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Azomethines, isoxazole, N-substituted pyrazoles and pyrimidine containing curcumin derivatives: Urease inhibition and molecular modeling studies

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ABSTRACT

Curcumin has shown large number of pharmacological properties against different phenotypes of various disease models. Different synthetic routes have been employed to develop its various derivatives for diverse biological functions. In this study, curcumin derived azomethine, isoxazole, pyrimidines and N-substituted pyrazoles were synthesized to investigate their urease enzyme inhibition. The structures of newly synthesized compounds were described by IR, MS, ^{1}H NMR and ^{13}C NMR spectral data. Urease enzyme inhibition was evaluated through in vitro assays in which compound 8b was found to be the most potent (IC_{50} = 2.44 ± 0.07 μM) among the tested compounds. The compounds with diazine ring system except the 4d showed better urease inhibition (IC_{50} = 11.43 ± 0.21–19.63 ± 0.28 μM) than the standard urease inhibitor thiourea (IC_{50} = 22.61 ± 0.23 μM). Similarly enzyme kinetics data revealed that compounds 3c-3e and 8b were competitive inhibitors with Ki values of 20.0, 19.87, 20.23 and 19.11 μM respectively while the compounds 4b, 4c and 4e were mixed type of inhibitors with Ki values 6.72, 19.69 and 6.72 μM respectively. Molecular docking studies were also performed to identify the plausible binding modes of the most active compounds.

1. Introduction

Chemically curcumin is diferuloylmethane which has been attracted extensively in biomedical research against various diseases due to its pharmacological safety. It exhibited wide range of biological activities such as antibacterial, antifungal, anti-inflammatory, antioxidant and cancer preventive properties [1–4]. Currently curcumin is acclaimed to be one of the most widely researched naturally occurring chemo preventive agent which is cytoprotective to healthy human cells. It has been tested in various disease models such as anti-amyloid β aggregation (Alzheimer's disease), arthritis, and cancer as both treatment and preventive agent [5–7].

In spite of important therapeutic application, limiting therapeutic utility concern is associated with curcumin because of its poor absorption and fast metabolism under physiological conditions [8]. Many efforts have been attempted to improve pharmacokinetics and pharmacological properties of curcumin through its structure modification [9,10]. Active methylene and keto moiety are believed to be responsible for its rapid metabolism. In order to circumvent the problem of rapid metabolism and to improve its pharmacokinetics profile, several synthetic modifications have been studied on carbonyl and active methylene moiety [11]. In the present study, azomethines (Schiff bases containing sulphonamides) with heterocyclic moieties, isoxazole, N-substituted pyrazoles and pyrimidine ring are incorporated in this focused segment of curcumin structure. The advantage of developing molecules around the curcumin scaffold is the lack of its toxicity [12].

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Nitrogen heterocyclic moieties containing derivatives gained considerable attention in medicinal chemistry for their broad spectrum pharmacological activities such as antimicrobial, anti-inflammatory, analgesic, enzyme inhibition, antioxidant and anti-cancer. These moieties have leading position in drug designing as important pharmacore [13–19].

Urease is a nickel based metalloenzyme belongs to super family of aminohydrolase and phosphotriesterase that accelerate the transformation of urea into carbon dioxide and ammonia. Continuous formation of ammonia enhances the gastric mucosa permeability that results in inflammation, ulcer, adenocarcinoma and lymphoma [20,21]. Urease is widely distributed among various types of bacteria and is well known for the pathologies induced by *Helicobacter pylori* (*H. pylori*). So, bacterial urease is virulent factor that cause urolithiasis, acute pyelonephritis and infection induced reactive arthritis. Major cause of peptic ulcer is now accepted by urease from *H. pylori*. So infection caused by *H. pylori* can be treated by inhibition of urease. For urease inhibition, quinolones, imidazoles, boronic acids, hydroxamic acids, heavy metal ions and phosphoramidates have already been investigated [22–24].

In earlier studies, curcumin containing pyrazoles/pyrimidine ring, isoxazole and azomethines have been found to demonstrate diverse biological activities such as antibacterial, antifungal, analgesic, anti-inflammatory, anticancer, antioxidant, anti-amyloid β aggregation, antimalarial and cyclooxygenase-2 inhibition [2,4,25–30].

In the best of our knowledge, this is a first report on curcumin containing pyrazoles/pyrimidine ring, isoxazole and azomethines as inhibitors of urease enzyme in which kinetics and molecular modeling studies were carried out to get insight into inhibition mode and binding conformations in urease enzyme.

2. Material and methods

2.1. Synthesis of curcumin derivatives

The systematic schemes for synthesis of azomethines (3a-3f, 4a-4f), isoxazole (5a), N-substituted pyrazoles (7a-7g) and pyrimidine (8a-8c) containing curcumin derivatives are described in supplementary material.

2.2. Chemistry

In present work, analytical grade chemicals were obtained from Central Chemicals-Lahore originate to Merck (Germany) and Sigma Aldrich (USA) and were used without further purification to synthesize desired compounds. The spectral studies were performed on FTIR-ATR spectrometer (Bruker-USA) and NMR spectrometer (^{1}HNMR, 400, 500 and 850 MHz whereas ^{13}CNMR, 100, 125 and 215 MHz, Bruker-USA). DMSO-d_6 was employed to obtain ^{1}HNMR, ^{13}CNMR spectra while TLC Silica gel 60 F_{254} (Merck, Darmstadt, Germany) was run to check the progress of reaction. MS 600H-1 (JEOL, USA) spectrometer with electron ionization interface was used for mass spectra. Flash 2000 HT elemental analyzer (Thermo Scientific, UK) was used for concentration of hydrogen (H), carbon (C), nitrogen (N) and sulfur (S) of synthesized compounds (analysis procedure can be seen from supplementary file) while the melting point was measured by Gallenkamp apparatus.

2.3. % Urease inhibition assay, IC_{50} and kinetics study protocol

Urease inhibition assay was performed as reported in literature with slight modifications [31]. Briefly, 100% initial activity well contained assay buffer (10 μL, K_2HPO_4, pH = 6.8–7.0), DMSO (10 μL) and urease enzyme solution (25 μL, jack bean urease 5 U/mL). Inhibitory well contained assay buffer (10 μL, K_2HPO_4, pH = 6.8–7.0), tested compounds (10 μL, 125–0.9677 μM) and urease enzyme solution (10 μL). Then plate was incubated at 37 °C for 10 min. After the incubation, 40 μL of substrate (urea, 20 mM) was added in each type of well and again incubated at 37 °C for 10 min. Then 40 μL of phenol reagent (phenol 1.0% w/v, sodium nitroprusside 0.005% w/v) and 40 μL of alkali reagent (sodium hydroxide 0.5%, 0.1% active chlorine from sodium hypochlorite) were added to each well and placed at room temperature for 50 min. Thiourea was used as reference standard in 125–0.9677 μM concentration. Absorbance of each well at 625 nm was noted by micro plate reader LT-4500 (Labtech International Ltd, UK) and urease inhibition (%) was calculated by following formula.

$$\%\text{Urease inhibition} = \left[1 - \frac{T}{C}\right] \times 100$$

Where, T is absorbance of inhibitory well and C is absorbance of 100% initial activity without inhibitor. All the assays were performed in triplicate and results are presented as mean ± SEM. IC_{50} values of each compound and reference was calculated by regression equation where 50% inhibition was observed. For kinetics studies, binding mechanism of each compound (inhibitor) was carried out that has IC_{50} values comparable with thiourea (reference compound). Five different inhibitor concentrations (0–20 μM) were reacted with different concentration of substrate (urea, 0.5–4.0 mM) to evaluate whether the inhibitor is competitive, non-competitive (mixed) and uncompetitive after determining the $K_{m\,(app)}$, $V_{max\,(app)}$ from Lineweaver Burk plot and K_i (inhibition constant) were also evaluated by Dixon plot using PRISM 7.0.

2.4. Molecular modeling studies

2.4.1. Protein and ligand preparation

Crystal structure of the jack bean urease enzyme (PDB ID = 4h9m) was retrieved from protein databank. Protein structure was prepared using the "protein preparation wizard" implemented in the Schrodinger software. Missing residues and their side chains were added using the Prime tool from the same software package. Similarly hydrogen atoms were added and crystallization reagents were removed. Water molecules were also removed from the structure as no one is involved in the reaction mechanism of urease enzyme. Then restrained minimization of prepared structure up to 0.3 Å from original structure was performed to remove all steric clashes. For minimization OPLSA 2005 force field was used. Similarly most active compound was sketched and prepared using ligprep module implemented in the Schrodinger software package. Different tautomers of the compound 8b were generated along with different conformations.

2.4.2. Molecular docking protocol

The prepared crystal structure of jack bean urease was used as receptor for molecular docking studies. For grid box generation, the active site was defined near the nickel ions. The X = 18.96 Å Y = −58.15 Å and Z = −23.99 Å coordinates were defined with 10 Å length in each dimension. Optional hydrogen bond or metal-ligand interaction constraint was defined for any of nickel ion during docking simulations. The hydrogen atoms of hydroxyl and thiol groups of active site amino acids were allowed to rotate during docking. Finally five top poses of the docked ligand was subjected to minimization and best pose was selected based on the highest glide score [32]. For docking simulations standard precision (sp) mode of glide was used.