C=S). ^{1}H NMR (300M Hz, $CDCl_3$): δ 11.24 (1H, OH), 9.54 (1H, NH), 4.09 (1H, N-CH), 4.01 (m, 2 H), 2.17 (m, 2 H), 1.73 (m, 2H), 2.52 (1H, CH), 1.16 (6H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.3 (C=S), 176.1 (C=OOH), 174.4 (C=O), 28.1-59.3 (C $_{Pyrrolidine}$).

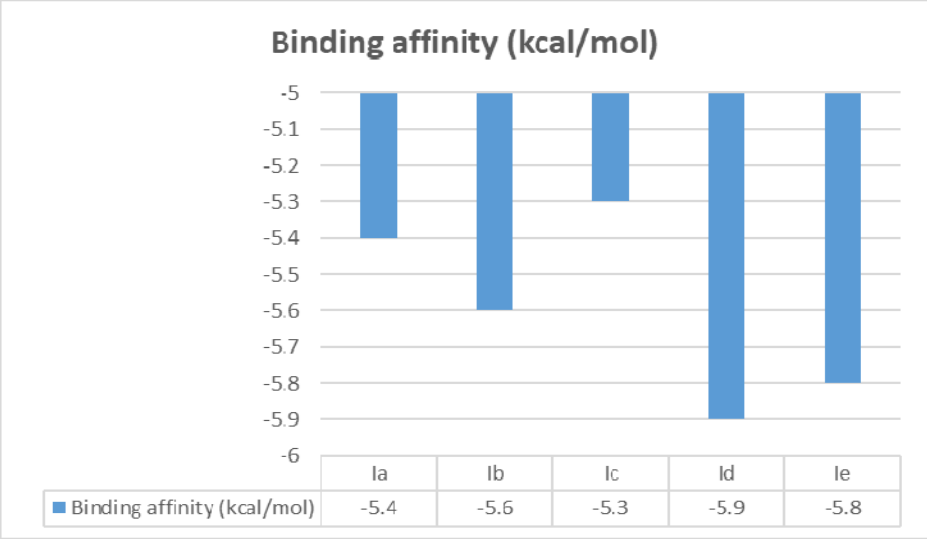


Fig. 1 – Binding energies (kcal/mol) of synthesized compounds with jack bean urease.

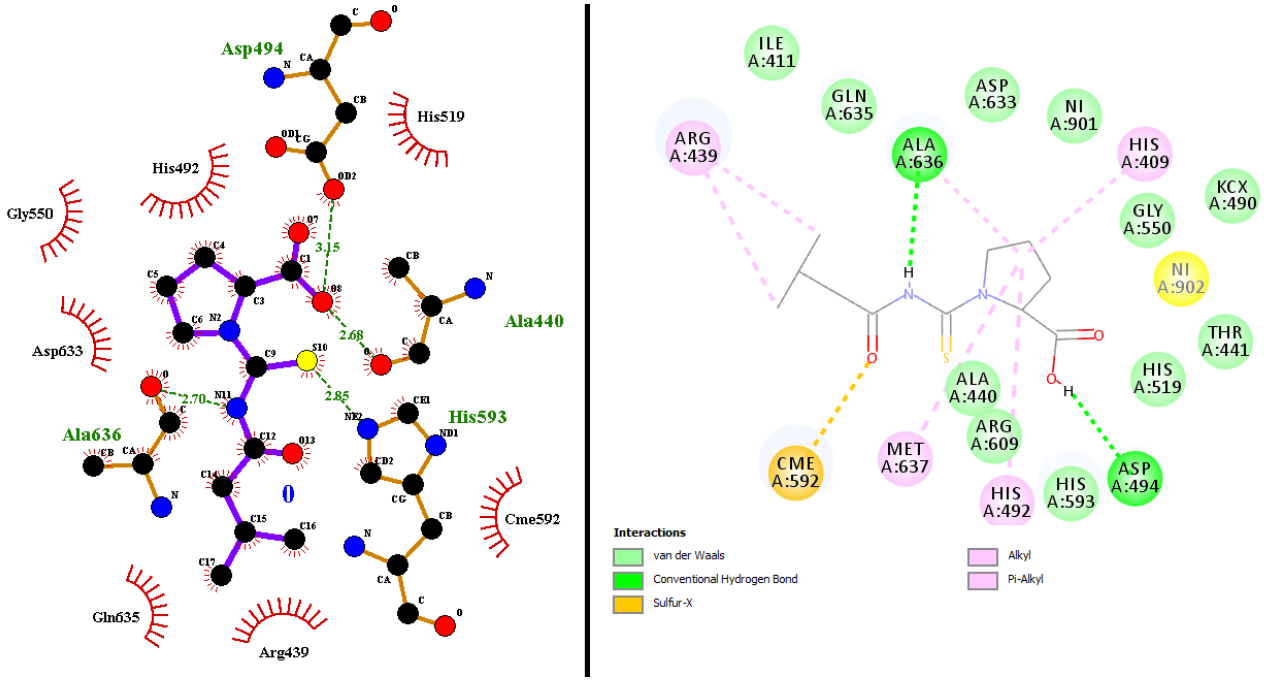


Fig. 2 – 2D representations of docking for **(I_d)** in the active site of jack bean urease.

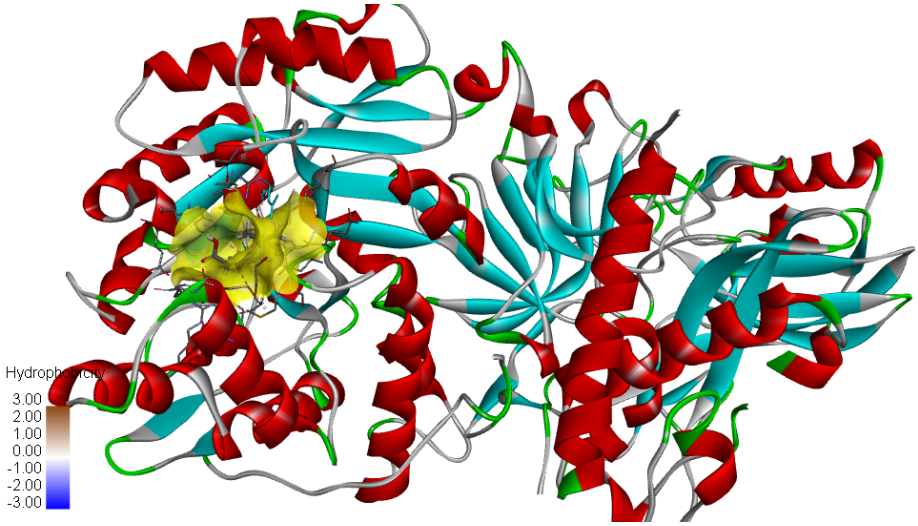
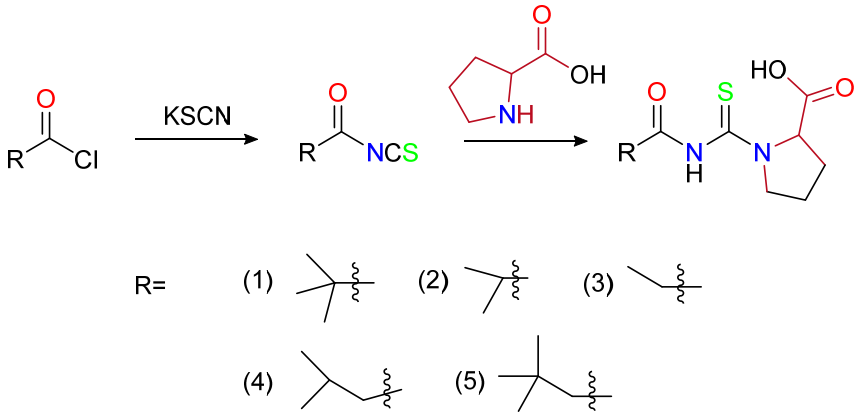


Fig. 3 – 3D representations of docking for **(I_d)** in the active site of jack bean urease.



Scheme 1 – General scheme for the synthesis of compounds (I_a-I_e).

Synthesis of 1-(propanamidomethanethioyl)pyrrolidine-2-carboxylic acid **(I_c)**

Propionyl chloride (0.44 mL, 5 mmol), KSCN (0.49 g, 5 mmol), and proline (0.58 g, 5 mmol) were used. Yield: 73 %. M.p.: 134 °C. Anal. Calcd for $C_9H_{14}N_2O_3S$ (230.28): C, 46.94; H, 6.13; N, 12.17; S, 13.92. Found: C, 46.95; H, 6.18; N, 12.15; S, 13.94%. IR (KBr pellets, ν cm^{-1}): 3419 (O-H), 3239 (N-H), 2945 (C-H $_{alp}$), 1712 (C=O $_{carboxylic}$), 1673 (C=O $_{amide}$), 1353 (C-N), 784 (C=S). ^{1}H NMR (300M Hz, $CDCl_3$): δ 11.28 (1H, OH), 9.87 (1H, NH), 4.24 (1H, N-CH), 4.06 (m, 2 H), 2.12 (m, 2 H), 1.76 (m, 2H), 2.34 (2H, CH_2), 1.05 (3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 188.5 (C=S), 179.2 (C=OOH), 175.6 (C=O), 26.8-59.2 (C $_{Pyrrolidine}$).

Synthesis of 1-[(3-methylbutanamido) methanethioyl]pyrrolidine-2-carboxylic acid **(I_d)**

3-methylbutanoyl chloride (0.61 mL, 5 mmol), KSCN (0.49 g, 5 mmol), and proline (0.58 g, 5 mmol) were used. Yield: 69 %. M.p.: 142 °C. Anal. Calcd for $C_{11}H_{18}N_2O_3S$ (258.34): C, 51.14; H, 7.02; N, 10.84; S, 12.41. Found: C, 51.13; H, 6.97; N, 10.81; S, 12.43 %. IR (KBr pellets, ν cm^{-1}): 3416 (O-H), 3258 (N-H), 2961 (C-H $_{alp}$), 1719 (C=O $_{carboxylic}$), 1698 (C=O $_{amide}$), 1376 (C-N), 765 (C=S). ^{1}H NMR (300M Hz, $CDCl_3$): δ 11.61 (1H, OH), 9.63 (1H, NH), 4.18 (1H, N-CH), 4.08 (m, 2 H), 2.17 (m, 2 H), 1.75 (m, 2H), 2.15 (2H, CH_2), 2.04 (1H, CH), 0.99 (6H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.1 (C=S), 176.6 (C=OOH), 172.7 (C=O), 27.1-59.8 (C $_{Pyrrolidine}$).

Synthesis of 1-[(3,3-dimethylbutanamido) methanethioyl]pyrrolidine-2-carboxylic acid **(I_e)**

3,3-dimethylbutanoyl chloride (0.70 mL, 5 mmol), KSCN (0.49 g, 5 mmol), and proline (0.58 g, 5 mmol) were used. Yield: 65 %. M.p.: 156 °C. Anal. Calcd for $C_{12}H_{20}N_2O_3S$ (272.36): C, 52.92; H, 7.40; N, 10.29; S, 11.77. Found: C, 52.94; H, 7.37; N, 10.26; S, 11.75%. IR (KBr pellets, ν cm^{-1}): 3467 (O-H), 3261 (N-H), 2973 (C-H $_{alp}$), 1733 (C=O $_{carboxylic}$), 1671 (C=O $_{amide}$), 1358 (C-N), 775 (C=S). ^{1}H NMR (300M Hz, $CDCl_3$): δ 11.48 (1H, OH), 9.36 (1H, NH), 4.13 (1H, N-CH), 4.04 (m, 2 H), 2.08 (m, 2 H), 1.78 (m, 2H), 2.07 (2H, CH_2), 0.98 (9H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 187.4 (C=S), 178.9 (C=OOH), 174.1 (C=O), 26.9-59.4 (C $_{Pyrrolidine}$).

2. Biological studies

Agar tube dilution protocol[20] and Agar well diffusion methods[21] were used for the preliminary screening of the **(I_a-I_e)** against different strains of fungi and bacteria. By following published protocols, the synthesized compounds were also tested for its antiurease[22–24] activity.

3. Molecular docking study

Autodock Vina (ver. 1.1.2) was used to study the molecular docking of the most active compound in in-vitro

analysis, by using 3D crystal structure of Jack bean urease **(PDB code: 4H9M)**, which is downloaded from the Protein Data Bank (www.pdb.org). All the hetroatoms were removed and the protein was then converted to pdbqt format using Autodock Tools (1.5.6).[25] The 2D chemical structures of the synthesized compounds **(I_a-I_e)** were sketched using Chem Draw Professional 13.0 and then energy minimized and converted to 3D format by Chem 3D 13.0. The Autodock Tool was then used to prepare the pdbqt format of the ligands.[25] The docking analysis was performed using the following parameters: center x = 19.748; center y = -57.479; center z = -22.485; size x = 15; size y = 15; size z = 15. The docking results were established with DS visualizer Software, Version 4.0[26] and ligplot.[27]

CONCLUSIONS

Thiourea based carboxylic acids **(I_a-I_e)** have been successfully synthesized and characterized by using spectroscopic analyses such as NMR (^{1}H, ^{13}C), FTIR, combined with elemental analysis. The synthesis of **(I_a-I_e)** is repeated several times therefore synthetic method is authentic. Our results indicate that these compounds **(I_a-I_e)** ensure favorable antibacterial and antifungal properties. The enzyme inhibition data exhibits that the target compounds **(I_a-I_e)** display promising results. Nevertheless, additional investigation is required to assess the compounds and also to understand its mode of mechanism of action comprehensively. However, these compounds might be considered for the drug designing programs in pharmaceutical domains.

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