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Dihydropyrimidine based hydrazine dihydrochloride derivatives as potent urease inhibitors

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ABSTRACT

Four series of heterocyclic compounds 4-dihydropyrimidine-2-thiones **7**–**12** (series A), *N*,*S*-dimethyl-dihydropyrimidines **13**–**18** (series B), hydrazine derivatives of dihydropyrimidine **19**–**24** (series C), and tetrazolo dihydropyrimidine derivatives **25**–**30** (series D), were synthesized and evaluated for *in vitro* urease inhibitory activity. The series B–D were first time examined for urease inhibition. Series A and C were found to be significantly active with IC_{50} values between 34.7–42.9 and 15.0–26.0 μM, respectively. The structure–activity relationship showed that the free S atom and hydrazine moiety are the key pharmacophores against urease enzyme. The kinetic studies of the active series A (**7**–**12**) and C (**19**–**24**) were carried out to determine their modes of inhibition and dissociation constants K_i. Compounds of series A (**7**–**12**) and series C (**19**–**24**) showed a mixed-type of inhibition with K_i values ranging between 15.76–25.66 and 14.63–29.42 μM, respectively. The molecular docking results showed that all the active compounds of both series have significant binding interactions with the active sites specially Ni-ion of the urease enzyme. Cytotoxicity of all series A–D was also evaluated against mammalian mouse fibroblast 3T3 cell lines, and no toxicity was observed in cellular model.

1. Introduction

The first synthesis of dihydropyrimidinones was reported by Biginelli in 1893 [1]. Pyrimidinones or dihydropyrimidinones (DHPMs) posses a broad range of biological activities. Several compounds containing dihydropyrimidine backbone (Fig. 1), including monastrol (**1**), were found to have potent anticancer and HIV gp-120-CD4 inhibition activities [2]. Trimethoprim (**2**) is a antibacterial drug which contain a pyrimidine core [3]. In 1,4-dihydropyridine class (DHPs), nitrendipine (**3**) is the most potent drug for the treatment of cardiovascular diseases, which functions as calcium channel modulators [4]. Predominantly dihydropyrimidines posses antihypertensive, antimalarial and anti-inflammatory activities, as well as neuropeptide γ-antagonists, and α-1a-antagonists properties [4,5].

Urease (urea amido-hydrolase EC 3.3.1.5) decomposes urea into ammonia and carbamate through a hydrolysis reaction. The carbamate at the physiological pH hydrolyzes spontaneously to carbonic acid and yield another molecule of ammonia [6].

Urease is well known for the pathologies induced by *Helicobacter pylori*. It plays a crucial role in the pathogenesis of gastric and peptic ulcers and cancer by facilitating the survival of *H. pylori* in the acidic environment of stomach. It is reported that ureases also cause urolithiasis. The urolithiasis is infectious stone formation in kidney, caused by *Yersinia enterocolitica* and *Proteus mirabilis*. The urease is also responsible for the development of infection-induced reactive arthritis and acute pyelonephritis [7]. The medicines available against infections caused by ureolytic bacteria proved to be largely ineffective [8], and only a few in the combination have been approved through clinical trials. Thus need of alternative or novel treatment is greatly felt.

Urea is the main nitrogenous waste of biological system, which is quickly metabolized by the action of microorganisms. The urease enzymes are present in a number of fungi, bacteria, and plants. This enzyme is plays an important role in nitrogen cycle as it supplies

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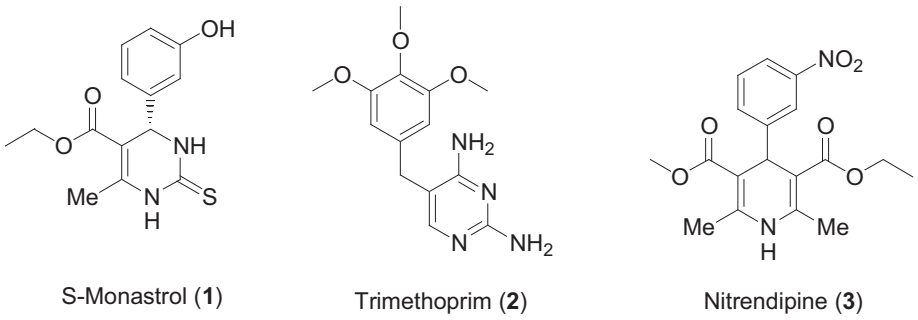

S-Monastrol (**1**) Trimethoprim (**2**) Nitrendipine (**3**)

Fig. 1. Dihydropyrimidine based drug molecules.

nitrogen for the growth of microorganisms by catalyzing the hydrolysis of urea [6]. In agriculture, urease inhibitory activity in soil for increasing crop yields is employed [9]. The high urease activity causes a number of environmental and economical problems [10]. Therefore, the discovery of effective and secure urease inhibitors is an important area in pharmaceutical and plant science due to the participation of ureases in a large number of pathological conditions, as well as for agriculture applications.

We describe here the urease inhibitory activity of four series of dihydropyrimidine analogues, including 4-dihydropyrimidine-2-thiones **7**–**12** (series A), *N*,*S*-dimethyl-dihydropyrimidines **13**–**18** (series B), hydrazine derivatives of dihydropyrimidine **19**–**24** (series C), and tetrazolo dihydropyrimidine derivatives **25**–**30** (series D) by employing a biochemical mechanism-based assay. In addition to this, docking studies on all four series, and the kinetic studies on selected members of series A and C were also performed. These studies help in the identification of mode of inhibition of these compounds.

2. Results and discussion

2.1. Chemistry

In continuation of our efforts to discover diverse classes of bioactive compounds, we carried out an intensive study on 3,4-dihydropyrimidine-2-thiones, as this class was already reported for urease inhibition [11].

3,4-Dihydropyrimidine-2-thiones **7**–**12** (series A), which represent one of the most active class of pyrimidine derivatives, possess a wide range of biological activities. This series was synthesized in good to excellent yields at 10 mmol scale. The Biginelli reaction was used by the cyclization of three components, e.g., arylaldehydes (**4**), substituted β-ketoesters (**5**), and thiourea (**6**) in acetonitrile *via* reflux or microwave heating, following the literature [12,13]. The initially formed dihydropyrimidine-2-thiones **7**–**12** were transformed to double methylated adducts *via* conventional oil bath heating with good to excellent yields. After that all the dimethylated analogues **13**–**18** were successfully converted into the corresponding hydrazine dihydrochloride derivatives **19**–**24** with excellent yields. Subsequently tetrazolo dihydrochloride derivatives **25**–**30** were obtained in good yields. The detailed synthesis of these series A–D was reported in our recent publication [14]. These varying analogues were synthesized according to Scheme 1.

2.2. Bioassay studies

All four series: 3,4-dihydropyrimidine-2-thiones **7**–**12** (series A), *N*,*S*-dimethyl-dihydropyrimidines **13**–**18** (series B), hydrazine derivatives of dihydropyrimidine **19**–**24** (series C), and tetrazolo dihydropyrimidine derivatives **24**–**30** (series D) were evaluated for *in vitro* urease inhibition according to the literature protocol [15]. Series A and C were found significantly active (Table 1). The urease inhibitory activity of series A was recently reported by our group. The docking studies showed that the S atom in series A interact with both the Ni atoms of the enzyme, in a similar manner as standard thiourea interacts [11].

In the preliminary screening, 3,4-dihydropyrimidine-2-thiones **7**–**12** (series A) showed a significant urease inhibitory activity with IC_{50} values between 34.7 and 42.9 μM. The activities of these compounds may be due to the Sulfur atom, which can interact with Ni-atoms in the active site of urease. The various substituted aromatic rings (Ar) at DHPM backbone may also influence the activities of these compounds. Compound **11** having *meta*-hydroxyl phenyl as Ar was found to be the most active compound in this series (IC_{50} = 34.7 μM). The significant activity of this compound appears due to the hydroxyl moiety, which may further form hydrogen bonding with amino acid residue at the active site of urease. Compounds **7** and **9** showed similar activities having IC_{50} values of 35.6 and 36.0 μM, respectively. Both these compounds have identical phenyl ring (Ar), while having ethyl and methyl differentiation at R_1 position, respectively. Similar type of activity was also observed in compounds **8** and **10**. Both compounds have *para*-tolyl substitution as Ar, while ethyl and methyl substituent at R_1 position, accordingly. Again both of these compounds showed same level of activity with IC_{50} values of 42.9 and 41.3 μM, respectively. Compound **12** has a *para*-nitro phenyl as Ar substituent and ethyl as R_1; showed IC_{50} of 40.5 μM. It is concluded in this series, substituted aromatic ring (Ar) of DHPM backbone may influence the activities, while R_1 substitution apparently play no significant role.

Afterwards double methylation of compounds in series A yielded *N*,*S*-dimethyl-dihydropyrimidine (series B). When the urease inhibitory activity of this series (B) was examined, interestingly all compounds **13**–**18** of this series were found to be inactive (Table 1). The possible reason for this lack of activity may be due to the methylation of S atom, as S atom interacts with the Ni atoms in the active site of urease. After methylation of S atom, chelation with Ni atoms is apparently abandoned. This experimentally strengthen the assumption that free S atom is necessary for urease inhibition.

In next step, all the compounds were converted into hydrazine dihydrochloride derivatives **19**–**24** (series C). After screening of urease inhibitory activity of series (C), all compounds were found to be significantly active, even two times more active than series A. It is already established that hydrazide is an active pharmacophore for urease inhibition [16–19]. All hydrazine dihydrochloride compounds **19**–**24** of this series showed urease inhibitory activity with IC_{50} values in the range 15.0–26.0 μM, comparable to standard thiourea (IC_{50} = 21.0 μM). Compounds **19** and **22**–**24** showed higher activity than the standard thiourea (Table 1). Compound **24** having a *para*-nitro phenyl ring (Ar) at DHPM backbone was found most active with IC_{50} value of 15.0 μM. Compound **23** with *meta*-hydroxy phenyl ring (IC_{50} = 17.4 μM) and **22** having *para*-tolyl ring (IC_{50} = 17.8 μM) were also found to be active. When