Bioorganic Chemistry 76 (2018) 37–52

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

5-Acetyl-6-methyl-4-aryl-3,4-dihydropyrimidin-2(1*H*)-ones: As potent urease inhibitors; synthesis, *in vitro* screening, and molecular modeling study

Shahbaz Shamim [a], Khalid Mohammed Khan [a,*], Uzma Salar [a], Farman Ali [a], Muhammad Arif Lodhi [b], Muhammad Taha [c], Farman Ali Khan [b], Sajda Ashraf [d], Zaheer Ul-Haq [d], Muhammad Ali [a], Shahnaz Perveen [e]

[a] *H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan*
[b] *Department of Biochemistry, Abdul Wali Khan University, Mardan, KP, Pakistan*
[c] *Department of Clinical Pharmacy, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, P.O. Box 31441, Dammam, Saudi Arabia*
[d] *Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan*
[e] *PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi 75280, Pakistan*

ARTICLE INFO

Article history:
Received 16 September 2017
Revised 23 October 2017
Accepted 31 October 2017
Available online 2 November 2017

Keywords:
Dihydropyrimidinone
Synthesis
Urease enzyme
In vitro
Structure-activity relationship (SAR)
In silico

ABSTRACT

5-Acetyl-6-methyl-4-aryl-3,4-dihydropyrimidin-2(1*H*)-ones **1**–**43** were synthesized in a "one-pot" three component reaction and structurally characterized by various spectroscopic techniques such as ^{1}H, ^{13}C NMR, EI-MS, HREI-MS, and IR. All compounds were evaluated for their *in vitro* urease inhibitory activity. It is worth mentioning that except derivatives **1**, **11**, **12**, and **14**, all were found to be more potent than the standard thiourea (IC_{50} = 21.25 ± 0.15 μM) and showed their urease inhibitory potential in the range of IC_{50} = 3.70 ± 0.5–20.14 ± 0.1 μM. Structure-activity relationship (SAR) was rationalized by looking at the varying structural features of the molecules. However, molecular modeling study was performed to confirm the binding interactions of the molecules (ligand) with the active site of enzyme.

1. Introduction

Urease (EC 3.5.1.5) is a nickel structured enzyme from the super family of amidohydrolase and phosphotriesterase that catalyzes the conversion of urea to carbon dioxide and ammonia. Hyperactivity of urease enzyme leads to interminable formation of ammonia which results in the increase of gastric mucosa permeability [1]. Urease plays a pivotal role in the nitrogen metabolism of cattle and various other animals [2]. The severity of some bacterial pathogens is associated with this enzyme and it has a prominent role in the pathogenesis of several diseases [3]. Urease enzyme facilitates the *Helicobacter pylori* (HP) to survive at low pH of stomach and plays a significant role in the development of gastric and peptic ulcer which may eventually cause cancer [4]. Continuous release of ammonia is directly responsible for several metabolic disorders and destructs the GIT epithelium by its action with human immune system. Therefore, it is the matter of dire necessity to identify more urease inhibitors which have significant stability, bioavailability, and very low toxicity [5].

Dihydropyrimidone is a six membered heterocyclic compound having nitrogen at position 1 and 3 [6]. Dihydropyrimidones are synthesized by multi-component reaction from aryl aldehyde, ethyl acetoacetate, and urea known as Biginelli reaction [7]. Among the five principle bases in nucleic acids, three of them are pyrimidine derivative including cytosine, thymine, and uracil. Cytosine is present in both DNA and RNA (uracil in RNA and thymine in DNA). Their presence in DNA confer a significant recognition and give a discernible attraction in the field of synthetic organic chemistry [8]. Dihydropyrimidones are known to possess a wide range of pharmacological properties such as anticancer, antitumor, antiviral, and antihypersensitive activities [9]. They are also recognized for calcium channel modulator and potential on immunorestoring agents in tumor effected organs [10].

Our research group has identified a number of lead compounds for their potential urease inhibitory activity [11–18] and has reported dihydropyrimidine derivatives such as 4-dihydropyrimidine-2-thiones (**A**), *N*,*S*-dimethyl-dihydro-pyrimidines (**B**), hydrazinyl dihydropyrimidines (**C**), tetrazolo dihydropyrimidine (**D**), and

* Corresponding author.
E-mail address: khalid.khan@iccs.edu (K.M. Khan).

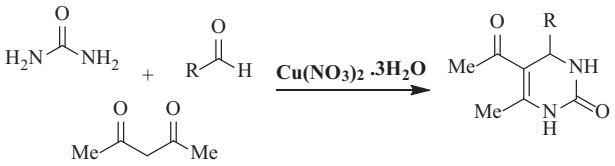
https://doi.org/10.1016/j.bioorg.2017.10.021
0045-2068/

substituted thiourea derivatives (**E**, **F**) as potent urease inhibitors. It is worth-noting that our currently synthesized compounds have structure similarity with the already identified inhibitors and standard thiourea (Fig. 1). In addition to that we also hypothesized that being more electronegative, the carbonyl oxygen may interact more strongly with the Ni atom of urease enzyme than the sulfur atom of thiocarbonyl moiety in the already reported compounds and standard thiourea. Thus, we intended to evaluate the synthetic compounds (**1–43**) based on dihydropyrimidone scaffold for urease inhibitory activity by adopting *in vitro* and *in silico* studies.

2. Results and discussion

2.1. Chemistry

5-Acetyl-6-methyl-4-aryl-3,4-dihydropyrimidin-2(1*H*)-one derivatives **1–43** were synthesized by reacting urea, acetylacetone, and aryl aldehyde in "one pot". Copper nitrate trihydrate ($Cu(NO_3)_2{\cdot}3H_2O$) was used as catalyst and the reaction was carried out in solvent free condition at 80–90 °C (Scheme 1). Reaction was heated and vigorously mixed till the solidification of the reaction mixture. Progression of reaction was monitored by thin layer chromatography (TLC). As the reaction completed, solid product was washed with excess of distilled water. The obtained crude product was then crystallized from ethanol in order to get pure products. All compounds were completely soluble in $(CH_3)_2SO$ (dimethyl sulfoxide) and $(CH_3)_2CO$ (acetone), partially soluble in CH_3OH (methanol), CH_3CH_2OH (ethanol), CH_3Cl (chloroform), and CH_2Cl_2 (dichloromethane), however, on heating get completely soluble in CH_3OH and CH_3CH_2OH. Compounds **1–43** (Table 1) were structurally characterized by various spectroscopic techniques including ^1H, ^{13}C NMR, EI-MS, HREI-MS, and IR.



Scheme 1. Synthesis of 5-acetyl-6-methyl-4-aryl-3,4-dihydropyrimidin-2(1*H*)-one (1 –4 3).

2.2. In vitro urease inhibitory activity

All synthetic 5-acetyl-6-methyl-4-aryl-3,4-dihydropyrimidin-2(1*H*)-one derivatives **1–43** were screened for *in vitro* urease inhibitory activity. It is noteworthy that except compounds **1**, **11**, **12**, and **14**, all other derivatives were found to be significantly urease inhibitors with IC_{50} values 3.70 ± 0.5 to 20.14 ± 0.1 μM as compared to the standard thiourea (IC_{50} = 21.25 ± 0.15 μM) (Table 1) which shows that all structural features such as pyrimidone ring, acetyl group, methyl group, and aryl ring (R) are cordially taking part in the activity and any variation in the activity is attributed by the different substitutions on ring (R) (Fig. 2).

2.3. Structure-activity relationship (SAR)

Surprisingly, compound **1** with unsubstituted benzene ring was found to be completely inactive and many compounds with substituted benzene ring except few were found to be active. It shows that substitution on benzene ring is playing an important role in the urease inhibitory activity.

Amongst the hydroxy containing compounds, compound **2** (IC_{50} = 6.23 ± 0.5 μM) and **3** (IC_{50} = 6.31 ± 0.7 μM) with mono hydroxy substitution were showed much closed and potent inhibitory activity as compared to the standard thiourea (IC_{50} = 21.25 ± 0.15 μM). It shows that hydroxy at *ortho* and *para* positions are equally contributing in the inhibition. However, the dihydroxy substituted derivative **4** (IC_{50} = 6.71 ± 0.5 μM) showed slightly lower inhibitory activity. Slightly reduced activity might be due to the competition between two hydroxy groups to interact with the active site of urease enzyme. Combination of other groups with hydroxy such as compound **5** (IC_{50} = 4.95 ± 0.7 μM) with bromo and hydroxy groups *ortho* to each other also showed potent and four-fold enhanced activity as compared to the standard thiourea. Its good activity might be due to the presence of two polar groups adjacent to each other which might be cordially interacted with the active site of enzyme. Incorporation of methoxy group *ortho* to hydroxy as in the case of compound **6** (IC_{50} = 5.58 ± 0.1 μM), also showed potent urease inhibitory activity. It was observed that switching the positions of both groups as in case of compound **7** (IC_{50} = 9.10 ± 0.1 μM), activity decreased which shows that positions

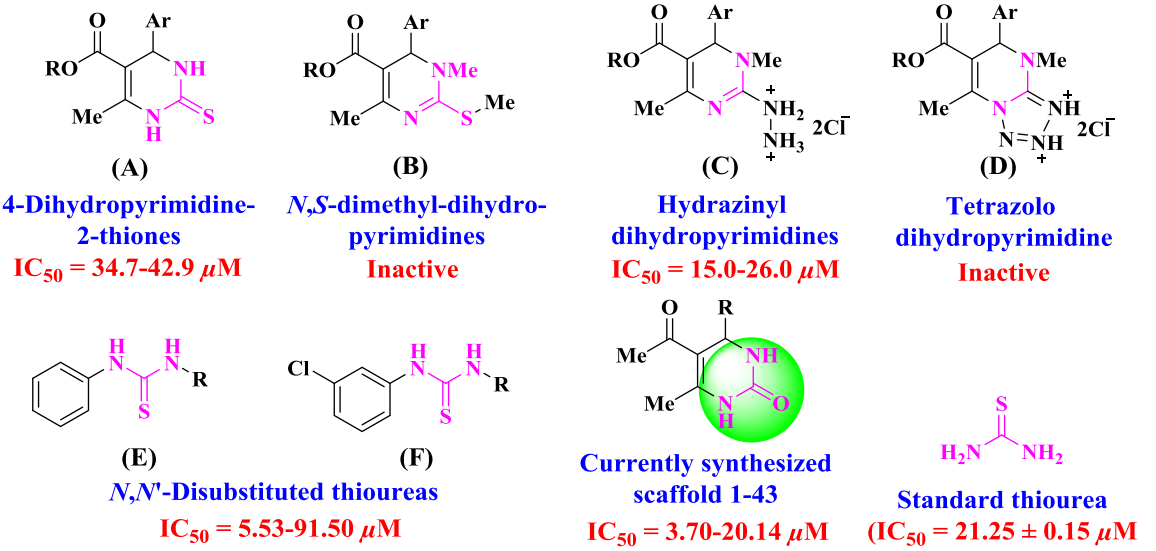


Fig. 1. Rationale of the current study.