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Inhibitors of bacterial and plants urease. A review.

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ABSTRACT

Urease is an important virulence factor for *Helicobacter pylori* and *Proteus mirabilis* as well as in environmental transformations of certain nitrogenous compounds. Urea hydrolysis caused by these microorganisms leads to increased pH and ammonia toxicity and enables bacterial colonization of the human gastric mucosa and urinary tract formation of struvite and carbonate-apatite stones. Due to the possibility of medical applications the development of novel, selective and efficient classes of urease inhibitors which satisfy the low toxicity requirement for human health and have low environmental impact is necessary. In this article are described the various urease inhibitors used so far by researchers, especially in the last few years.

KEY WORDS: urea, urease, inhibitor, ulcer disease, kidney stones

Introduction

Urease (urea amidohydrolase EC3.5.1.5) is a large heteropolymeric enzyme which belongs to the super family of amidohydrolase (Sander *et al.* 1997). It catalyses the hydrolysis of urea to ammonia and carbamate. High concentration of ammonia arising from the reaction, as well as the accompanying pH elevation, has important negative effects in the fields of medicine and agriculture (Polacco *et al.* 1995). Many microorganisms utilize urea as a source of nitrogen for augmentation, for example: bacteria, fungi, algae. Urease plays a pivotal role in nitrogen metabolism of plant during the germination process (Polacco *et al.* 2002; Zhu *et al*. 2009).

The order of amino acids and their enzymatic mechanism in all the ureases is preserved. The X-ray crystallographic studies on bacterial ureases derived from *Sporosarcina pasteurii* (*Bacillus pasteurii*), *Klebsiella aerogenes* and *Helicobacter pylori* gave a detailed insight into the structure of its active site. These investigations have enabled to propose the general mechanism of urea hydrolysis (Ciurli *et al.* 2013; Mangani *et al.* 1996; Karplus *et al.* 1995). It was found, that inside the active center two nickel ions are held in separation bridged by carboxylate group of carbamylated Lys and hydroxide originated from the water molecule. Both ions are further coordinated by two histidines, while one of them forms extra bond with Asp. Binuclear metallocenter is additionally stabilized by hydroxide cluster that fills active site cavity of the native enzyme (Mangani *et al.* 1999).

Urease activity is widely distributed in soil and aquatic environments, where it plays a significant role in nitrogen metabolism (Burns 1978). In many environments the level of available nitrogen compounds is inadequate for optimal plant production. Therefore,

fertilizers are applied which can be converted to a form of nitrogen that plants can assimilate (Beaton 1978; Hausinger *et al.* 1989). High concentrations of ureases cause significant environmental and economic problems by releasing enormous amounts of ammonia into the atmosphere during urea fertilization (the most widely used fertilizer). It induces plant damage by depriving of their essential nutrients and secondly through ammonia toxicity and carbon dioxide release which increase the pH of the soil (Bremner 1995). Therefore effective methods are needed to solve the problems encountered in the use of urea as fertilizer (Balasubramanian & Ponnuraj 2010). One of the approaches is the inhibition of the urea hydrolysis. Urease inhibitors could have a practical value as the active additives to nitrogen fertilizers, that could regulate the excessive rates of ureolysis in soil (Schwedt *et al*. 1993).

Ureases are important enzymes in some human and animal pathogenic states, they are responsible for kidney stones entailed in urolithiasis that contributes toward the acute pyelonephritis with other urinary tract infection. Furthermore urease contributes to arthritis and gastric intestinal infections and ultimately the urease imbalance lead to peptic ulcers. This pathologies are caused by *Helicobacter pylori* and *Proteus mirabilis*. The obvious remedy for treating bacterial infection are antibiotics, however often proved to be unsuccessful (Polacco *et al.* 1995; Choudhary *et al.* 2008; Hausinger *et al.* 1995; Hausinger *et al.* 1989).

Strategies based on urease inhibition are the main treatment of diseases caused by urease producing bacteria. Enzyme inhibition is an important area of pharmaceutical research. Studies in this field have already led to the discovery of wide variety of drugs useful in a number of diseases and have been used for treating a number of physiological conditions. Specific inhibitors interact with enzymes and block their activity towards their corresponding natural and synthetic substrates (Choudhary *et al.* 2002).

The standard of urease inhibitors

Urease inhibitors belong to different chemical classes and many of them have been investigated in the past decades, for example hydroxamic acids, which was found to be effective against a wide range of bacterial ureases and is the best recognized urease inhibitors (Polacco *et al*. 1995; Uehare *et al.* 1962; Williamson *et al*. 2003). Phosphoramidates are the most potent compounds, which have found applications in agriculture as soil urease inhibitors (Choudhary *et al.* 2002; Orlinska *et al.* 2001). Hydroxamic acids and phosphoroamide compounds create tetrahedral intermediate with a structural similarity to the tetrahedral intermediate postulated to occur during urea hydrolysis. However, because of teratogenicity of hydroxamic acid in rats (Schmidt *et al*. 1968) and degradation of phosphoramidates at low pH (Garcia-Mina *et al.* 2008)prevented their use as a drug in vivo. Boric and boronic acids are suggested to form a complex with nickel ion(s) (Breitenbach & Hausinger 1988). Quinone derivatives are the another class of compounds that showed enzyme inhibitory activities. Heavy metal ions interact with thiolgroups of cysteine residues. The reaction is analogous to the formation of metal sulphide (Krajewska 1991). A promising group of urease inhibitors constitute polyphenols, widely used as biologically active food additives due to their high antioxidant properties. The example is gallocatechin, a polyphenol which is extracted from green tea, naturally occurring flavonoids have been reported as inhibitors of *Helicobacter pylori* urease (Sugimura *et al.* 2003; Kim *et al.* 2005). Thiols are not potent inhibitors however the presence of other charged groups has a significant effect on the inhibition constant (Todd & Hausinger 1989). Ureases have long been known for their sensitivity to the inhibition by heavy metal ions. The inhibition of ureases by bismuth compounds has been mainly tested for their potential application in the treatment of peptic

ulcers and Helicobacter pylori infections, because bismuth compounds are widely used as bactericidal agents (Zhang *et al.* 2006; Krajewska 2009). The relative effectiveness of the heavy metal ions as inhibitors of jack been urease has been reported to decrease in the following approximate order: $Hg^{2+}>Ag^{+}>Cu^{2+}>Ni^{2+}>Cd^{2+}>Zn^{2+}>Co^{2+}>Fe^{3+}>Pb^{2+}>Mn^{2+}$(Krajewska 2008).

These are only examples of urease inhibitors. The part of them cannot be used in vivo because of their toxicity or instability, therefore the development of novel classes of selective and efficient urease inhibitors which satisfy the low toxicity requirement for human health and have low environmental impact is necessary. In the last few years, many potent inhibitors have been obtained and reported in the literature.

The new class of urease inhibitors

Recently, a series of new and novel Schiff base derivatives showed significant inhibitory activity against Jack bean urease (Zhu *et al*. 2007) due to the similarity of their basic skeleton with urease substrate. The most potent inhibitors werecompounds with K_i=0.09 μM and K_i=0.122 μM (Figure 1, compound 1 and 2). All of the compounds showed competitive mechanism of inhibition (Iqbala *et al.* 2011). Schiff base hydrazones are well known class of compounds, possess various activities like antimicrobial (Blandini *et al.* 1995), antimycobacterial (Kandefer-Szerszen *et al.* 2007), antitumor, anti-inflammatory (Soares *et al.* 2006), antimalarial (Wang *et al*. 2006) and antidiabetic activities (Sharma *et al.* 2012). Scaffold of Schiff base urease inhibitors can be utilized in further optimization to improve potency and selectivity by variations in the basic skeleton.

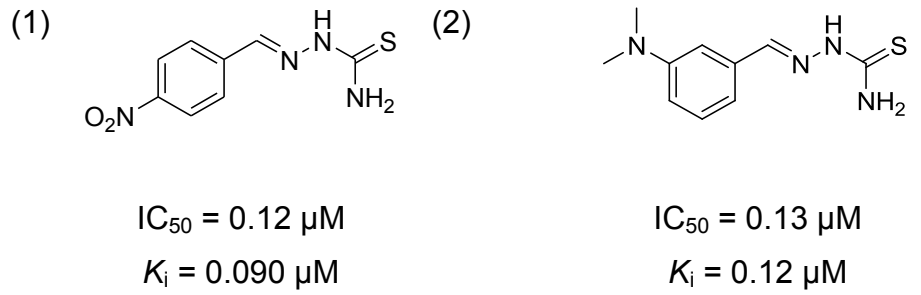

Figure 1. The structure of Jack bean urease inhibitors.

It is very interesting that, the study of urease inhibitors from natural products has attracted a lot of attention (Choudhary *et al*. 2012; Lateef *et al*. 2012). It is well known that structural diversity and complexity within natural products are unique and the functional complexity found in natural products is difficult to invented de novo in the laboratory (Häbich *et al*. 2006). In 2001, Bae *et al*. found flavonoids having weak inhibitory activity against *Helicobacter pylori* urease (Zhu *et al.* 2011). Based on these studies, Zhu-Ping Xiao and his co-workers synthesized and evaluated nineteen derivatives of flavonoids against *Helicobacter pylori* urease. Analysis of structure activity relationship disclosed that 4-deoxy analogues are more potent than other products. Out of them, 4',7,8-trihydroxyl-2-isoflavene (Figure 2) was found to be the most active with IC_{50} of 0.85 μM, being over 20-fold more potent than the commercial available urease inhibitor, acetohydroxamic acid (Hai-Liang *et al.* 2013; Janser *et al.* 2013).

In 2003, Kawase *et al*. reported for the first time that several α,β-unsaturated ketones are

inhibitors for jack bean urease. The most potent compounds were cyclic and of low-molecular weight, e.g. 2-cyloheptene-1-one (IC_{50} = 0.16 mM), 2-cyclohexene-1-one (IC_{50} = 0.69 mM), 2-cyclopentene-1-one (IC_{50} = 0.97 mM) (Tani *et al*. 2003). The result of studies conducted by Kawase *et al*. in 2003 and then by Ingo Janser and his co-workers demonstrated that ethacrynic acid is potent inhibitory activity against jack bean urease, even at low concentrations (Tani *et al.* 2003; Krajewska 2009). Ethacrynic acid and a series of its analogues were synthesized and subsequently evaluated for their inhibitory effect on urease. The highest inhibitory activity was found for compound (5) (IC_{50} = 0.05 mM), compound (6) (IC50 = 0.07 mM), compound (4) (IC_{50} = 0.08 mM), and compound (7) (IC_{50} = 0.10 mM) (Figure 3). It is noteworthy that all four compounds possess a methoxy group at the aromatic system. They demonstrated that the α,β-unsaturated carbonyl unit of this compounds is mandatory to inhibit the enzyme. These studies require further follow-up (Janser *et al*. 2013).

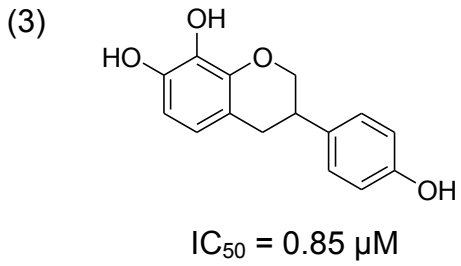

Figure 2. The structure of 4',7,8-trihydroxyl-2-isoflavene.

Of all the above mentioned classes of compounds, amides and phosphoric acid esters represents the group of the most exploited inhibitors towards both bacterial and plant ureases (Krajewska 2009). Several phosphorodiamidates and their thiophosphoric analogues were successfully introduced to agriculture to control hydrolysis of urea in soil and diminish nitrogen loss. Unfortunately, their possible therapeutical use is limited by low hydrolytic stability of P-N bond in acidic pH (Marzadori *et al*. 2009; Garcia-Mina *et al*. 2011; Berlicki *et al*. 2008).

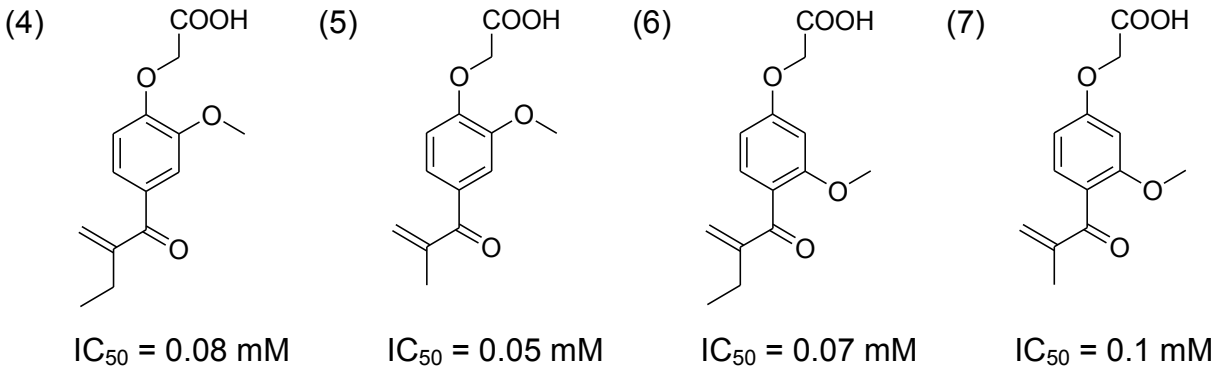

Figure 3. The analogues of ethacrynic acid.

Inhibitory properties of phosphinic and phosphonic acid derivatives towards urease for a long time are synthesized and analyzed in Wroclaw University of Technology in the Bioorganic Chemistry group (Figure 4).The idea of using this compound as urease

inhibitor corresponds to its structural similarity to the transition state of urea hydrolysis as well as to phosphorodiamidate, which is one of the most potent urease inhibitor. The research is also based on the assumption that, in comparison to hydrolytically unstable phosphorodiamidic acid, the phosphinic acid and its derivatives remained stable even at acidic pH due to the presence of highly inert P-C linkages (Berlicki *et al*. 2008; Berlicki *et al*. 2010; Kosikowska & Berlicki 2012).

The computer aided design using crystal structures of *Sporosarcinia pasteurii* urease allowed the development of the novel and potent inhibitors, *P*-methyl phosphinic acids, which example is the most active *N*-(N′-benzyloxycarbonylglycyl)aminomethyl(*P*-methyl)phosphinothioic acid with K_i= 170 nM and 45 nM against *Sporosarcinia pasteurii* and *Proteus vulgaris* enzyme, respectively (compound 8) (Berlicki *et al*. 2008). Introduction of *P*-hydroxymethyl group into the molecule resulted in considerable increase of the inhibitory activity against urease isolated from *Sporosarcinia pasteurii* and *Proteus vulgaris* as compared with their *P*-methyl counterparts obtained previously. The most potent inhibitors in this group of compounds is *N*-methylaminomethyl-*P*-hydroxymethylphosphinic acid with K_i=430 nM and K_i = 360 nM against *Sporosarcinia pasteurii* and *Proteus vulgaris* urease, respectively (compound 9) (Berlicki *et al*. 2010). In order to improve affinity of inhibitor structure to selected bacterial ureases were explored the potential of aminomethylphosphonic and *P*-methyl-aminomethylphosphinic acids as novel inhibitors. The *N*,*N*-dimethyl derivative both mentioned structures were the most effective withK_i=13 ± 0.8 µM and 0.62 ± 0.09 µM, respectively (compounds 10 and 11). This structures offer the possibility of various modifications, which might provide improved physicochemical and inhibitory properties (Kosikowska & Berlicki 2012).

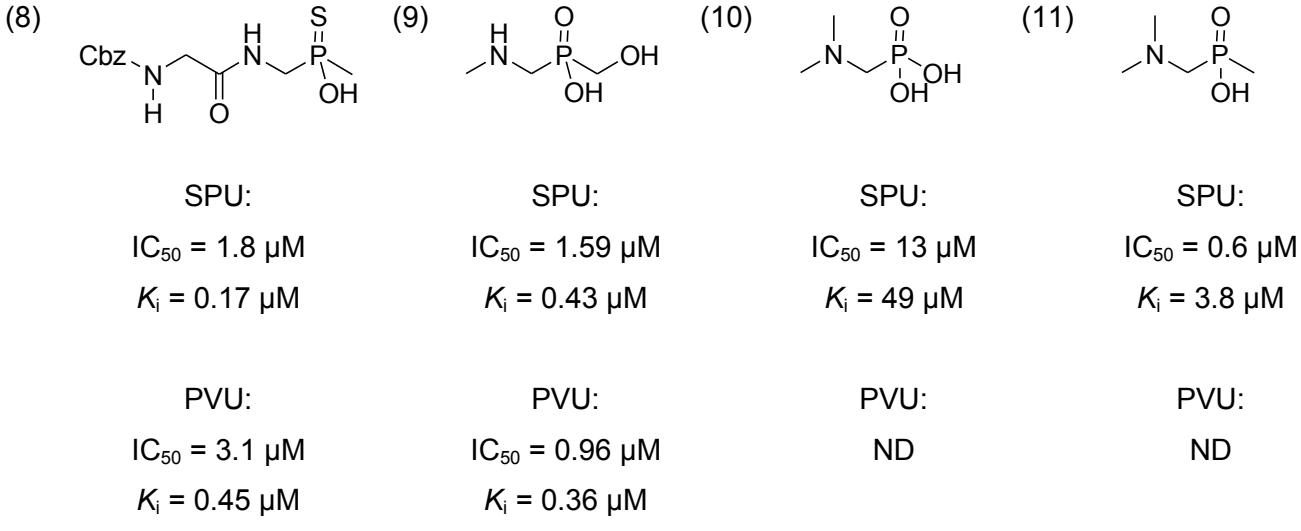

Figure 4. The structure of phosphinic and phosphonic urease inhibitors (SPU - Sporosarcinia pasteurii urease, PVU - Proteus vulgaris urease, ND - not determined).

Conclusions

Catalyzed hydrolysis of urea plays an important role as virulence factor for the urinary tract infections and gastrointestinal infections. Urease inhibition has become a growing area of research at the interface of the biomedical sciences, such as biology, chemistry, biophysics and biotechnology. A lot of potent inhibitors has been reported in literature. All the above mentioned research led to the synthesis of structures with low

structural complexity, high hydrolytic stability and satisfactory biological activity against bacterial and plants urease (*Proteus vulgaris* and *Proteus mirabilis*, *Sporosarcinia pasteurii*, *Helicobacter pylori*). Urease inhibitors are expected to bring interesting discovery in pharmaceutical, agricultural and environmental fields.

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