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Original article

Development of bis-thiobarbiturates as successful urease inhibitors and their molecular modeling studies

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ABSTRACT

Bis-thiobarbiturate derivatives **1**–**15** have been synthesized, characterized by ^{1}HNMR and EI-MS and screened for urease inhibition. All compounds showed various degree of urease inhibitory activity with IC_{50} values ranging $7.45 \pm 0.12 - 74.24 \pm 0.81$ μmol/L while the standard thiourea behaved normally ($IC_{50} = 21.10 \pm 0.12$). Compounds **1** ($IC_{50} = 7.45 \pm 0.12$ μmol/L), **9** ($IC_{50} = 18.17 \pm 1.03$ μmol/L) and **13** ($IC_{50} = 8.61 \pm 0.45$ μmol/L) showed excellent urease inhibitory activity in the series. Molecular modeling studies were performed to understand the binding site with the bimetallic nickel center of the enzyme. Structure-activity relationship has also been established for these compounds. This study identified bis-thiobarbiturate as a novel class of urease inhibitors.

1. Introduction

Urease (EC 3.5.1.5, urea amidohydrolase) is a nickel-dependent metalloenzyme that catalyzes the hydrolysis of urea to ammonia and CO_2 or carbamate [1]. A variety of ureases are found in bacteria, fungi, higher plants and soil as a soil enzyme [2]. Activity of urease has been shown to be a prominent virulence determinant in the pathogenesis of many clinical conditions, which are detrimental for human and animal health as well as for agriculture [3]. Urease is known to be one of the major causes of diseases induced by *Helicobacter pylori*, thus allow them to survive at low pH inside the stomach. It also plays an important role in the pathogenesis of gastric and peptic ulcer [4]. Urease is directly involved during the formation of infectious stones and contributes to the pathogenesis of urolithiasis, pyelonephritis and hepatic encephalopathy, hepatic coma and urinary catheter encrustation [5]. Urease is responsible for urinary tract and gastrointestinal infections [6], possibly causing severe diseases such as peptic ulcers and stomach cancer as in the case of *H. pylori* [7]. In agriculture, high urease activity causes significant environmental and economic problems through releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization [3]. Moreover, it induces plant damage primarily by depriving plants of their essential nutrients and secondarily by ammonia toxicity, increasing the pH of the soil [8]. Due to the diverse functions of this enzyme, its inhibition by potent and specific compounds could provide an invaluable addition for the treatment of infections and secondary complexes such as gastritis and gastric ulcer caused by *H. pylori* [9,10].

Barbiturates are well-known compounds of hypnotic properties and are used as an active moiety on central nervous system. Thiobarbituric acid is different from barbituric acid due to the presence of a sulfur atom instead of an oxygen atom. Two active methylene hydrogen atoms at carbon-5 flanked between the two carbonyl carbons due to which their acidity further increased and

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their derivatives show significance biological activities [11]. Thiobarbituric acid analogs have been also reported as antifungal [12], antiurease [13], antimicrobial [14], Adiponectin expression [15], herbicides [16], anti-convulsing [17], anti-sclerosis's agents [18] and antidiabetic and antibacterial agents [19]. Thiobarbituric acid analogs also showed anti-cancer and anti-viral activities [20].

2. Experimental

^{1}H NMR spectra were performed in DMSO-d_6 on an Avance Bruker AM 300–500 MHz Instrument and TMS was used as an external reference. Chemical shifts values are given in δ (ppm). Electron impact mass spectra (EI-MS) were characterized on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

2.1. General procedure for synthesis of bis-thiobarbiturate derivatives

Bis-thiobarbiturate derivatives **1**–**15** have been synthesized by the reactions of 1, 3 diethyl-2-thioxodihydropyrimidine-4, 6(1*H*, 5*H*)-dione (*N, N*-diethylthiobarbituric acid, 2 mmol) with different aromatic aldehydes (1 mmol) in the presence of 5–10 mL of EtOH (Scheme 1). Reaction mixture was stirred in ethanol for about 3 h. The reaction completion was monitored by periodic TLC analysis. After the completion of the reactions, the mixture was poured over crushed ice followed by acidification with dill. HCl. Solid precipitates were collected by filtration, dried and recrystallized from ethanol to give the pure product in excellent yield. The structures of all synthetic compounds **1**–**15** were confirmed through EI-MS and ^{1}H NMR.

5, 5′-((3,5-Dimethoxyphenyl)methylene)bis(1,3-diethyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione) **(1):** ^{1}H NMR: (400 MHz, DMSO-d_6): δ 7.2 (s, 2H, H-2/6), 6.8 (s, 1H, H-4), 3.7 (m, 4H, CH_2), 3.5 (m, 4H, CH_2), 3.4 (s, 6H, OMe), 3.1 (m, 1H, CH), 1.2 (m, 12H, CH_3); EI-MS: *m/z* (rel. int. %): 548 (M^+, 42), 534 (45), 350 (100), 152 (34), 99 (56).

5,5′-(Quinolin-2-ylmethylene)bis(1,3-diethyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione) (**2**): ^{1}H NMR: (400 MHz, DMSO-d_6): δ 7.8 (d, 1H, $J_{7,8}$ = 6.7 Hz, H-7), 7.6 (d, 1H, $J_{8,7}$ = 6.7 Hz, H-8), 7.3 (d, 1H, $J_{3,4}$ = 7.1 Hz, H-3), 7.1 (d, 1H, $J_{6,5}$ = 7.5 Hz, H-6), 6.8 (m, 2H, H-4/5), 3.6 (m, 4H, CH_2), 3.4 (m, 4H, CH_2), 3.0 (m, 1H, CH), 1.6 (m, 12H, CH_3); EI-MS: *m/z* (rel. int. %): 539 (M^+, 41), 524 (75), 351 (56), 152 (100).

5,5′-((6-(Benzyloxy)-1*H*-indol-3-yl)methylene)bis(1,3-diethyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione) (**3**): ^{1}H NMR: (400 MHz, DMSO-d_6): δ 7.8 (s, 1H, H-2), 7.6 (d, 1H, $J_{4,5}$ = 8.1 Hz, H-4), 7.3 (d, 1H, $J_{5,4}$ = 7.5 Hz, H-5), 7.1 (s, 1H, H-7), 6.8 (m, 5H, H-2′/3′/4′/5′/6′), 3.5 (m, 4H, CH_2), 3.2 (m, 4H, CH_2), 2.8 (m, 1H, CH), 1.4 (m, 12H, CH_3); EI-MS: *m/z* (rel. int. %): 633 (M^+, 56), 514 (75), 351 (100), 162 (100).

5,5′-((3,4-Dihydroxyphenyl)methylene)bis(1,3-diethyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione) (**4**): ^{1}H NMR: (400 MHz, DMSO-d_6): δ 8.35 (s, 1H, H-2), 7.76 (dd, 1H, $J_{6,5}$ = 8.4, $J_{6,2}$ = 2 Hz, H-6), 7.0 (d, 1H, $J_{6,5}$ = 8.4 Hz, H-5), 3.7 (m, 4H, CH_2), 3.5 (m, 4H, CH_2), 3.4 (s, 6H, OMe), 3.1 (m, 1H, CH), 1.2 (m, 12H, CH_3); EI-MS: *m/z* (rel. int. %): 520 (M^+, 34), 414 (45), 363 (100), 151 (37), 99 (57).

2.2. Urease inhibition assay

Reaction mixtures having one unit of urease enzyme (*Bacillus pasteurii*) solution and 55 μL of buffers containing 100 mmol/L urea were incubated with 5 μL of test compounds (1 mmol/L) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring the ammonia production using the indophenol's method [21]. Briefly, 45 μL of the phenol reagent and 70 μL of the alkali reagent were added to each well. The increased absorbance at 630 nm was measured after 50 min, using a micro-plate reader (Molecular Devices, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed using the Soft-Max Pro s4.5.Software (Molecular Devices, USA).

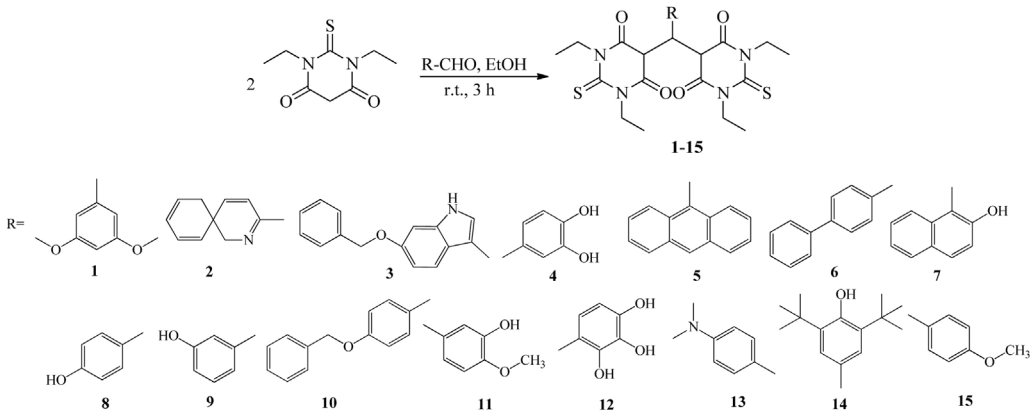
3. Results and discussion

3.1. Chemistry

A set of 15 bis-thiobarbiturate derivatives **1**–**15** were synthesized by the reactions of *N, N*-diethylthiobarbituric acid with different aromatic aldehydes in ethanol as a solvent. The structures of all the synthesized compounds **1**–**15** were confirmed on the basis of spectral data. ^{1}H NMR data of new bis-thiobarbiturate analogues were recorded and several generalizations could be made. Observation of a multiplet at 3.1 ppm is attributed to the bridged CH proton and is a clear indication of the product formation. A deshielded singlet with a two-proton integration appeared at 7.2 ppm, which could be assigned to the aromatic protons (H-2/6). The molecular ion peak was observed in the mass spectra of all the synthesized compounds, which confirmed their molecular masses.

3.2. Urease inhibition studies

Bis-thiobarbiturate derivatives **1**–**15** were screened against the urease enzyme according to the literature protocol [21]. All



Scheme 1. Synthetic protocol for *bis*-thiobarbiturate derivatives (**1**–**15**).