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Long chain 1-acyl-3-arylthioureas as jack bean urease inhibitors, synthesis, kinetic mechanism and molecular docking studies

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ABSTRACT

The current research work reports the synthesis of novel long chain acyl thiourea derivatives as inhibitors of jack bean ureas. The title compounds were synthesized by the conversion of long chain carboxylic acids into corresponding acid chlorides followed by reaction with potassium thiocyanate to obtain a key reactive intermediate isothiocyanate, the latter was treated with suitably substituted aromatic anilines to afford the title 1-(substituted)phenyl-3-tetradecanoylthioureas. All of the compounds showed higher urease inhibitory activity than the standard thiourea. The compound **5f** exhibited excellent enzyme inhibitory activity with IC_{50} 0.0391 ± 0.0028 µM while IC_{50} of thiourea is 18.195 ± 0.382 µM. The kinetic mechanism analyzed by Lineweaver–Burk plots showed that compound **5f** is a non-competitive type inhibitor. Docking studies suggested that Asp494, Ala636, His593, Ala636, Lue494, Asp521 and Arg439 are the major interacting residues in the binding site of the protein and may have an instrumental role in the inhibition of enzyme's function. Synthesized acyl thioureas **5a–5k** showed good docking score (−8.2 to −6.9 Kcal/mol) and efficacy of lead molecules was investigated by carrying out pharmacokinetic studies, Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) assessment justified that these novel synthesized compounds showed good lead like potential with little hepatotoxic and skin sensitive effects. Chemo-informatics properties were evaluated by computational approaches and it was found that synthesized compounds mostly obeyed the Lipinski's rule.

1. Introduction

The inhibition of enzymes by small molecular weight organic molecules have fascinated the synthetic and medicinal chemists as a valuable strategy in drug discovery leading to the development of effective drugs. Urease enzyme hydrolysis urea into ammonia and carbon dioxide, the amount of ammonia released by the hydrolysis results in the enhancement of basic medium which paves the way for the survival of *Helicobacter pylori* a harmful bacteria. *H. Pylori* infection causes several human disorders, including gastritis, peptic ulcer disease and gastric cancers beside being resistant to several drugs. Moreover, the urease is actively involved in the formation of kidney stones and may also lead into the development of urolithiasis, pyelonephritis, and hepatic encephalopathy [1–4].

In addition the urease enzyme also hampers the soil fertility. Urea is one of the most widely utilized fertilizers for the soil growth as it furnishes the nitrogen in high percent (46%), besides economic and easy to handle [5,6]. Therefore urease inhibition is of prime importance and has attracted much attention due to multiple applications in pharmaceutical and drugs [7–9].

Thioureas possess slight structural similarity to the protein of urease enzyme, thereby exhibiting significant activity against jack bean urease [10–12]. Acyl thioureas are endowed with vast medicinal applications and broad synthetic utility [13–25]. Furthermore, these are valuable synthons in the synthesis of a wide variety of heterocycles. The chemical reactivity and biological applications of acyl thioureas have recently comprehensively reviewed by Saeed et al. [26,27]. The current research work aims to provide the synthesis of new acyl thioureas derived from myristic acid, their bioevaluation as jack bean urease inhibitors and free radical scavengers. All the synthesized derivatives possess significant potential of inhibi-

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tion against jack bean urease and molecular docking and kinetic mechanism also delineate their binding affinity and binding mode of inhibition.

2. Experimental

2.1. Methods and materials

Melting points were recorded using a digital Gallenkamp (Tokyo, Japan) model MPD.BM 3.5 apparatus and are uncorrected. ^{1}H NMR spectra were determined as $CDCl_3$ solutions at 300 MHz using a Bruker AM-300 spectrophotometer using TMS as an internal reference and ^{13}C NMR spectra were determined at 75 MHz using a Bruker 75 MHz NMR spectrometer in DMSO solution. FT-IR spectra were recorded on an FTS 3000 MX spectrophotometer. Elemental analyses were conducted using a LECO-183 CHNS analyzer.

2.2. General method for the synthesis of 1-aryl-3-tetradecanoylthiourea derivatives

A solution of myristic acid 1.14 g (5 mmol) and DMF (0.05 mL) in thionyl chloride 0.50 ml (7 mmol) was stirred under reflux for 3 h to afford corresponding acid chloride. A solution of KSCN 0.48 g (5 mmol) in dry acetone (10 ml) was added drop wise to a reaction mixture. The reaction mixture was refluxed for half an hour, after cooling, a solution of aniline (5 mmol) in dry acetone (10 ml) was added and resulting mixture was stirred at 50 °C for 8–9 h The progress of reaction was monitored through TLC. The reaction mixture was then poured onto crushed ice and the precipitates were collected by filtration, dried, and recrystallized from ethanol to afford the 1-aryl-3-tetradecanoylthiourea derivatives with excellent yield.

2.3. Urease inhibition assay

The Jack bean urease activity was determined by measuring amount of ammonia produced with indophenols method described by Weatherburn and Raza et al (2017). The reaction mixtures, comprising 20 µL of enzyme (Jack bean urease, 5 U/mL) and 20 µL of compounds in 50 µL buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA and 0.01 M LiCl, pH 8.2), were incubated for 30 min at 37 °C in 96-well plate. Briefly, 50 µL each of phenol reagents (1%, w/v phenol and 0.005%, w/v sodium nitroprusside) and 50 µL of alkali reagent (0.5%, w/v NaOH and 0.1% Sodium hypochlorite NaOCl) were added to each well. The absorbance at 625 nm was measured after 10 min, using a microplate reader (OPTI $_{Max}$, Tunable). All reactions were performed in triplicate. The urease inhibition activities were calculated according to the following formula:

$$\text{Urease inhibition activity (\%)} = \left(OD_{control} - OD_{sample} \times 100\right)/OD_{control}$$

Where $OD_{control}$ and OD_{sample} represents the optical densities in the absence and presence of sample, respectively. Thiourea was used as the standard inhibitor for urease.

2.4. Kinetic study

Kinetic analysis was carried out to determine the mode of inhibition. Compound **5f** was selected on the basis of most potent IC_{50} values. Kinetics were carried out by varying the concentration of urea in the presence of different concentrations of compound **5f** (0.0, 0.015, 0.030, and 0.06 µM). Briefly the urea concentration was changed from 100, 50, 25, 12.5, 6.25, 3.12 mM for urease kinetics studies and remaining procedure was same for all kinetic studies as describes in urease inhibition assay protocol. Maximal initial velocities were determined from initial linear portion of absorbances up to 10 min after addition of enzyme at per minute's interval. The inhibition type on the enzyme was assayed by Lineweaver-Burk plot of inverse of velocities (1/V) versus inverse of substrate concentration 1/ [S] mM^{-1}. The EI dissociation constant *Ki* was determined by secondary plot of 1/V versus inhibitor concentration. Urease activity was determined by measuring ammonia production using the indophenol method as reported previously (Saeed et al 2015). The results (change in absorbance per min) were processed by using SoftMaxPro.

2.5. Free radical scavenging assay

Radical scavenging activity was determined by modifying already reported method 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [23]. The assay solution consisted of 100 µL of DPPH (150 µM), 20 µL of increasing concentration of test compounds and the volume was adjusted to 200 µL in each well with DMSO. The reaction mixture was then incubated for 30 min at room temperature. Ascorbic acid (Vitamin C) was used as a reference inhibitor. The assay measurements were carried out by using a micro plate reader (OPTI $_{Max}$, Tunable) at 517 nm. The reaction rates were compared and the percent inhibition caused by the presence of tested inhibitors was calculated. Each concentration was analyzed in three independent experiments run in triplicate.

2.6. Computational methodology

2.7. Retrieval of target protein from PDB

The Jack bean urease structure was retrieved from Protein Data Bank (PDB) (www.rcsb.org) with PDBID 4H9M. The selected crystal structure of urease was minimized by using UCSF Chimera 1.10.1 tool [28]. Furthermore, the stereo-chemical properties of urease structure and Ramachandran plot and values were generated by Molprobity server [29] and Protparam [30], respectively. The Discovery Studio 4.1 Client was used to generate the hydrophobicity graph of target protein [31]. The protein architecture and statistical percentage values of receptor proteins helices, beta-sheets, coils and turn were predicted from online server VADAR 1.8 [32].

2.8. Chemo-informatics and ADMET properties of synthesized compounds

The synthesized ligands (**5a-k**) were evaluated on the basis of chemo-informatics and ADMET properties. Multiple online servers like Molinspiration (http://www.molinspiration.com/), and Molsoft (http://www.molsoft.com/) were employed to check the chemo-informatics and biological properties of these ligands (**5a-k**). Lipinski's rule of five was analyzed using Molinspiraion and Molsoft. Furthermore, their ADMET properties were evaluated by pkCSM online tool [32].

2.9. Molecular docking

The synthesized ligands (**5a–k**) were sketched in ACD/ChemSketch tool and minimized by UCSF Chimera 1.10.1 tool. The molecular docking experiments were performed using PyRx docking through VINA wizard [33]. The grid box center values were adjusted as for $X = 10.48$, $Y = -55.22$ and $Z = -26.48$), respectively. While, the size parameters values for $X = 66.60$, $Y = 60.08$, and $Z = 56.84$ were also focused to get the better conformational binding poses. The default exhaustiveness value was used to maxi-

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