



## Review

# A review on the development of urease inhibitors as antimicrobial agents against pathogenic bacteria <sup>☆</sup>



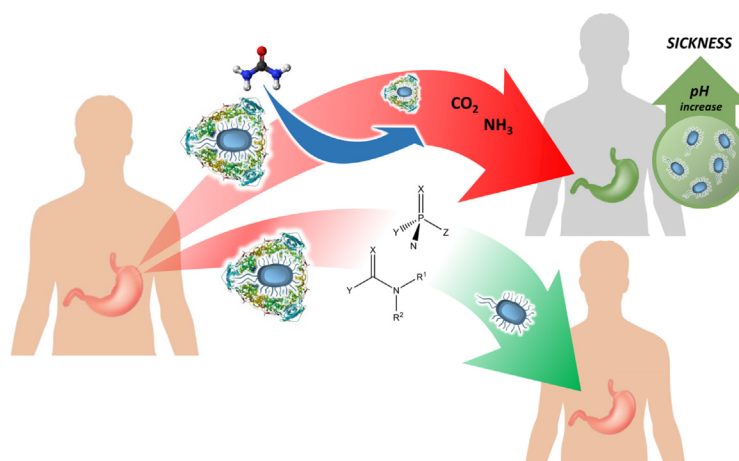
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## GRAPHICAL ABSTRACT



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## ABSTRACT

Ureases are enzymes that hydrolyze urea into ammonium and carbon dioxide. They have received considerable attention due to their impacts on living organism health, since the urease activity in microorganisms, particularly in bacteria, are potential causes and/or factors contributing to the persistence of some pathogen infections. This review compiles examples of the most potent antiurease organic substances. Emphasis was given to systematic screening studies on the inhibitory activity of rationally designed series of compounds with the corresponding SAR considerations. Ureases of *Canavalia ensiformis*, the usual model in antiureolytic studies, are emphasized. Although the active site of this class of hydrolases is conserved among bacteria and vegetal ureases, the same is not observed for allosteric site. Therefore, inhibitors acting by participating in interactions with the allosteric site are more susceptible to a potential lack of association among their inhibitory profile for different ureases. The information

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about the inhibitory activity of different classes of compounds can be useful to guide the development of new urease inhibitors that may be used in future in small molecular therapy against pathogenic bacteria. © 2018 Production and hosting by Elsevier B.V. on behalf of Cairo University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

Urease, an enzyme that strictly depends on nickel ions ( $\text{Ni}^{2+}$ ) [1], is a type of hydrolase that accelerates the rate of urea hydrolysis to ammonia ( $\text{NH}_3$ ) and carbamic acid, which disproportionates into ammonia and carbon dioxide ( $\text{CO}_2$ ), by one hundred trillion-fold (Scheme 1) [2–4]. Since its discovery in plants [5], *Canavalia ensiformis* (Fabaceae) urease has been exhaustively used as a model to develop new urease inhibitors for use in clinical and agricultural applications [6] and has become the milestone in biochemistry as the first enzyme to be crystallized [7]. The versatile uses of the purified urease from *C. ensiformis* in the discovery of new urease inhibitors is in part due to the similarity of amino acid sequences among ureases from multiple species [8], suggesting the presence of a common ancestor of this enzyme. The first complete three-dimensional structure of a urease was reported by Jabri and coworkers in 1995 from crystallography studies performed with urease from *Klebsiella aerogenes* [9]. Later, other structures were disclosed for ureases from *Bacillus pasteurii* [10], *Helicobacter pylori* [11] and, most recently, *C. ensiformis* [12]. Indeed, the elucidation of the urease structure from a legume was crucial to obtain a better understanding of the requirements for the ureolytic activity of this class of enzymes in different organisms [12].

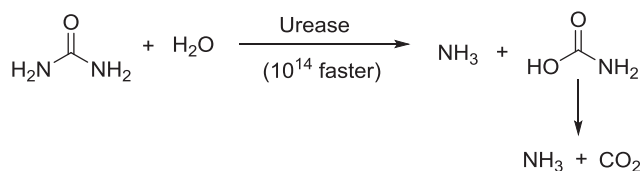
The increase in medium pH caused by the accumulation of  $\text{NH}_3$  is a urease trait of tremendous medical importance [4]. Urine and/or gastrointestinal infections by ureolytic bacteria cause health complications in humans and animals, including kidney stone formation, pyelonephritis, hepatic encephalopathy and, ultimately, hepatic coma [4,13]. Therefore, major public health issues are related to *Helicobacter pylori*, a gram-negative bacterial species that is able to survive in an acidic environment, such as the stomach (pH 1–2). Consequently, *H. pylori* infections induce gastric inflammation and increase the risk of developing duodenal and gastric ulcers, gastric adenocarcinoma and gastric lymphoma [4,14]. Approximately 50% of the global population is infected with *H. pylori*. This bacteria species can persist in the stomach throughout the life of infected individuals without causing disease symptoms. The high prevalence of *H. pylori* in the human population indicates that this microorganism has developed mechanisms of resistance against host defenses [14]. The urease enzyme in the cytoplasm and/or bound to the *H. pylori* surface is the main virulence factor of this human pathogen [15,16]. Urease represents up to 10% of the total protein content of *H. pylori* [17]. Moreover, the lysis of some pathogen-infected cells leads to the release of cytosolic ureases that bind to the surface of intact bacterial cells and cause the hydrolysis of urea, which is present in the human gut at a concentration of 3 mM. The resulting production of  $\text{NH}_3$  increases the medium pH, creating a permissive environment that promotes *H. pylori* survival [15,18]. During the past 20 years, the recommended first-line therapy for *H. pylori* eradication consists of a combination

of the antibiotics amoxicillin and clarithromycin with omeprazole, a proton pump cell inhibitor. However, the increase in *H. pylori* resistance to these antibiotics (particularly to clarithromycin) has rendered these therapeutics an ineffective option in recent years [3,19,20]. Indeed, other treatment strategies have emerged to fight *H. pylori* infection, including the use of bismuth salts (a metal with antiurease properties [21]) combined with a proton pump inhibitor or combinations of other classes of antibiotics as fluoroquinolones, aminopenicillins, and tetracyclines [3,20,22].

Urease is also produced by most strains of *Proteus mirabilis* and *Staphylococcus saprophyticus* [23]. *P. mirabilis*, a gram-negative bacteria, causes a variety of community- or hospital-acquired illnesses, including urinary tract, wound, and bloodstream infections [24]. One of the major factors known to be involved in *Proteus mirabilis*-induced urinary crystal formation is the bacterial urease, a well-known virulence factor of this microorganism [25–27]. Indeed, the *P. mirabilis* urease increases the pH of the urinary tract and causes the local supersaturation and formation of carbonate apatite and struvite crystals [28]. In addition, the ability of a urease-negative mutant of *P. mirabilis* urease to colonize the urinary tract is approximately 100-fold less than the parent strain [26,29]. *S. saprophyticus*, a spherical bacterium of the gram-positive coccus group, is also a frequent cause of urinary tract infections [30]. Among the virulence factors in *S. saprophyticus*, urease is a major factor contributing to the invasiveness of this bacteria [31], particularly in the bladder tissue, whereas its persistence in the urinary tract and nephropathogenicity are governed by factors other than urease [32]. Indeed, *P. mirabilis* and *S. saprophyticus* are some of the primary etiological agents related to urinary tract infections [33,34], and urease is one of the key virulence factors that allows these pathogens to successfully infect the urinary tract [34–36].

Another urease-dependent human pathogen is *Yersinia enterocolitica*, a well-known enteric pathogen that causes yersiniosis [37–39], is an invasive enteric pathogen that gains access to the body via the oral route through the consumption of contaminated food or water [40,41]. This bacteria causes a wide spectrum of clinical disorders, ranging from self-limiting gastroenteritis to mesenteric lymphadenitis, visceral abscesses, septicemia in immunocompromised hosts, and reactive arthritis [40–42]. Although *Y. enterocolitica* grows optimally at a pH of approximately 7.0 to 8.0, these bacteria remains viable in acidic conditions (pH 4.4) for 48 h [43]. The ability of certain *Y. enterocolitica* strains to survive the high acidity of some foods and *in vitro* acidic conditions suggests that these bacteria are relatively acid tolerant [44,45]. The mechanism underlying the acid tolerance of *Y. enterocolitica* has been proposed to be due to the urease activity present in this species [44,46].

Due the tremendous medical importance of urease, urease inhibitors with improved stability and low toxicity may be an effective therapy against diseases caused by urease-dependent pathogenic microorganisms. Here, we present an overview of the most relevant organic substances that exert antiureolytic inhibitory effects on ureases. The urease inhibitors presented here are organized into five classes according to their chemical structures, namely: (thio) urea derivatives, five- and six-membered heterocycles, barbituric analogues and phosphoramidated substances. Urease inhibitors derived from natural products and metal complexes will not be addressed in this review since very good reviews of these compounds have been published elsewhere [6,47].



**Scheme 1.** Representation of urea hydrolysis catalyzed by ureases.

## Organic substances as urease inhibitors

### (Thio)urea derivatives

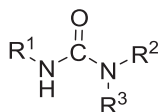
The development of enzyme inhibitors based on the molecular structure of the native substrate is an approach commonly used in rational drug design. Several systematic screens of urease inhibitors designed based on the urea structure have been conducted, particularly in the last 10 years.

In one of the first of these studies, a series of  $N^1, N^2$ -di- and tri-substituted urea derivatives were synthesized, and their inhibitory activities against *Canavalia ensiformis* urease were tested [48].  $N^1, N^2$ -diaryl derivatives containing nitro groups at both phenyl rings (like compound **1**, Scheme 2) show low micromolar inhibition of *C. ensiformis* urease.

More recently, Mustafa and co-workers identified several novel urease inhibitors [49]. The authors designed and synthesized  $N^1$ -toluoyl,  $N^2$ -substituted urea derivatives and evaluated them using *in vitro* enzyme-inhibition assays that included *C. ensiformis* urease. Three compounds containing a methoxy group in the phenyl ring [compounds **2**, **3** and **4** (Series A), Scheme 3] exhibited the strongest inhibition of the urease enzyme (47 to 59%). Notably, each of the three abovementioned inhibitors contains its tolyl moiety with different substitution patterns (*ortho*, *meta* or *para* position), suggesting that the inhibitory activity is not substantially affected by the position of  $R^1$ .

In 2002, Uesato et al., based on the urease inhibiting capacity of hydroxyurea, synthesized  $N^1$ -hydroxy- $N^2$ -substituted derivatives and evaluated their inhibitory activity towards urease using hydroxyurea as a substrate (Scheme 4) [50]. *Ortho*- and *para*-substitutions at the phenyl ring decreased the inhibitory activity, possibly because of the steric hindrance provided by the groups at these positions, which might diminish the hydroxamic acid connection with the active site. A few years later, using the same motivation, Rajic and co-workers synthesized hydroxamic acid derivatives, tested their antiurease activity and found that only the derivatives bearing a hydroxyl group inhibited urease activity [51].

More recently, many investigations examining thiourea-based urease inhibitors have developed thiourea derivatives that present higher inhibitory potency than their urea counterparts. Khan and co-workers have synthesized a variety of substituted thioureas and screened their urease inhibitory activity [52]. Substitutions with functional groups attached to the phenyl or heterocyclic ring around the thiourea core and compounds with substituents containing lone electron pairs exert a decisive effect on the urease inhibitory activity. The strongest inhibitory activity ( $IC_{50}$  8.43  $\mu$ M)



$$R^1 = iC_6H_4, 4-(NO_2)C_6H_4, 2,4-(Cl)C_6H_3$$

$$R^2 = H, CH_3, C_4H_9, C_2H_5$$

$$R^3 = CH_3, C_3H_7, C_4H_9, C_2H_5, C_6H_5, 4-NO_2-C_6H_4, 2-Pyridinyl, 3-Pyridinyl, 4-Pyridinyl, Morpholinyl, (C_2H_4)C_6H_5.$$

Standard inhibitor: thiourea ( $IC_{50} = 21.0 \mu$ M)

Series: 14 compounds ( $IC_{50} = 1.25$  to  $55.6 \mu$ M)

Best inhibitor: **1** ( $IC_{50} = 1.25 \mu$ M;  $R^1 = 4-NO_2-C_6H_4$ ;  $R^2 = H$ ;  $R^3 = 4-NO_2-C_6H_4$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 2.** Chemical structures of  $N^1, N^2$ -di- and tri-substituted urea derivatives reported as potential urease inhibitors.



$$R^1 = 2-CH_3, 3-CH_3, 4-CH_3$$

$$R^2 = \text{pyrrolidin-2-one, mesityl, ethylbenzene,}$$

$$4\text{-methoxyphenyl, benzothiazolyl, 1-(o-toluy)ethanone}$$

Standard inhibitor: thiourea (Inhibition = 98.2 %)

Series A: 14 compounds (Inhibition= 0.30 to 59.1 %)

Series B: 03 compounds (Inhibition= 19.9 to 37.7 %)

Best inhibitors: **2 (A)** (Inhibition= 59.1 %;  $R^1 = 4-CH_3$ ;  $R^2 = \text{anisole}$ ),

**3 (A)** (Inhibition = 53.2 %;  $R^1 = 3-CH_3$ ;  $R^2 = \text{anisole}$ ),

**4 (A)** (Inhibition= 47.5 %;  $R^1 = 2-CH_3$ ;  $R^2 = \text{anisole}$ ).

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 3.** Chemical structures of  $N^1$ -toluoyl- $N^2$ -substituted urea derivatives described as urease inhibitors.

was observed for the derivative bearing a 3-pyridyl substituent (compound **8**, Scheme 5).

Taha and co-workers screened a series of symmetrical bis-thiourea derivatives bearing a disulfide moiety [53] (Scheme 6). Compounds with different substituted phenyl rings at both terminal nitrogens were evaluated as inhibitors of *C. ensiformis* urease. The presence of a fluorine atom at the phenyl groups, regardless of the position, lead to high inhibition (compounds **8**, **9** and **13**; Scheme 6). Comparable inhibitory activity ( $IC_{50}$  ranging between 0.4 and 1.7  $\mu$ M) was observed for derivatives containing *para*-Cl-phenyl (**14**) *para*-CF<sub>3</sub>-phenyl (**12**) or electron-releasing substituents, such as methyl and methoxy groups at *para* or *ortho* positions (compounds **10**, **11** and **15**). In addition, these compounds were considered nontoxic, based on a cytotoxicity assay.

The introduction of the benzoyl moiety at the thiourea nitrogen atom was extensively probed in the literature in studies involving  $N^1$ -benzoyl,  $N^2$ -aryl substituted derivatives (Scheme 7) [54–58]. The beneficial effect of the benzoyl group on the inhibitory activity towards *C. ensiformis* urease was evidenced in a direct comparison between extent of inhibition achieved by the monosubstituted N-benzoyl thiourea and thiourea [54]. Additionally, kinetic experiments designed to probe the mechanism of urease inhibition suggested that the benzoyl thiourea derivatives acted as mixed-type inhibitors that bound to either catalytic or allosteric sites of the enzyme [54]. In the same paper, the screen of the inhibitory activities revealed eight title derivatives showing percent inhibition values (51 to 72%) comparable to the positive control hydroxyurea (74%). Compounds containing electron-donating and electron-withdrawing substituents on the phenyl ring showed variable inhibitory activities [56]. Nevertheless, the presence of *p*-tertbutyl, *o*-NO<sub>2</sub>, (*m*- or *p*-)Cl or (*m*- or *p*-)Br at the phenyl ring attached to  $N^2$  of the thiourea core enhanced the inhibitory activity [54]. Similarly, Rauf and co-workers (2016) revealed that benzoylthioureas bearing the 2,4,6-trichlorophenyl group as a substituent (compound 19 – Series A; Scheme 7) ( $IC_{50}$  1.67  $\mu$ M) and derivatives with 2,4-dichlorophenyl (compound 17 – Series A; Scheme 7) and 2,3-dichlorophenyl groups (compound 18 – Series A; Scheme 7) ( $IC_{50}$  1.34 and 1.92  $\mu$ M, respectively) were much more active than the standard inhibitor (thiourea,  $IC_{50}$  22.3  $\mu$ M) [56].

The effect of a phenyl ring bearing either *para*-ethyl benzoate [58] or *para*-sulfanilamide [57] at  $N^2$  nitrogen of benzoylthioureas



Series A: 13 compounds ( $IC_{50}$  = 12.7 to 533.3  $\mu$ M) [50]

$R^1$  = F,  $CH_3$ ,  $OCH_3$ ,  $NO_2$

Standard inhibitor: Hydroxyurea ( $IC_{50}$  = 22.3  $\mu$ M)

Best inhibitors: **5** ( $IC_{50}$  = 12.7  $\mu$ M;  $R^1$  = 3- $CH_3$ ); **6** ( $IC_{50}$  = 16.2  $\mu$ M;  $R^1$  = 3- $OCH_3$ )

Series B: 18 compounds ( $IC_{50}$  = 37.4  $\mu$ M to no activity) [51]

$R^2$  =  $CH(CH_3)$ -[4- $CH_2CH(CH_3)_2$ - $C_6H_4$ ],  $CH(CH_3)$ (3- $OC_6H_5$ - $C_6H_4$ ),  $CH(CH_3)$ (3- $COC_6H_5$ - $C_6H_4$ ),  $CH_2$ -[2- $NH(2,6$ -Cl- $C_6H_3)$ - $C_6H_4$ ], 3- $CH_2$ -[1,2,5-[CO(4-Cl- $C_6H_4$ )]- $CH_3$ - $OCH_3$ -indol],  $CH(CH_3)$ -(3- $CH_2C_6H_5$ - $C_6H_4$ )

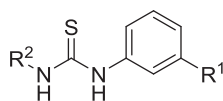
$R^3$  = H,  $CH_3$

$R^4$  = H,  $CH_3$ ,  $CH_2CH_3$ ,  $CH_2C_6H_5$

Best inhibitor: **7** ( $IC_{50}$  = 37.4  $\mu$ M;  $R^2$  =  $CH_2$ -[2- $NH(2,6$ -Cl- $C_6H_3)$ - $C_6H_4$ ];  $R^3$  = H;  $R^4$  = H)

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 4.** Chemical structures of  $N^1$ -hydroxy- $N^2$ -substituted derivatives described as urease inhibitors.



$R^1$  = H, Cl

$R^2$  = Aryl or Alkyl

Standard inhibitor: thiourea ( $K_i$  = 20.01  $\pm$  0.020  $\mu$ M)

Series: 38 compounds ( $K_i$  = 8.6  $\pm$  0.024 to 19.29  $\pm$  0.026  $\mu$ M)

Best inhibitor: **8** ( $K_i$  = 8.6  $\pm$  0.024  $\mu$ M;  $R^1$  = H;  $R^2$  = 3-pyridyl)

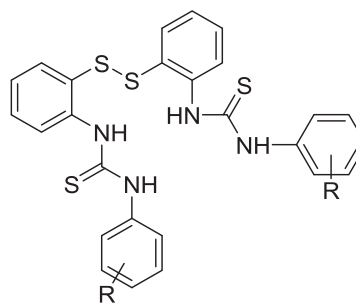
\*Values of  $K_i$  determined with urease from *Canavalia ensiformis*

**Scheme 5.** Chemical structures of  $N^1$ -aryl- $N^2$ -(aryl or alkyl)-substituted thiourea derivatives that possess antiureolytic activity.

was evaluated in the studies developed by Saeed and co-workers. The *in vitro* assays using *C. ensiformis* urease showed very high inhibitory activities for most of the tested derivatives. Among the  $N^2$ -*para*-benzoate series, compounds bearing a 4-methoxy group (compound **22** Series A; **Scheme 8**) and 3,4-dimethoxy substituent (compound **21** Series A; **Scheme 8**) at the benzoyl group showed the best urease inhibition, with  $IC_{50}$  values of 0.21 and 0.13  $\mu$ M, respectively. Notably, compounds derived from 3-chloro and 2,4-dichloro benzoic acid also showed comparable  $IC_{50}$  values [58].

Among the series of sulfanilamide thioureas, the most active compounds contain 4-chloro (compound **23** Series B; **Scheme 8**) and 2-chloro-5-nitro (compound **24** Series B; **Scheme 8**) substituents on aryl group and showed  $K_i$  values of 0.20 and 0.44  $\mu$ M, respectively. According to the analysis of the structure–activity relationship, substituents with an electron-withdrawing group located at the 4-aryl group were the most potent inhibitors [57].

In 2017, Saeed and co-workers reported the synthesis of two series of *N*-acyl thioureas derived from myristic (Series B, **Scheme 9**) [59] and palmitic acids (Series A, **Scheme 9**) [60]. The



$R$  = H,  $CH_3$ , F, Br,  $OCH_3$ , 3- $NO_2$ , 4- $NO_2$ , 4- $CF_3$ , 3,4-Cl, 4-Cl-, 2-Cl.

Standard inhibitor: thiourea ( $IC_{50}$  = 19.46  $\mu$ M)

Series: 20 compounds ( $IC_{50}$  = 0.40 to 18.60  $\mu$ M)

Best inhibitors: **8** ( $IC_{50}$  = 0.40  $\mu$ M;  $R$  = 4-F);

**9** ( $IC_{50}$  = 0.70  $\mu$ M;  $R$  = 2-F); **10** ( $IC_{50}$  = 1.10  $\mu$ M;  $R$  = 2- $CH_3$ );

**11** ( $IC_{50}$  = 1.40  $\mu$ M;  $R$  = 4- $CH_3$ );

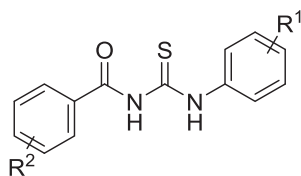
**12** ( $IC_{50}$  = 1.50  $\mu$ M;  $R$  = 4- $CF_3$ ); **13** ( $IC_{50}$  = 1.50  $\mu$ M;  $R$  = 3-F);

**14** ( $IC_{50}$  = 1.60  $\mu$ M;  $R$  = 4-Cl); **15** ( $IC_{50}$  = 1.70  $\mu$ M;  $R$  = 2- $OCH_3$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 6.** Chemical structures of symmetrical (*bis*)-thiourea which possesses antiurease activity.

inhibitory effects of substituted phenyl rings at the remaining nitrogen atom on *C. ensiformis* urease were evaluated. All tested compounds presented very low  $\mu$ M  $IC_{50}$  values (c.a. 0.01 to 0.09). One compound with a chlorine atom on the phenyl ring was identified as the most active inhibitor in each of the studied series (compounds **25** and **26**). Curiously, the chlorine atom of the most active acyl-thiourea derivative of palmitic acid [60] is attached at the *para* position, whereas the most active derivative of the myristic acid series [59] contains its chlorine at the *meta* position. Kinetic investigations of these two compounds indicated a non-competitive inhibitory profile for this class of compounds.

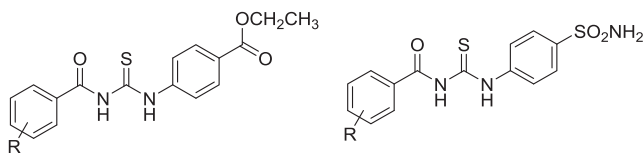


A or B

Series A: 64 compounds ( $IC_{50} = 1.23$  to  $74.91 \mu M$ ) [55, 56] $R^1 = H, 3\text{-Cl}$  $R^2 = H, \text{Mono, di and tetra-substituted: F, Cl, Br, NO}_2, \text{CF}_3, \text{CH}_3, \text{CH}_2\text{CH}_3, \text{CH}_2\text{CH}_2\text{CH}_3, \text{OCH}_3$ Standard inhibitor: thiourea ( $IC_{50} = 22.3 \mu M$ )Best inhibitors: **16** ( $IC_{50} = 1.23 \mu M$ ;  $R^1 = 3\text{-Cl}$ ;  $R^2 = 2,4,6\text{-CH}_3$ ),**17** ( $IC_{50} = 1.34 \mu M$ ;  $R^1 = 3\text{-Cl}$ ;  $R^2 = 2,4\text{-Cl}$ ),**18** ( $IC_{50} = 1.92 \mu M$ ;  $R^1 = 3\text{-Cl}$ ;  $R^2 = 2,3\text{-Cl}$ ),**19** ( $IC_{50} = 1.67 \mu M$ ;  $R^1 = H$ ;  $R^2 = 2,4,6\text{-Cl}$ )Series B: 65 compounds (% inhibition  $\leq 5$  to 73.9%) [54] $R_1 = \text{NO}_2, \text{OCH}_3, \text{Cl}$  $R_2 = H, F, Cl, Br, \text{CH}_3, \text{tBu, OH, OCH}_3$ 

Standard inhibitor: thiourea (% inhibition = 22.6);

Hydroxyurea (% inhibition = 73.6)

Best inhibitors: **20** (% inhibition = 73.9%;  $R^1 = H$ ;  $R^2 = 4\text{-Cl}$ )**Scheme 7.** Chemical structures of  $N^1$ -benzoyl,  $N^2$ -aryl substituted derivatives described as urease inhibitors.

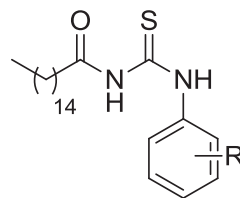
A

B

 $R = 4\text{-OCH}_3, 3,4\text{-OCH}_3, 4\text{-Cl, 2-Cl-5-NO}_2$ .Standard inhibitor: Thiourea ( $IC_{50} = 20.3 \mu M$  and  $K_i = 23.0 \mu M$ )Series A: 10 compounds ( $IC_{50} = 0.13$  to  $1.07 \mu M$ )Series B: 20 compounds ( $K_i = 0.20$  to  $53.0 \mu M$ )Best inhibitors: **21 (A)** ( $IC_{50} = 0.13 \mu M$ ;  $R = 3,4\text{-Cl}$ );**22 (A)** ( $IC_{50} = 0.21 \mu M$ ;  $R = 4\text{-OCH}_3$ ); **23 (B)** ( $K_i = 0.20 \mu M$ ;  $R = 4\text{-Cl}$ );**24 (B)** ( $K_i = 0.44 \mu M$ ;  $R = 2\text{-Cl-5-NO}_2$ )**Scheme 8.** Chemical structures of potential urease inhibitors based on benzoate or sulfanilamide thioureas.

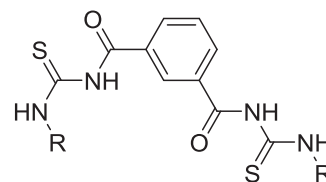
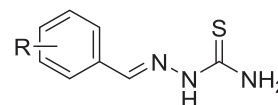
Jamil and co-workers reported the results of a screen for symmetrical isophthalyl-bis-(thioureas) (Scheme 10) [61]. The presence of an electron-withdrawing substituent at each terminal nitrogen was identified as a crucial factor determining the inhibitory activity of the four most active compounds (**27–30**).

Eighteen substituted benzylidene thiosemicarbazide derivatives synthesized by Aslam and co-workers (2011) [62] (Scheme 11) showed low  $IC_{50}$  values for *C. ensiformis* urease. Compounds bearing 3- $\text{NO}_2$  and 4- $\text{N}(\text{CH}_3)_2$  as substituents showed very good inhibitory activity (compounds **31** and **32**, respectively).



A

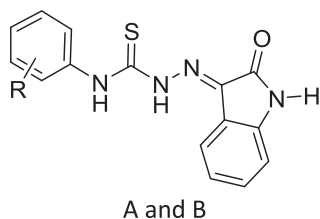
B

 $R = 4\text{-Cl, 2-Cl, 2,4-NO}_2, 2\text{-CN-4-NO}_2, 3\text{-Cl, 4-CH}_3, 4\text{-COOH, acetyl, 2,3-Cl, 4-NO}_2, 4\text{-SO}_3\text{H, naphthyl, mesityl}$ Standard inhibitor: thiourea ( $IC_{50} = 4.72$  and  $18.19 \mu M$ )Series A: 10 compounds ( $IC_{50} = 0.01$  to  $0.07 \mu M$ )Series B: 11 compounds ( $IC_{50} = 0.03$  to  $0.09 \mu M$ )Best inhibitors: **25 (A)** ( $K_i = 0.014 \mu M$ ;  $R = 4\text{-Cl}$ );**26 (B)** ( $K_i = 0.021 \mu M$ ;  $R = 3\text{-Cl}$ )\*Values of  $IC_{50}$  and  $K_i$  determined with urease from *Canavalia ensiformis***Scheme 9.** Chemical structures of  $N$ -acyl thioureas-urease inhibitors derived from myristic or palmitic acids. $R = \text{purine, 4-COOH-C}_6\text{H}_4, 3\text{-NO}_2\text{-C}_6\text{H}_4, 2\text{-NO}_2\text{-C}_6\text{H}_4$ .Standard inhibitor: thiourea ( $IC_{50} = 21.0 \mu M$ )Series: 10 compounds ( $IC_{50} = 26.3$  to  $31.1 \mu M$ )Best inhibitors: **27** ( $IC_{50} = 26.3 \mu M$ ;  $R = 4\text{-COOH-C}_6\text{H}_4$ );**28** ( $IC_{50} = 26.7 \mu M$ ;  $R = 3\text{-NO}_2\text{-C}_6\text{H}_4$ );**29** ( $IC_{50} = 28.4 \mu M$ ;  $R = \text{purine}$ );**30** ( $IC_{50} = 31.1 \mu M$ ;  $R = 2\text{-NO}_2\text{-C}_6\text{H}_4$ .)\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis***Scheme 10.** Chemical structures of symmetrical isophthalyl-bis-(thioureas) urease inhibitors. $R = 3\text{-NO}_2, 4\text{-N}(\text{CH}_3)_2$ Standard inhibitor: Thiourea ( $K_i = 19.6 \pm 0.45 \mu M$ )Series: 18 compounds ( $K_i = 0.090 \pm 0.06$  to  $0.910 \pm 0.11 \mu M$ )Best inhibitors: **31** ( $K_i = 0.090 \pm 0.06 \mu M$ ;  $R = 3\text{-NO}_2$ );**32** ( $K_i = 0.122 \pm 0.11 \mu M$ ;  $R = 4\text{-N}(\text{CH}_3)_2$ )\*Values of  $K_i$  determined with urease from *Canavalia ensiformis***Scheme 11.** Chemical structures of benzylidene thiosemicarbazides urease inhibitors.

Other derivatives with a halogen group at *ortho* position showed comparable activity, whereas compounds with a halogen group at *meta* and *para* positions showed lower activity.

Similarly, Pervez et al. examined the use of several isatin derivatives as potential urease inhibitors [63]. Overall, compounds with a methoxy substituent at the *para* position and compounds with a chloro substituent at the *ortho* position of the phenyl ring (compound **33** – Series A, Scheme 12) showed the most potent inhibitory effects of compounds in the present series, exhibiting relatively greater activity at the tested concentrations. Subsequently, the same research group reported the results for a novel series of *N*<sup>4</sup>-substituted isatin-3-thiosemicarbazones. In general, the addition of one, two or three substituents with inductive electron-withdrawing effects at different positions of the phenyl ring increased the urease inhibitory activity of the derivatives. For example, compounds bearing trifluoromethoxy and trifluoromethyl (compounds **34** and **35** – Series B, Scheme 12) substituents showed the highest inhibitory activity (41 to 78%) compared with compounds bearing methoxy and methyl substituents (8 to 29%). Additionally, substitutions in the phenyl ring with an electron-withdrawing group at *N*<sup>4</sup> position exerted a positive effect on enzyme activity, as compounds showed more potent urease inhibition ( $IC_{50} = 20.6 \mu M$ ) than the standard thiourea ( $IC_{50} = 21.0 \mu M$ ). [64].

Sharma and co-workers [65] also explored the isatin moiety in a study involving a comprehensive SAR analysis the urease inhibitory activities of 32 *N*-phenyl urea/thiourea compounds (Scheme 13). Substituents at the phenyl ring bearing halogen and methoxy groups were screened for their potency in inhibiting *C. ensiformis* urease. The presence of the double 3-(1-piperazinyl)-1, 2-benzisothiazole moiety and conjugation to the *N*-phenyl-urea/thiourea motif enhanced the inhibitory activity. Additionally, all compounds in the thiourea series (Series B – Scheme 13) were slightly more potent than their urea counterparts (Series A – Scheme 13). Moreover, fluoro and methoxy derivatives showed promising inhibitory activities and were more potent than the corresponding chlorinated or bromated structures. Finally, the addition of a methoxy group at the *para* or *ortho* position yielded the two most potent thioureas of the series (compounds **36** and **37**, respectively, both from Series B).



Series A:  $R = 4-CH_3, 2-Cl, 4-Cl$

Series A: 15 compounds (Inhibition (at  $100 \mu M$ ) = 6.28 to 43.66 %)

Series B:  $R = 2-F, 2-OCH_3, 4-OCH_3, 3-CF_3, 4-CF_3$

Series B: 13 compounds ( $IC_{50} = 20.6$  to  $47.9 \mu M$ )

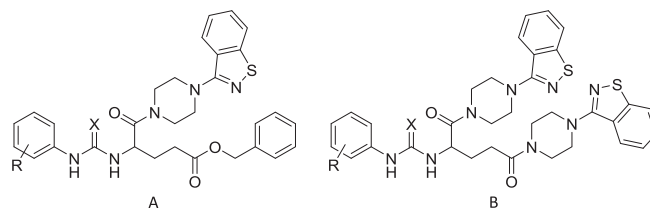
Standard inhibitor: thiourea ( $IC_{50} = 21.0 \mu M$ )

Best inhibitors: **33 (A)** (Inhibition = 43.66 %;  $R = 2-Cl$ );

**34 (B)** ( $IC_{50} = 20.6 \mu M$ ;  $R = 2-F$ );

**35 (B)** ( $IC_{50} = 20.6 \mu M$ ;  $R = 3-CF_3$ )

**Scheme 12.** Chemical structures of potential urease inhibitors based on isatin derivatives.



$X = S, O$

$R = Br, 3-Cl, 4-Cl, F, OCH_3, H$

Standard inhibitor: thiourea ( $IC_{50} = 21.5 \mu M$ )

Series A: 10 compounds ( $IC_{50} = 15.0$  to  $30.5 \mu M$ )

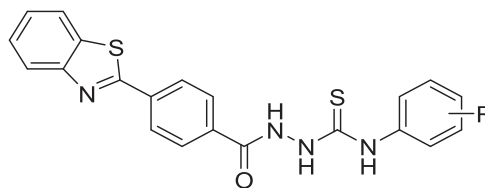
Series B: 21 compounds ( $IC_{50} = 4.5$  to  $44.5 \mu M$ )

Best inhibitors: **36 (B)** ( $IC_{50} = 4.6 \mu M$ ;  $X = S$ ;  $R = 4-OCH_3$ );

**37 (B)** ( $IC_{50} = 5.6 \mu M$ ;  $X = S$ ;  $R = 2-OCH_3$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 13.** Chemical structures of potential urease inhibitors bearing an isatin moiety.



$R = H, F, Cl, Br, 2-CH_3, OCH_3, 4-CH_3, 4-NO_2, 3-CH_3, 3-NO_2, 3,4-Cl, 4-CF_3$ .

Standard inhibitor: thiourea ( $IC_{50} = 19.46 \pm 1.20 \mu M$ )

Series: 20 compounds ( $IC_{50} = 34.48$  to  $1.4 \mu M$ )

Best inhibitors: **38** ( $IC_{50} = 1.4 \pm 0.10 \mu M$ ;  $R = 4-NO_2$ );

**39** ( $IC_{50} = 1.6 \pm 0.10 \mu M$ ;  $R = 4-CF_3$ );

**40** ( $IC_{50} = 2.1 \pm 0.24 \mu M$ ;  $R = 3,4-Cl$ );

**41** ( $IC_{50} = 2.5 \pm 0.10 \mu M$ ;  $R = 4-F$ )

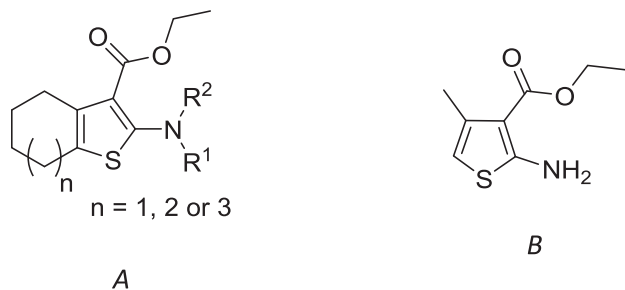
\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 14.** Chemical structures of hybrids benzothiazole thiosemicarbazides that present antiurease activities.

A hybridization strategy using benzothiazoles and thiosemicarbazides was also employed by Taha et al. to develop new antiurease agents [66]. Eighteen of the synthesized compounds (Scheme 14) exhibited  $IC_{50}$  values less than thiourea, revealing a trend in which substituted aromatic rings were more active than unsubstituted rings. Furthermore, when an electron-withdrawing group is present in the aryl motif, the polarizability and activity of the molecule towards urease increases [66].

#### Five-membered heterocycles

In 2010, Khan reported a potent series of inhibitors based on 2-aminothiophenes derivatives (Scheme 15) that were identified using molecular modeling and virtual screens against *Canavalia ensiformis* urease. According to the docking study, compounds with a single thiophene ring bind next to the nickel ions and exhibit better inhibitory potency than compounds with fused bulky rings or compounds with substitutions at the amino group. In the latter case, the thiophene ring is located at a distant site from the nickel ions and results in a loss of activity, apparently due to torsional



$R^1 = H, C(O)OCH_3, C(O)CH_2Cl, C(O)CHCl_2, C(O)CH_2Cl, C(S)SCH_3, C(O)Cl$

$R^2 = H, C(O)Cl$

Standard inhibitor: acetohydroxamic acid ( $IC_{50} = 24.1 \mu M$ )

Series A: 10 compounds ( $IC_{50} = 2.99$  to  $31.8 \mu M$ )

Series B: 1 compound ( $IC_{50} = 2.81 \mu M$ )

Best inhibitors: **42 (A)** ( $IC_{50} = 2.99 \mu M$ ;  $R^1 = R^2 = C(O)Cl$ );

**43 (B)** ( $IC_{50} = 2.81 \mu M$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 15.** Chemical structures of 2-aminothiophenes – a five-membered heterocycle-urease inhibitors.

strain [67]. For example, a compound with a methyl group at C-4 position was the most potent among the series and more active than the standard inhibitor. On the other hand, a similar derivative with fused ring showed a loss of activity against the enzyme.

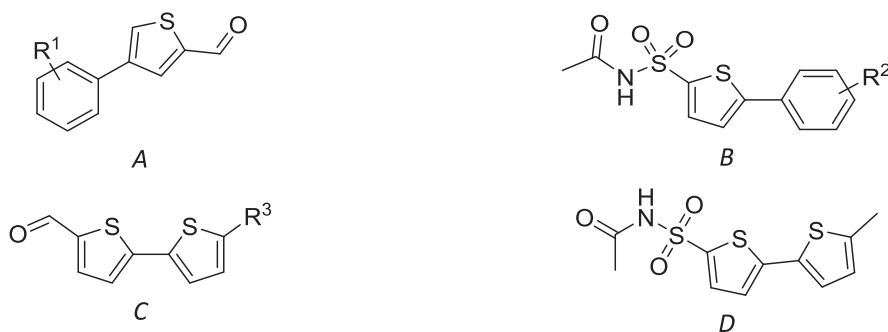
Ali and coworkers assessed the antiurease activity of diverse 5-aryl-thiophene-2-carbaldehydes [68]. The authors observed a trend in which electron-withdrawing groups were more active at inhibiting the enzyme than their electron-donating counterparts,

highlighting the di-halogenated compound (**44** – Series A; Scheme 16) as the most active of the series [68]. Noreen et al. also explored the potential of 5-aryl-thiophene-2-sulfonamides, three of which were more active than the positive control, namely compounds **48** and **49** (Scheme 16) [69].

Pyrazole derivatives have also been explored in an attempt to identify new urease inhibitors [70]. Harit et al. evaluated pyrazole dimers, forming nitrogen centered tripods (Scheme 17). When the pyrazole rings were fused through either a N-C or C-C linkage, the 4 resulting tripods were selective urease inhibitors compared with  $\alpha$ -chemotrypsin, cholinesterases, phosphodiesterase and  $\beta$ -glucuronidase. Dimerization also reduces the  $IC_{50}$  of the derivative by 50%, from  $94 \mu M$  to  $44 \mu M$ . Furthermore, the authors concluded that the nature of the side arm had no effect on urease inhibition.

Similarly, the imidazole motif has been extensively explored in medicinal chemistry investigations and specifically in screens for urease inhibitors. Naureen and coworkers [71] tested fifteen series of tetraaryl imidazole-indole compounds (Scheme 18) and showed that they exhibited comparable or better urease inhibitory activity than the positive control thiourea. The most potent inhibitors were the compounds containing disubstituted halogens (compounds **52**, **55**, and **56**; Scheme 18) or containing the trifluoromethyl moiety on the arylindole group (compounds **53** and **54**; Scheme 18) and displayed  $IC_{50}$  values as low as  $0.12 \mu M$ .

Some antimicrobial nitroimidazole drugs, namely metronidazole and secnidazole, also present antiurease activity. Encouraged by these properties, Mao and coworkers synthesized a series of hybrids of salicylates and metronidazole [72] or secnidazole [73]. Both hybrid series were active against urease, with secnidazoles being more active (Scheme 19). A synergistic effect was observed, since secnidazole alone yielded an  $IC_{50} = 156 \pm 10 \mu M$  [73] and the hybrids showed inhibition at the submicromolar level. Docking studies using both hybrids types showed that their binding generated a flap movement of  $\alpha 313$ - $\alpha 346$  residues, opening the enzyme's active site. The most active compound, **57** (Series D;



Series A: 10 compounds ( $IC_{50} = 32.2$  to  $27.1 \mu g/mL$ )

$R^1 = H, 3,5-CF_3, 3-CN-5-CF_3, 4-CH_3, 4-OCH_3, 3,5-CH_3, 3,4-Cl, 3-Cl-4-F$

Series C: 2 compounds ( $IC_{50} = 28.4 \mu g/mL$  to no activity)

$R^3 = CH_3, Cl$

Standard inhibitor: thiourea ( $IC_{50} = 27.5 \mu g/mL$ )

Best inhibitors: **44 (A)** ( $IC_{50} = 27.1 \mu g/mL$ ;  $R^1 = 3-Cl-4-F$ ); **45 (A)** ( $IC_{50} = 27 \mu g/mL$ ;  $R^1 = 4-CH_3$ ); **46 (C)** ( $IC_{50} = 28.4 \mu g/mL$ ;  $R^3 = Cl$ )

Series B: 7 compounds ( $IC_{50} = 218$  to  $17.1 \mu g/mL$ )

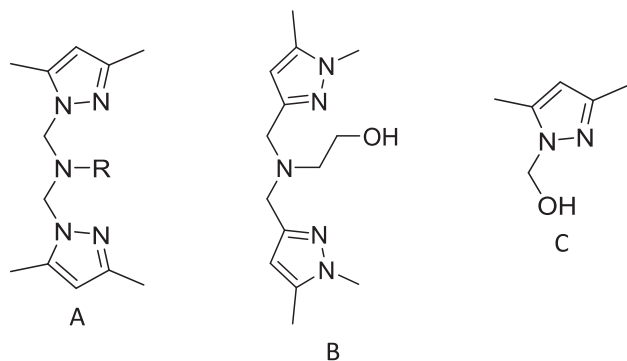
$R_2 = H, 3,5-CF_3, 4-Cl, 4-CH_3, 3,5-CH_3, 3,4-Cl$

Series D: 1 compound ( $IC_{50} = 17.1 \pm 0.15 \mu g/mL$ )

Standard inhibitor: thiourea ( $IC_{50} = 43 \pm 0.38 \mu g/mL$ )

Best inhibitors: **47 (D)** ( $IC_{50} = 17.1 \pm 0.15 \mu g/mL$ ); **48 (B)** ( $IC_{50} = 17.9 \pm 0.13 \mu g/mL$ ;  $R^2 = 3,5-CH_3$ ); **49 (B)** ( $IC_{50} = 23.3 \pm 0.21 \mu g/mL$ ;  $R^2 = 4-Cl$ )

**Scheme 16.** Chemical structures of urease inhibitors based on 5-aryl-thiophene-2-carbaldehydes scaffold.



R = 2-ethanol or cyclohexyl

Standard inhibitor: thiourea (Inhibition =  $21 \pm 0.011 \mu\text{M}$ )

Series A: 2 compounds (Inhibition = 44.66 to 43.46  $\mu\text{M}$ )

Series B: 1 compound (no Inhibition)

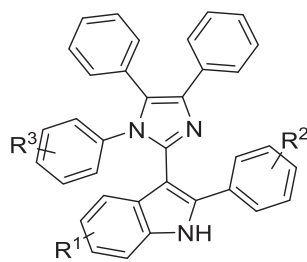
Series C: 1 compound ( $\text{IC}_{50} = 94.3 \pm 0.47 \mu\text{M}$ )

Best inhibitors: **50 (A)** ( $\text{IC}_{50} = 43.46 \pm 0.02 \mu\text{M}$ ; R = cyclohexyl);

**51 (A)** ( $\text{IC}_{50} = 44.66 \pm 0.03 \mu\text{M}$ ; R = ethanol)

\*Values of  $\text{IC}_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 17.** Chemical structures of urease inhibitors based on pyrazoles moiety.



$\text{R}^1 = \text{H}, \text{Br}$

$\text{R}^2 = \text{H}, \text{Cl}, \text{CF}_3, \text{Br}$

$\text{R}^3 = \text{H}, \text{Br}, \text{F}, \text{Cl}, \text{CH}_3, \text{OCH}_3$

Standard inhibitor: thiourea ( $\text{IC}_{50} = 21.25 \pm 0.15 \mu\text{M}$ )

Series: 18 compounds ( $\text{IC}_{50} = 0.12$  to 29.12  $\mu\text{M}$ )

Best inhibitors: **52** ( $\text{IC}_{50} = 0.15 \pm 0.04 \mu\text{M}$ ;  $\text{R}^1 = 5\text{-Br}$ ,  $\text{R}^2 = \text{H}$ ,  $\text{R}^3 = 4\text{-Br}$ );

**53** ( $\text{IC}_{50} = 0.12 \mu\text{M}$ ;  $\text{R}^1 = \text{H}$ ;  $\text{R}^2 = 4\text{-CF}_3$ ;  $\text{R}^3 = 4\text{-CH}_3$ );

**54** ( $\text{IC}_{50} = 0.16 \mu\text{M}$ ;  $\text{R}^1 = \text{H}$ ;  $\text{R}^2 = 4\text{-CF}_3$ ;  $\text{R}^3 = 4\text{-F}$ );

**55** ( $\text{IC}_{50} = 0.37 \mu\text{M}$ ;  $\text{R}^1 = \text{H}$ ;  $\text{R}^2 = 4\text{-Cl}$ ;  $\text{R}^3 = \text{H}$ );

**56** ( $\text{IC}_{50} = 0.44 \mu\text{M}$ ;  $\text{R}^1 = \text{H}$ ;  $\text{R}^2 = 4\text{-Cl}$ ;  $\text{R}^3 = 4\text{-F}$ )

\*Values of  $\text{IC}_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 18.** Chemical structures of urease inhibitors based on imidazole-indole moieties.

**Scheme 19**), also showed hydrogen bonding between the phenolic oxygen and Thr135 and His417 in addition to hydrophobic interactions with Phe195, Ala246 and Phe273 [73].

Along with thiazoles, thiazolidine aliphatic esters (**Scheme 20**) were also described as potential antiurease agents by Lodhi et al.

[74]. A screen against *C. ensiformis* and *B. pasteurii* ureases identified nine esters that were more active than thiourea, and the heptyl ester **62** (**Scheme 20**) was the most active inhibitor of both enzymes. Molecular docking studies showed the interaction of the carbonyl oxygen atom with a nickel atom, forming a pseudotetrahedral geometry responsible for the principal interaction between the thiazolidines and urease. The authors inferred that the observed increase in activity with increase of chain length represented an inductive effect that accumulates to a greater extent than steric hindrance until the octyl ester [74]. These theories agree with observed decrease in the potency of compounds containing branched chains and heteroatoms.

In contrast to the abovementioned related heterocycles, a screen of the urease inhibitory activity of several difunctionalized oxazolones revealed that only compounds possessing a phenyl ring at C2 displayed antiureolytic activity, but was 3 times less active than thiourea (compounds **65** and **66**, **Scheme 21**) [75].

Some researchers have explored the use of sulfur heterocycles, mainly benzothiazoles. Araujo and coworkers [76] synthesized a number of 2-arylbenzothiazoles (**Scheme 22**) with the aim of obtaining urease inhibitors. Among the synthesized compounds, three stand out as being as potent as known urease inhibitors: compound **67**, which is comparable to hydroxyurea (62% inhibition), and compounds **68** and **69**, which are equivalent to thiourea (26%). The inhibitory mechanism of compound **67** was investigated using kinetic experiments revealing that this benzothiazole binds either to the free urease or the enzyme-substrate complex; thus, it represents a mixed-type inhibitor. The dissociation constants obtained from those experiments showed a  $K_i$  for the urease-compound **67** of  $1.02 \pm 0.04 \text{ mM}$  and a  $K_i$  for the urease-urea-compound **67** of  $3.17 \pm 0.69 \text{ mM}$ , indicating that the affinity of compound **67** for the active site is approximately three times that of urea.

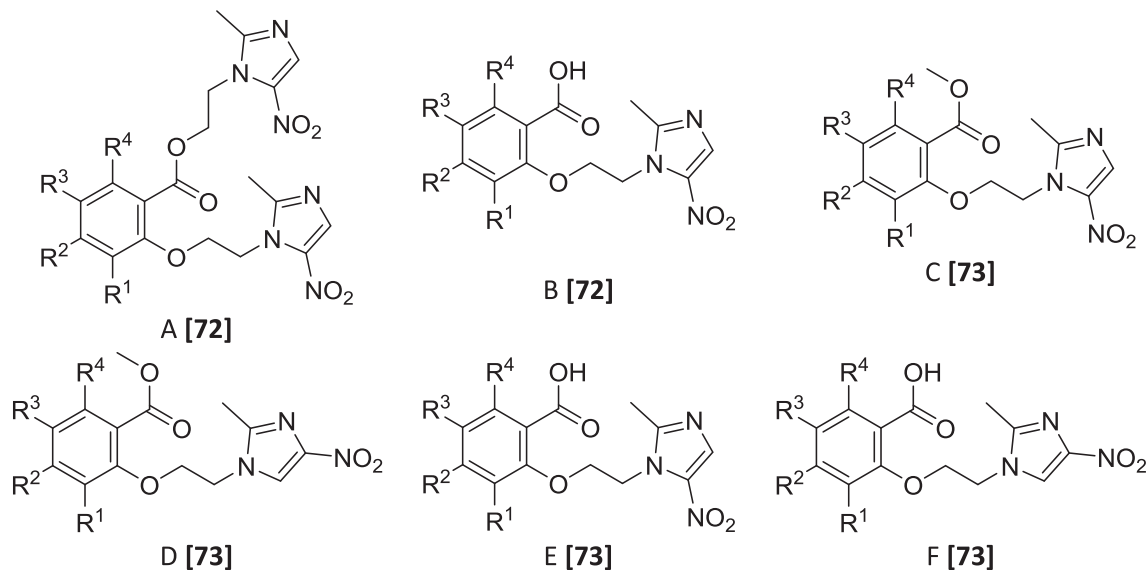
Conjugation of benzothiazole and acyl thiourea cores was also exploited by Gull et al. [77] to obtain hybrid 6-aryl-2-acetamidobenzothiazoles (**Scheme 23**). All compounds displayed similar range of inhibitory activity, with an  $\text{IC}_{50}$  of approximately 18  $\mu\text{g/mL}$ , and were more active than thiourea. The addition of an electron-donating group in the *para* position of the aryl group resulted in a small gain in potency compared to an electron-withdrawing group. *In silico* docking studies of compound **70** (R = *p*-tolyl, **Scheme 23**) in both active sites, A and B, showed hydrophobic interactions with the residues His593, Met637, Ala636, Gln635 and Asp494 and a hydrogen bond with the phosphate group at site A. At site B, cation- $\pi$  type interactions were observed between compound **70** and Lys208, Asp206, Thr158, Glu254, Phe182, Lys156 and Asp183, along with hydrogen bonds with Glu252 and Lys156. All compounds interacted better with site B than site A of *H. pylori* urease, indicating that these molecules participate in a stronger bond with site B than with site A. Furthermore, an inversely proportional linear correlation between the number of hydrogen bonds and the  $\text{IC}_{50}$  was noted [77].

Akhtar and coworkers synthesized chiral-substituted 1,2,4-triazoles (**Scheme 24**) and assessed their activity [78]. Substituents with differently sized chiral moieties showed an insignificant influence on urease inhibition. In a subsequent paper, the same authors [79] synthesized 5-aryl-1,2,4-triazole-3-thiones with different halogen patterns at the 5-aryl moiety (**Scheme 25**). The bromo-substituted rings in compounds **72** and **73** were more active than their chlorinated or fluorinated analogues and thiourea itself.

Similarly, Özil et al. synthesized 1,2,4-triazole derivatives (**Scheme 26**) by modifying groups attached to the central benzene ring and  $\text{N}^2$ -atom from triazole rings. Of all compounds, derivatives **74** and **75** were the most active [80].

The urease inhibition potential of disubstituted 1,2,4-triazole-3-thiones (Series A, **Scheme 27**) were evaluated by Khan and cowork-





$R^1 = H, CH_3, NO_2, I, Br, Cl$

$R^2 = H, Cl, CF_3, NH_2, OCH_3, CH_3$

$R^3 = H, Br, I, Cl, CH_3, OCH_3, SO_3H, NO_2$

$R^4 = H, CH_3$

Standard inhibitor: acetohydroxamic acid ( $IC_{50} = 17 \mu M$ , IP = 91,2% [72];  $16 \mu M$  [73])

Series A: 8 compounds (IP = 38.3% to 95.3%)

Series B: 6 compounds (IP = 38.3% to 56.8%)

Series C: 9 compounds ( $IC_{50} = 51$  to  $1.7 \mu M$ )

Series D: 8 compounds ( $IC_{50} = 37$  to  $1.0 \mu M$ )

Series E: 5 compounds ( $IC_{50} = 29$  to  $2 \mu M$ )

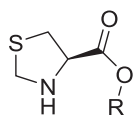
Series F: 8 compounds ( $IC_{50} = 45$  to  $19 \mu M$ )

Best inhibitors: **57 (D)** ( $IC_{50} = 1.0 \pm 0.4 \mu M$ ;  $R^1 = H$ ;  $R^2 = Cl$ ;  $R^3 = H$ ;  $R^4 = H$ ); **58 (A)** ( $IC_{50} = 26 \mu M$ ;  $R^1 = H$ ;  $R^2 = H$ ;  $R^3 = Br$ ;  $R^4 = H$ ); **59 (A)** ( $IC_{50} = 12 \mu M$ ;  $R^1 = H$ ;  $R^2 = H$ ;  $R^3 = Cl$ ;  $R^4 = H$ ); **60 (C)** ( $IC_{50} = 1.7 \pm 0.5 \mu M$ ;  $R^1 = H$ ;  $R^2 = H$ ;  $R^3 = H$ ;  $R^4 = H$ ); **61 (E)** ( $IC_{50} = 2.0 \pm 1 \mu M$ ;  $R^1 = H$ ;  $R^2 = H$ ;  $R^3 = CH_3$ ;  $R^4 = H$ )

\*IP = inhibition percentage at 1mM

\*Values of  $IC_{50}$  determined with urease from *Helicobacter pylori*

**Scheme 19.** Chemical structures of urease inhibitors based on hybrids of salicylates and metronidazole or secnidazole.



$R = H, CH_3, C_2H_5, CH_2CH_2CH_3, CH(CH_3)_2, CH_2(CH_2)_2CH_3, C(CH_3)_3, CH_2(CH_2)_4CH_3, CH_2(CH_2)_5CH_3, CH(CH_3)CH_2(CH_2)_3CH_3, CH_2(CH_2)_6CH_3, CH(CH_3)CH_2(CH_2)_4CH_3, CH_2(CH_2)_7CH_3, CH_2CH_2Cl$

Standard inhibitor: thiourea ( $IC_{50} = 21.01 \mu M$ ), pH = 5.0

Series: 14 compounds ( $IC_{50} = 180.99$  to  $0.29 \mu M$ )

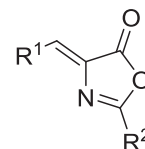
Best inhibitors: **62** ( $IC_{50} = 0.29 \pm 0.012 \mu M$ ;  $R = CH_2(CH_2)_5CH_3$ );

**63** ( $IC_{50} = 0.40 \pm 0.003 \mu M$ ;  $R = CH_2(CH_2)_4CH_3$ );

**64** ( $IC_{50} = 0.34 \pm 0.60 \mu M$ ;  $R = CH(CH_3)CH_2(CH_2)_3CH_3$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 20.** Chemical structures of thiazoles, thiazolidines aliphatic esters - potential urease inhibitors.



$R^1 = \text{phenyl}, CH_3$

$R_2 = 3\text{-NO}_2\text{-4-Cl-C}_6\text{H}_3, 2\text{-(ethoxy)phenyl}, 3,5\text{-Br-4-OH-C}_6\text{H}_2, 4\text{-OCH}_3\text{-cinnamyl}$

Standard inhibitor: thiourea ( $IC_{50} = 21.4 \mu M$ )

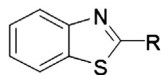
Series: 6 compounds ( $IC_{50} = 65.3$  to  $58 \mu M$ )

Best inhibitors: **65** ( $IC_{50} = 58 \mu M$ ;  $R^1 = \text{phenyl}$ ;  $R^2 = 2\text{-(ethoxy)phenyl}$ );

**66** ( $IC_{50} = 62 \mu M$ ;  $R^1 = \text{phenyl}$ ;  $R^2 = 4\text{-OCH}_3\text{-cinnamyl}$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 21.** Chemical structures of oxazolones: a class of urease inhibitors.



R = phenyl, 4-NO<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>, 4-CN-C<sub>6</sub>H<sub>4</sub>, 4-N(CH<sub>3</sub>)<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>, 4-SCH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>, 4-OCH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>, 3-OCH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>, 4-F-C<sub>6</sub>H<sub>4</sub>, 4-OH-C<sub>6</sub>H<sub>4</sub>, 3-OH-C<sub>6</sub>H<sub>4</sub>, 2-OH-C<sub>6</sub>H<sub>4</sub>, 2-COOH-C<sub>6</sub>H<sub>4</sub>, 3,5-OCH<sub>2</sub>O-C<sub>6</sub>H<sub>4</sub>, 2-thiophenyl, 2-pyrrolyl, 2-furyl, 4-pyridinyl, 3-pyridinyl, cyclohexyl.

Standard inhibitor: thiourea (26% inhibition)

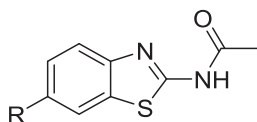
Series A: 18 compounds

Best inhibitors: **67** ( $K_i = 1.02 \pm 0.04$  mM; R = 3-pyridinyl);

**68** (R = 4-pyridinyl); **69** (R = 2-COOH-C<sub>6</sub>H<sub>4</sub>)

\*Values of % inhibition and  $K_i$  determined with urease from *Canavalia ensiformis*

**Scheme 22.** Chemical structures of benzothiazoles – an interesting class of urease inhibitors.



R = phenyl, 3,5-CF<sub>3</sub>-C<sub>6</sub>H<sub>3</sub>, 4-Cl-C<sub>6</sub>H<sub>4</sub>, 4-CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>, 3,5-CH<sub>3</sub>-C<sub>6</sub>H<sub>3</sub>, 3,4-Cl-C<sub>6</sub>H<sub>3</sub>, 4-OCH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>, 5-methyl-thiophen-2-yl.

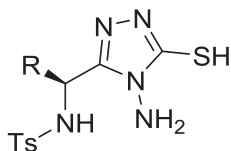
Standard inhibitor: thiourea ( $IC_{50} = 23.1$  μg/mL)

Series: 8 compounds ( $IC_{50} = 19.2$  to  $16.5$  μg/mL)

Best inhibitor: **70** ( $IC_{50} = 16.5$  μg/mL; R = 4-CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>)

\*Values of  $IC_{50}$  determined with urease from *Helicobacter pylori*

**Scheme 23.** Chemical structures of urease inhibitors based on 6-aryl-2-acetamidobenzothiazoles.



R = CH<sub>3</sub>, (CH<sub>3</sub>)<sub>2</sub>CH, (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>, (CH<sub>3</sub>)CHCH<sub>2</sub>CH<sub>3</sub>, CH<sub>2</sub>OH, CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, CH<sub>2</sub>CH<sub>2</sub>SCH<sub>3</sub>, (imidazol-4-yl)methyl, (indol-3-yl)methyl

Standard inhibitor: thiourea ( $IC_{50} = 21.0 \pm 0.1$  μM)

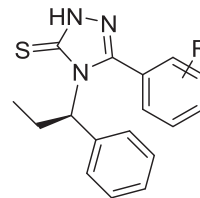
Series: 9 compounds ( $IC_{50} = 22.0$  to  $43.8$  μM)

Best inhibitor: **71** ( $IC_{50} = 22.0 \pm 0.5$  μM; R = (CH<sub>3</sub>)<sub>2</sub>CH)

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 24.** Chemical structures of urease inhibitor bearing the 1,2,4-triazole core.

ers, which were more active than the thiodiazole analogue (Series B, [Scheme 27](#)). A suitable structure–activity relationship was established for these compounds. The presence of one nitro group in the *meta* position of the aryl ring at position 5 of the triazole (compound **76** – Series A; [Scheme 27](#)) enhanced the inhibitory potency compared to an unsubstituted phenyl ring. The substitution pat-



R = 2-Cl, 3-Cl, 4-Cl, 2-Br, 3-Br, 4-Br, 2-F, 3-F, 4-F

Standard inhibitor: thiourea ( $IC_{50} = 21.0 \pm 0.1$  μM)

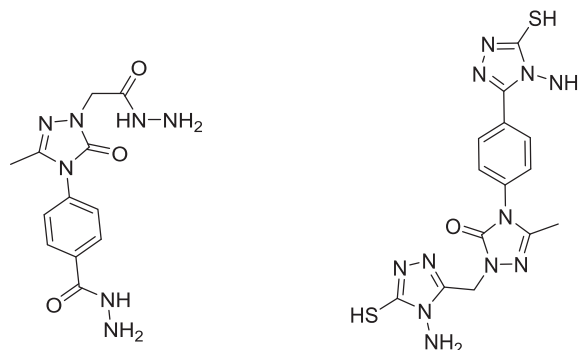
Series: 10 compounds ( $IC_{50} = 15.0$  to  $30.5$  μM)

Best inhibitors: **72** ( $IC_{50} = 7.8 \pm 0.2$  μM; R = 2-Br);

**73** ( $IC_{50} = 12.4 \pm 0.2$  μM; R = 3-Br)

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 25.** Chemical structures of urease based on 5-aryl-1,2,4-triazole-3-thiones.



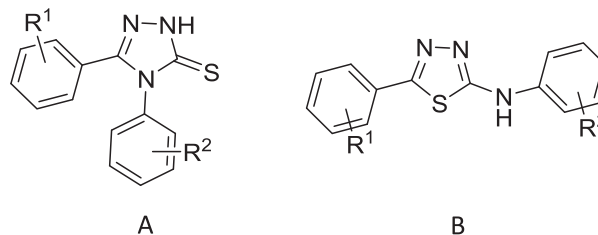
**74** ( $IC_{50} = 26.54 \pm 2.01$  μg/mL)

**75** ( $IC_{50} = 77.09 \pm 8.66$  μg/mL)

Standard inhibitor: thiourea ( $IC_{50} = 98.18 \pm 2.13$  μg/mL)

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 26.** Chemical structures of 1,2,4-triazole derivatives – potent substances described as urease inhibitor.



R<sup>1</sup> = 4-CH<sub>3</sub>, 2-CH<sub>3</sub>, 2-Cl, 3-Cl, 4-Cl, 3-NO<sub>2</sub>, 4-NO<sub>2</sub>, H

R<sup>2</sup> = 2,3-CH<sub>3</sub>, 2,4-CH<sub>3</sub>, 2,6-CH<sub>3</sub>

Standard inhibitor: thiourea ( $IC_{50} = 21.0 \pm 0.11$  μM)

Series A: 10 compounds ( $IC_{50} = 459.5$  to  $45.6$  μM)

Series B: 8 compounds ( $IC_{50} = 483.5$  to  $214.7$  μM)

Best inhibitors: **76 (A)** ( $IC_{50} = 45.60 \pm 0.04$  μM; R<sup>1</sup> = 3-NO<sub>2</sub>, R<sup>2</sup> = 2,4-CH<sub>3</sub>);

**77 (A)** ( $IC_{50} = 86.0 \pm 0.04$  μM; R<sup>1</sup> = H, R<sup>2</sup> = 2,3-CH<sub>3</sub>)

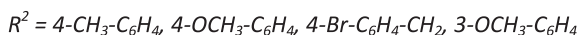
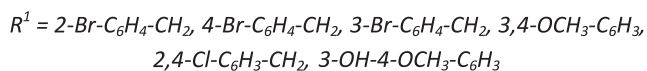
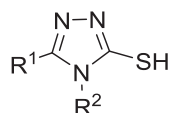
\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 27.** Chemical structures of urease inhibitor bearing the 1,2,4-triazole-3-thione moiety.

tern of the ring attached to the nitrogen atom at position 4 also had a clear influence on the inhibitory capacity. Small polarizable groups as a methyl group, in the *para* position favored inhibitory activity compared to groups placed at the *meta* and *ortho* positions [81].

Abid et al. [82] screened a series of triazole derivatives (Scheme 28) that were synthesized by varying benzyl and phenyl groups and contained halogen atoms, methyl or methoxy moieties. The compound with a bromine atom at the *meta* position in the benzyl moiety (compound **78**; Scheme 28) was the most active derivative, whereas inhibitors with electron-donating groups at the *para* or *meta* positions of the phenyl group were the least active compounds of the series against urease.

Oxadiazoles and their derivatives have also been reported to function as urease inhibitors. Akhtar and coworkers screened 2-arylamino-5-aryloxyalkyl-1,3,4-oxadiazoles (Scheme 29) for urease-inhibiting activity [83]. The influence of the nature of the 2-



Standard inhibitor: thiourea (Inhibition =  $21.0 \pm 0.01 \mu\text{M}$ )

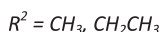
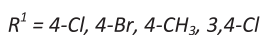
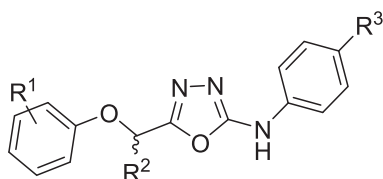
Series: 14 compounds ( $IC_{50} = 170.6$  to  $10.7 \mu\text{M}$ )

Best inhibitors: **78** ( $IC_{50} = 10.66 \pm 0.16 \mu\text{M}$ ;  $R^1 = 3\text{-Br-C}_6\text{H}_4\text{-CH}_2$ ;  $R^2 = 3\text{-OCH}_3\text{-C}_6\text{H}_4$ );

**79** ( $IC_{50} = 18.10 \pm 0.09 \mu\text{M}$ ;  $R^1 = 3\text{-Br-C}_6\text{H}_4\text{-CH}_2$ ;  $R^2 = 4\text{-CH}_3\text{-C}_6\text{H}_4$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 28.** Chemical structures of triazole derivatives described as potential urease inhibitors.



Standard inhibitor: thiourea ( $IC_{50} = 22.0 \pm 1.2 \mu\text{M}$ )

Series: 21 compounds ( $IC_{50} = 57.2$  to  $6.0 \mu\text{M}$ )

Best inhibitors: **80** ( $IC_{50} = 6.03 \pm 0.02 \mu\text{M}$ ;  $R^1 = 4\text{-Br}$ ,  $R^2 = \text{Et}$ ,  $R^3 = \text{NO}_2$ );

**81** ( $IC_{50} = 6.21 \pm 0.04 \mu\text{M}$ ;  $R^1 = 4\text{-Br}$ ,  $R^2 = \text{CH}_3$ ,  $R^3 = \text{F}$ );

**82** ( $IC_{50} = 7.42 \pm 0.03 \mu\text{M}$ ;  $R^1 = 4\text{-CH}_3$ ,  $R^2 = \text{Et}$ ,  $R^3 = \text{F}$ );

**83** ( $IC_{50} = 7.65 \pm 0.06 \mu\text{M}$ ;  $R^1 = 4\text{-Br}$ ,  $R^2 = \text{Et}$ ,  $R^3 = \text{F}$ )

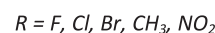
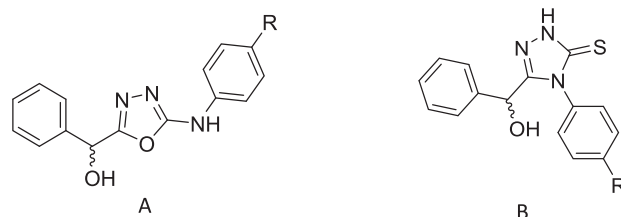
\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 29.** Chemical structures of oxadiazoles described as potential urease inhibitors.

arylamino substituent was the most relevant finding, whereas compounds containing a methyl group or chlorine or bromine atoms at the *para* position represented the least active molecules. However, the oxadiazoles with a fluorine or nitro group were the most active inhibitors (compounds **80–83**, Scheme 29).

Similar results were obtained for analogue compounds [84], where a *p*-methyl substituent in the aminophenyl (compound **86** – Series B, Scheme 30) group increased activity. 1,3,4-Oxadiazoles-2-thione analogues containing methoxylated aromatic groups (compounds **89** and **90**, Scheme 31) also showed high antiurease activity [85]. Although halogenated derivatives generally showed the lowest activity, the *para*-chlorobenzyl analogue was the most active tested inhibitor ( $IC_{50} 1.15 \pm 0.2 \mu\text{M}$ ).

The oxadiazole core was also explored by Shahzada and coworkers, who evaluated different substituted phenyl rings and aliphatic chains at position 5 of the oxadiazole rings (Scheme 32) [86]. The activity of halogenated rings decreases from *ortho* to *para* patterns, the exception being a bromo-substituted ring, where the *para*-bromo displays the highest activity of all synthesized com-



Standard inhibitor: thiourea ( $IC_{50} = 21.0 \pm 0.011 \mu\text{M}$ )

Series A: 5 compounds ( $IC_{50} = 80.3$  to  $16.1 \mu\text{M}$ )

Series B: 5 compounds ( $IC_{50} = 131.7$  to  $16.7 \mu\text{M}$ )

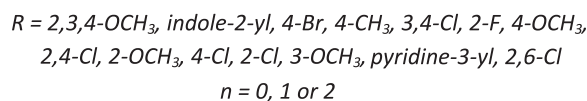
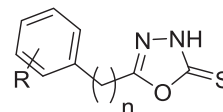
Best inhibitors: **84 (A)** ( $IC_{50} = 16.1 \pm 0.12 \mu\text{M}$ ;  $R = \text{F}$ );

**85 (B)** ( $IC_{50} = 18.9 \pm 0.188 \mu\text{M}$ ;  $R = \text{Cl}$ );

**86 (B)** ( $IC_{50} = 16.7 \pm 0.178 \mu\text{M}$ ;  $R = \text{Me}$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 30.** Chemical structures of aminophenyl benzylalcohols which possesses antiurease properties.



Standard inhibitor: thiourea ( $IC_{50} = 22.3 \pm 1.2 \mu\text{M}$ )

Series: 18 compounds ( $IC_{50} = 42.4$  to  $1.15 \mu\text{M}$ )

Best inhibitors: **87** ( $IC_{50} = 1.15 \pm 0.2 \mu\text{M}$ ;  $R = 4\text{-Cl}$ ,  $n = 1$ );

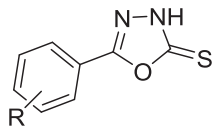
**88** ( $IC_{50} = 3.27 \pm 0.3 \mu\text{M}$ ;  $R = 2\text{-F}$ ,  $n = 1$ );

**89** ( $IC_{50} = 5.61 \pm 0.3 \mu\text{M}$ ;  $R = 4\text{-OCH}_3$ ,  $n = 2$ );

**90** ( $IC_{50} = 5.60 \pm 0.6 \mu\text{M}$ ;  $R = 2\text{-OCH}_3$ ,  $n = 1$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 31.** Chemical structures of 1,3,4-oxadiazoles-2-thione analogues described as potent inhibitors of urease enzyme.



R = 2-OH, 4-OCH<sub>3</sub>, 3-NO<sub>2</sub>, 4-NO<sub>2</sub>, 3,4,5-OCH<sub>3</sub>, 2-Cl, 3-Cl, 4-Cl, 4-F, 2-Br, 3-Br, 4-Br, 3-CH<sub>3</sub>, 4-CH<sub>3</sub>, 2-NH<sub>2</sub>, 3-(CH<sub>3</sub>)-4(NO<sub>2</sub>)

Standard inhibitor: thiourea (IC<sub>50</sub> = 21.00 μM)

Series: 18 compounds (IC<sub>50</sub> = 166.35 to 12.60 μM)

Best inhibitors: **91** (IC<sub>50</sub> = 13.03 ± 1.80 μM; R = 4-Br);

**92** (IC<sub>50</sub> = 13.62 ± 1.49 μM; R = 3-CH<sub>3</sub>-4-NO<sub>2</sub>);

**93** (IC<sub>50</sub> = 12.60 ± 0.92 μM; R = 2-Br);

**94** (IC<sub>50</sub> = 15.55 ± 2.37 μM; R = 3-NO<sub>2</sub>)

\*Values of IC<sub>50</sub> determined with urease from *Canavalia ensiformis*

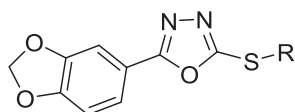
**Scheme 32.** Chemical structures of substances based on oxadiazole platform that has antiureolytic properties.

pounds. Molecules with nitro-substituted rings displayed maximum activity when positioned at the *meta* carbon, whereas the compound was inactive if attached to the *para* position. The introduction of a methyl group at position 3 on the *para*-nitrophenyl moiety decreased the IC<sub>50</sub> to 13.6 μM. The *n*-octyl (not shown) chain was as active as the control, thiourea. The authors inferred that activity decreases as steric hindrance increases, probably due to diminished interactions with nickel ions.

Rheman and coworkers [87] built a thioether version of the 1,3,4-oxadiazole coupled to the 3,4-methylenedioxyphenyl group (Scheme 33). Of the synthesized compounds, derivative **95** containing a bromine atom in *meta* position was the most active inhibitor against urease, displaying an IC<sub>50</sub> in the same range as thiourea.

Fused heterocycles, such as the 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole derivatives developed by Rafiq and coworkers (Scheme 34), were assembled for antiureolytic purposes. The assay of the compounds against urease identified inhibitors that were more active than thiourea, and compound **97** was the most potent derivative [88].

Pyrazolotriazines were hybridized with sulfonamides (Scheme 35) and presented high antiurease potency (IC<sub>50</sub> from 0.037 to 0.084 μM) [89]. Remarkably, the most active inhibitor was the chiral compound containing a (*S*)-2-hydroxy-1-methylethylamine substituent (compound **100**; Scheme 35), but its enantiomer displayed the lowest inhibition. A kinetic study



R = CH<sub>2</sub>CH<sub>2</sub>Br, CH<sub>2</sub>CH<sub>2</sub>Cl, (2-ethoxycarbonyl)-methyl, benzyl, 2-methylbenzyl, 2-bromobenzyl, 3-bromobenzyl or 4-bromobenzyl

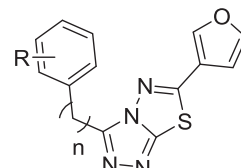
Standard inhibitor: thiourea (IC<sub>50</sub> = 21.28 ± 0.11 μM)

Series: 8 compounds (IC<sub>50</sub> = 313.49 to 30.10 μM)

Best inhibitors: **95** (IC<sub>50</sub> = 30.10 ± 0.65 μM; R = 3-bromobenzyl);

**96** (IC<sub>50</sub> = 46.20 ± 0.85 μM; R = 2-Methylbenzyl)

**Scheme 33.** Chemical structures of substances based on 1,3,4-oxadiazole moiety coupled to 3,4-methylenedioxyphenyl group.



R = F, OCH<sub>3</sub>, Cl, 3-Br, 4-Br

n = 1 or 0

Standard inhibitor: thiourea (IC<sub>50</sub> = 26 ± 5 μM)

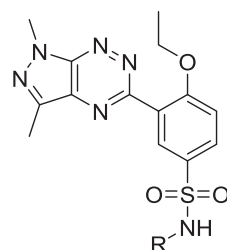
Series: 10 compounds (IC<sub>50</sub> = 32 to 0.764 μM)

Best inhibitors: **97** (IC<sub>50</sub> = 0.764 ± 0.03 μM; R = 4-OCH<sub>3</sub>; n = 0);

**98** (IC<sub>50</sub> = 1.55 ± 0.16 μM; R = 4-F; n = 1);

**99** (IC<sub>50</sub> = 2.19 ± 0.05 μM; R = 3-F; n = 1)

**Scheme 34.** Chemical structures of antiureolytic substances based on 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole platform.



R = (*S*)-CH(CH<sub>3</sub>)CH<sub>2</sub>OH, (*R*)-CH(CH<sub>3</sub>)CH<sub>2</sub>OH, (*S*)-CH(CH<sub>2</sub>OH)CH(CH<sub>3</sub>)CH<sub>3</sub>, (*R*)-CH(CH<sub>2</sub>OH)CH(CH<sub>3</sub>)CH<sub>3</sub>, (*S*)-CH<sub>2</sub>CH(OH)CH<sub>3</sub>, (*R*)-CH<sub>2</sub>CH(OH)CH<sub>3</sub>, (*S*)-CH<sub>2</sub>CH(OH)CH<sub>2</sub>OH, (*R*)-CH<sub>2</sub>CH(OH)CH<sub>2</sub>OH, (*S*)-CH(CH<sub>2</sub>OH)CH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>3</sub>, (*R*)-CH(CH<sub>2</sub>OH)CH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>3</sub>

Standard inhibitor: thiourea (IC<sub>50</sub> = 20.7 ± 0.45 μM)

Series: 10 compounds (IC<sub>50</sub> = 0.084 to 0.037 μM)

Best inhibitors: **100** (K<sub>i</sub> = 0.01 μM; R = (*S*)-CH(CH<sub>3</sub>)CH<sub>2</sub>OH);

**101** (IC<sub>50</sub> = 0.044 ± 0.130 μM; R = (*R*)-CH<sub>2</sub>CH(OH)CH<sub>3</sub>);

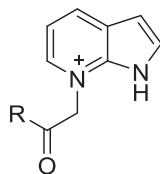
**102** (IC<sub>50</sub> = 0.042 ± 0.012 μM; R = (*S*)-CH(CH<sub>2</sub>OH)CH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>3</sub>)

\*Values of IC<sub>50</sub> and K<sub>i</sub> determined with urease from *Canavalia ensiformis*

**Scheme 35.** Chemical structures of hybrids pyrazolotriazine-sulfonamidas described as potential urease inhibitors.

of compound **100** was performed and exhibited a mixed type inhibitory behavior with a K<sub>i</sub> = 0.01 μM. Saify et al. reported 7-azaindole derivatives (Scheme 36), whose IC<sub>50</sub> values ranged from 2.19 to 255.11 μM [90]. The analogue **103** (Scheme 35) possessing a 4-methoxyphenacyl moiety presented the highest inhibition with an IC<sub>50</sub> of 2.19 ± 0.37 μM, whereas the second best inhibitor, compound **104**, only had an IC<sub>50</sub> = 133.31 ± 0.46 μM.

Selenium compounds are also reported to possess antiureolytic activity, such as ebselen (**105**, Scheme 37, IC<sub>50</sub> = 3.3 μM) and its derivatives described by Macegoniuk et al.; ebselen was reported to exhibit antiulcer properties and inhibits gastric secretion [91,92]. In an attempt to further derivatize ebselen, Macegoniuk et al. evaluated the activity of compounds bearing different groups on the nitrogen atom. The presence of a carboxylic acid group decreased significantly the activity of the inhibitors (IC<sub>50</sub> of 25.4 μM to inactive). The activity returned to the previous order of magnitude for the corresponding methyl ester counterpart [IC<sub>50</sub> 3.3 μM (**106**) to 4.07 μM (**107**); Scheme 37]. Compounds containing phe-



R = 3,4-OH-C<sub>6</sub>H<sub>3</sub>, 3-NO<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>, 2-NO<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>, 4-NO<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>, 2,4-F-C<sub>6</sub>H<sub>3</sub>, 4-F-C<sub>6</sub>H<sub>4</sub>, 4-Cl-C<sub>6</sub>H<sub>4</sub>, 4-OCH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>, 2-naphthyl

Standard inhibitor: thiourea (IC<sub>50</sub> = 21 μM)

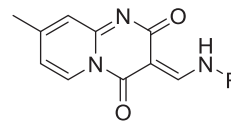
Series: 13 compounds (IC<sub>50</sub> = 255.11 to 2.19 μM)

Best inhibitors: **103** (IC<sub>50</sub> = 0.764 μM; R = 4-OCH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>),

**104** (IC<sub>50</sub> = 133.31 ± 0.46 μM; R = 2-naphthyl)

\*Values of IC<sub>50</sub> determined with urease from *Canavalia ensiformis* and *Bacillus pasteurii*

**Scheme 36.** Chemical structures of 7-azaindoles stated as urease inhibitors.



R = 2-C(O)CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>, 3-C(O)CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>, 4-C(O)CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>, 2,4,6-(CH<sub>3</sub>)<sub>3</sub>-C<sub>6</sub>H<sub>2</sub>, 2-F-C<sub>6</sub>H<sub>4</sub>, 3-F-C<sub>6</sub>H<sub>4</sub>, 4-F-C<sub>6</sub>H<sub>4</sub>, 2-SH-C<sub>6</sub>H<sub>4</sub>, 3-CH<sub>3</sub>-pyridyl, 4-CH<sub>3</sub>-pyridyl, 4-NO<sub>2</sub>-pyridyl, NHC(O)-4-NO<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>, NHC(O)C<sub>6</sub>H<sub>5</sub>, NHC(O)-4-Br-C<sub>6</sub>H<sub>4</sub>, NHC(O)-4-Cl-C<sub>6</sub>H<sub>4</sub>, NHC(O)-4-OH-C<sub>6</sub>H<sub>4</sub>

Standard inhibitor: Thiourea (94% inhibition in 100 μM concentration)

Series: 16 compounds (0.2 to 97% inhibition in 100 μM concentration)

Best inhibitors: **108** (97% inhibition in 100 μM concentration; R = 2-SH-C<sub>6</sub>H<sub>4</sub>);

**109** (87% inhibition in 100 μM concentration; R = 4-NO<sub>2</sub>-pyridyl)

\*Values of IC<sub>50</sub> determined with urease from *Canavalia ensiformis*

**Scheme 38.** Chemical structures of pyridopyrimidine-based urease inhibitors.

nyl group, including ebsalen (**105**; Scheme 37), and methyl ester derivatives were the most active inhibitors, with IC<sub>50</sub> values as low as 3.3 μM.

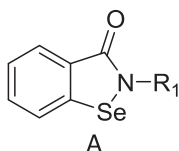
#### Six-membered heterocycles

Due to the wide range of biological activities of six-membered heterocycles, such as pyridinones [93–98], pyridopyrimidine and other compounds, [99–109] several studies aiming to develop urease inhibitors based on these structural motifs have been reported. Rauf et al. [110] evaluated the urease inhibitory activity of pyridopyrimidine derivatives. According to the researchers, the presence of a metal-chelating group such as –SH or the moiety 4-nitrobenzohidrazide determines the activity of these compounds, which could explain the inhibitory activity of compounds **108** and **109** (Scheme 38) towards urease. Based on the results from tautomerization studies, an increase in the negative charge of the heteroatoms of the compound correlates with the increase in urease inhibition.

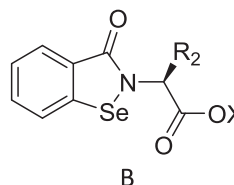
Bektas et al. envisaged a strategy to connect moieties of known bioactive 1,2,4-triazoles and oxazolidinones to the linezolid structure, developed analogues, and screened their antiurease activity. All synthesized hybrid compounds (Scheme 39) exhibited good inhibition of the urease enzyme [111].

Oliveira et al. synthesized several pyridinone derivatives and evaluated their inhibitory potential against urease (Scheme 40) [112]. Although an apparent relationship between the presence of an alkyl substituent at the bridge carbon and urease inhibition was not observed, the presence of an electron-releasing group at the *para* position of the benzene ring increased the biological activity of the compounds, which explained the high inhibition percentage of compounds **111** and **112** (Scheme 40). Additionally, hyperconjugation of ethyl and methyl groups and electron-releasing groups at the *meta* position of the benzene ring are not associated with increased inhibitory activity.

Recently, Iftikhar et al. reported progress in their fruitful research on dihydropyrimidine (DHPM) by screening 15 new 5-C-substituted (Scheme 41) analogues for their urease inhibition potential [113]. The SAR studies based on urease inhibition assays showed that thiosemicarbazides and isatin derivatives were more potent inhibitors. Molecular docking showed that compound **113**



A



B

R<sub>1</sub> = Phenyl, (R)-1-(1-phenylethyl), (S)-1-(2-hydroxy-1-phenylethyl), (CH<sub>2</sub>)<sub>n</sub>COOH

R<sub>2</sub> = CH<sub>3</sub>, CH(CH<sub>3</sub>)<sub>2</sub>, CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, CH<sub>2</sub>COOH, (CH<sub>3</sub>)CHCH<sub>2</sub>CH<sub>3</sub>

X = H or CH<sub>3</sub>

Standard inhibitor: Acetohydroxamic acid (K<sub>i</sub> = 3 300 ± 400 nM)

Series A: 6 compounds (K<sub>i</sub> = 2.1 ± 0.18 to 1210 ± 120 nM)

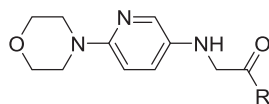
Series B: 7 compounds (K<sub>i</sub> = 32.6 ± 2.6 to 2410 ± 190 nM)

Best inhibitors: **105 (A)** (K<sub>i</sub> = 2.11 ± 0.18 nM; R = phenyl); **106 (B)** (K<sub>i</sub> = 76.3 ± 5.2 nM; R =

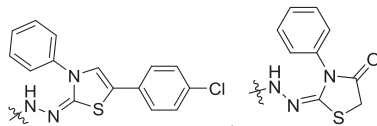
CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>; X = CH<sub>3</sub>); **107 (B)** (K<sub>i</sub> = 164.2 ± 9.3 nM; R = (CH<sub>3</sub>)CHCH<sub>2</sub>CH<sub>3</sub>; X = CH<sub>3</sub>)

\*Values of K<sub>i</sub> determined with urease from *Bacillus pasteurii*

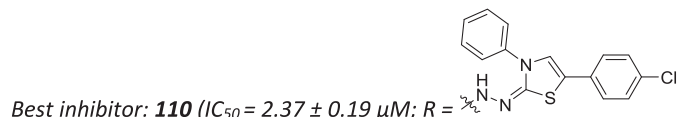
**Scheme 37.** Chemical structures of containing selenium atom urease inhibitors.



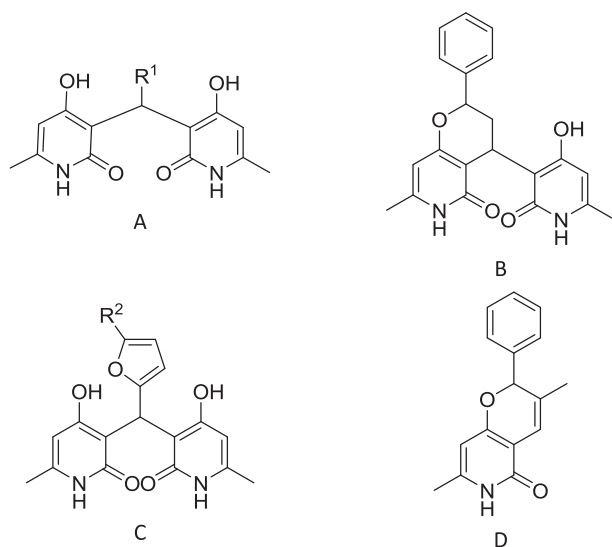
$R = \text{OCH}_2\text{CH}_3, \text{NHNH}_2, \text{NH}(\text{N})\text{CH}(4\text{-Br-C}_6\text{H}_4), \text{NH}(\text{N})(\text{CH})_3\text{C}_6\text{H}_5, (\text{NH})_2\text{CSNHC}_6\text{H}_5,$



Series: 7 compounds ( $\text{IC}_{50} = 2.37 \pm 0.19$  to  $13.23 \pm 2.25 \mu\text{M}$ )



**Scheme 39.** Chemical structures of urease inhibitors derived from linezolid core.



$R^1 = \text{H}, \text{CH}_3, \text{CH}_2\text{CH}_3, \text{CH}_2\text{CH}_2\text{CH}_3, \text{CH}_2\text{CH}(\text{CH}_3)_2, \text{C}_6\text{H}_5, \text{CH}_2\text{C}_6\text{H}_4,$   
 $2\text{-NO}_2\text{-C}_6\text{H}_4, 3\text{-NO}_2\text{-C}_6\text{H}_4, 4\text{-NO}_2\text{-C}_6\text{H}_4, 3\text{-CN-C}_6\text{H}_4, 4\text{-CN-C}_6\text{H}_4,$   
 $3\text{-F-C}_6\text{H}_4, 4\text{-F-C}_6\text{H}_4, 3\text{-OH-C}_6\text{H}_4, 4\text{-N}(\text{CH}_3)_3\text{-C}_6\text{H}_4, 3\text{-OCH}_3\text{-C}_6\text{H}_4,$   
 $4\text{-OCH}_3\text{-C}_6\text{H}_4, 3\text{-CH}_3\text{-C}_6\text{H}_4, 4\text{-CH}_2\text{CH}_3\text{-C}_6\text{H}_4, 3,4\text{-Br}_2\text{-OCH}_3\text{-C}_6\text{H}_3,$   
 $4\text{-Br-C}_6\text{H}_4, 3\text{-Cl-C}_6\text{H}_4, 4\text{-Cl-C}_6\text{H}_4, 4\text{-CF}_3\text{-C}_6\text{H}_4, 4\text{-NHCOCH}_3\text{-C}_6\text{H}_4,$   
 $3,4\text{-(OCH}_2\text{O)-C}_6\text{H}_4$

$R^2 = \text{H}, 4\text{-Br-C}_6\text{H}_4, 4\text{-Cl-C}_6\text{H}_4, 2\text{-NO}_2\text{-C}_6\text{H}_4$

Standard inhibitor: Thiourea ( $22.1 \pm 0.4\%$  of inhibition)

Series A: 27 compounds ( $-4.6 \pm 1.8$  to  $49.8 \pm 1.6\%$  of inhibition)

Series B: 1 compound ( $13.9 \pm 0.8\%$  of inhibition)

Series C: 4 compounds ( $5.3 \pm 0.9$  to  $19.4 \pm 1.2\%$  of inhibition)

Series D: 1 compound ( $36.2 \pm 2.0\%$  of inhibition)

Best inhibitors: **111 (A)** ( $49.8 \pm 1.6\%$  of inhibition;  $R^1 = 4\text{-OCH}_3\text{-C}_6\text{H}_4$ );

**112 (A)** ( $42.0 \pm 1.2\%$  of inhibition;  $R^1 = 3\text{-Br-4-OCH}_3\text{-C}_6\text{H}_3$ )

\*Values of  $\text{IC}_{50}$  determined with urease from *Canavalia ensiformis*

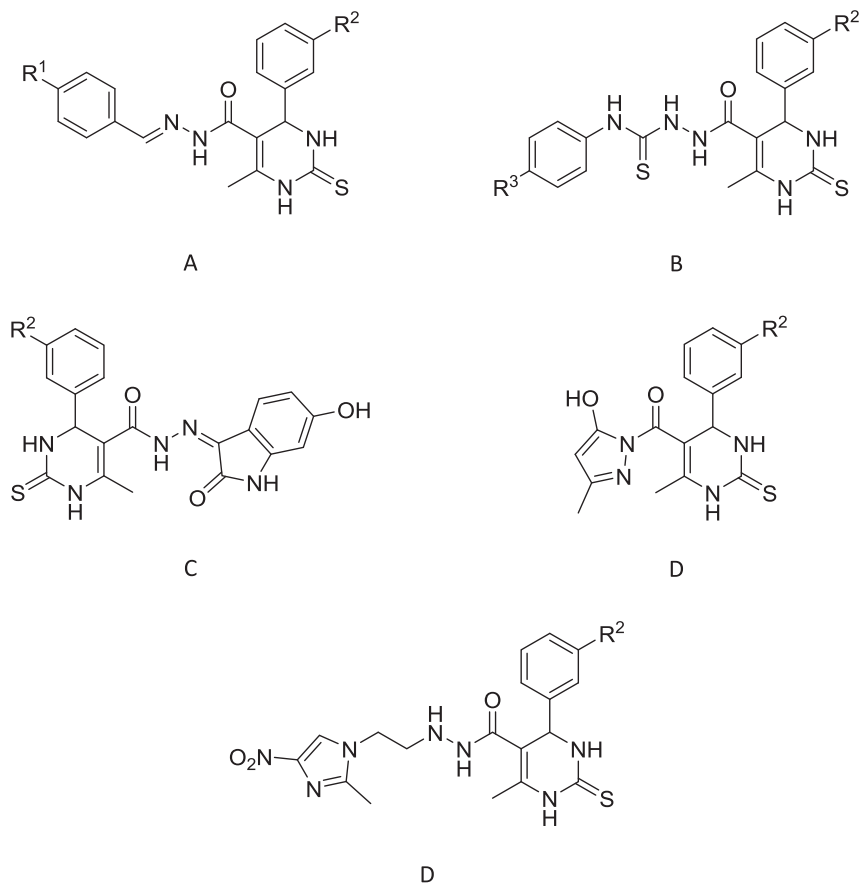
**Scheme 40.** Chemical structures of urease inhibitors based on pyridinones.

(Scheme 41) coordinates with the bis-nickel center via its 4-methoxy group at phenyl ring of semithiocarbamide; the NH group of pyrimidine forms a hydrogen bond with CME592 at the entrance of binding pocket. The aromatic ring of pyrimidine forms arene-cation interaction with Arg439, also at the entrance of binding pocket. The best result obtained for compound **116** (Scheme 41) among the other compounds was explained by the presence of 3 typical hydrogen bonds with His409, KCX490 and Asp633, in addition to the formation of hydrogen bonds with active site flap CME592 [113].

Khan et al. also reported studies on the urease-inhibiting activities of DHPM analogues, but the most active compounds in this study were the hydrazine derivatives (Series B, Scheme 42) [114]. Kinetic studies and molecular docking analyses of this class of substances suggested a mixed-type inhibition profile. The compounds participated in strong interactions with amino acids residues and the nickel center in the active site of the enzyme; the strongest interactions were observed for hydrazine derivatives, probably due to their polar nature [114]. On the other hand, the investigation conducted by Rashid et al. indicated that 3,4-dihydropyrimidine-2-ones and particularly 3,4-dihydropyrimidine-2-thiones (Series A, Scheme 42) were an active series of urease inhibitors [115]. Products with substituents at position 3 of the benzene ring showed higher inhibitory activity. According to the molecular docking studies, the free S atom and the hydrazine moiety were the main substituents responsible for the inhibitory capacity of the compounds through interactions with the nickel center of the enzyme [115].

Quinolone derivatives are another interesting class of compounds, due to some of their pharmacological properties [116–119]; these compounds were synthesized and screened as urease inhibitors. The studies conducted with sparfloxacin (Series A, Scheme 43) [120] and 8-nitroflouroquinolone (Series B, Scheme 43) [121] derivatives revealed moderate urease inhibition activities.

The diverse applicability of xanthenes and xanthenes in areas such as technology, photochemistry and biology [122–128] motivated the Khurana group to develop derivatives as potent urease inhibitors [129]. Based on the results of the urease inhibition assay, compounds with aryl groups carrying electron-donating groups at the para position exhibited the best inhibitory activity (Scheme 44).



$R^1 = H, OH, O(CH_2)_3CH_3$

$R^2 = Br, OH, NO_2$

$R^3 = H, OCH_3, Br$

Standard inhibitor: Thiourea ( $IC_{50} = 21 \pm 0.011 \mu M$ )      Series C: 2 compounds ( $IC_{50} = 0.23$  and  $5.2 \mu M$ )

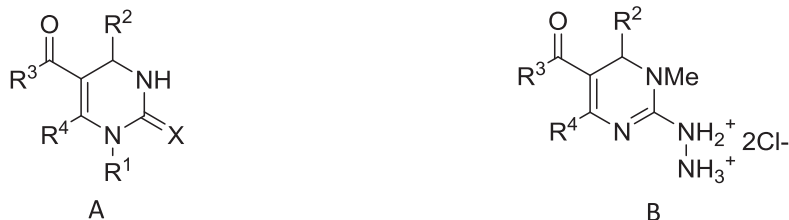
Series A: 4 compounds ( $IC_{50} = 33.71$  to  $111 \mu M$ )      Series D: 2 compounds ( $IC_{50} = 10.87$  and  $12.3 \mu M$ )

Series B: 4 compounds ( $IC_{50} = 0.58$  to  $1.05 \mu M$ )      Series E: 3 compounds ( $IC_{50} = 10.54$  to  $16.7 \mu M$ )

Best inhibitors: **113 (B)** ( $IC_{50} = 0.58 \pm 0.03 \mu M$ ;  $R^2 = Br$ ;  $R^3 = OMe$ ); **114 (B)** ( $IC_{50} = 0.61 \pm 0.19 \mu M$ ;  $R^2 = Br$ ;  $R^3 = Br$ ); **115 (B)** ( $IC_{50} = 0.79 \pm 0.05 \mu M$ ;  $R^2 = Br$ ;  $R^3 = H$ ); **116 (C)** ( $IC_{50} = 0.23 \pm 0.01 \mu M$ ;  $R^2 = Br$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 41.** Chemical structures of urease inhibitors based on dihydropyrimidines.



Series A: 25 compounds ( $IC_{50} = 5.36 \mu M$  to no activity) [115]

$R_1 = H, CH_3, CH_2C_6H_5$ ;  $R_2 = C_6H_5, 3-Br-C_6H_4, 3-OCH_3-C_6H_4, 3-OH-C_6H_4, 3,4-OCH_3, OH-C_6H_3, 4-OCH_3-C_6H_4, 4-N(CH_3)_2-C_6H_4, 3-thienyl, 4-OH-C_6H_4, 4-Cl-C_6H_4, 3-pyridyl$ ;  $R_3 = CH_3, C_6H_5, OCH_2CH_3$ ;  
 $R_4 = CH_3, OCH_2CH_3, CF_3, C_6H_5$ ;  $X = O, S$

Standard inhibitor: Thiourea ( $IC_{50} = 21.0 \pm 0.1 \mu M$ )

Best inhibitors: **117 (A)** ( $IC_{50} = 5.36 \mu M$ ;  $R_1 = H$ ;  $R_2 = 3-Br-C_6H_4$ ;  $R_3 = OCH_2CH_3$ ;  $R_4 = CH_3$ ;  $X = S$ );

**118 (A)** ( $IC_{50} = 5.4 \mu M$ ;  $R_1 = H$ ;  $R_2 = 3-OCH_3-C_6H_4$ ;  $R_3 = OCH_2CH_3$ ;  $R_4 = CH_3$ ;  $X = S$ )

Series B: 6 compounds ( $K_i = 14.63 \pm 0.02$  to  $29.42 \pm 0.00 \mu M$ ) [114]

Standard inhibitor: Thiourea ( $K_i = 20.01 \pm 0.02 \mu M$ )

$R_1 = H$   
 $R_2 = C_6H_5, 4-CH_3-C_6H_4, 3-OH-C_6H_4, 4-NO_2-C_6H_4$   
 $R_3 = OCH_3, OCH_2CH_3$   
 $R_4 = CH_3$   
 $X = S$

Best inhibitor: **119 (B)** ( $K_i = 14.63 \pm 0.02 \mu M$ ;  $R_2 = 4-NO_2-C_6H_4$ ;  $R_3 = OCH_2CH_3$ ;  $R_4 = Me$ )

\*Values of  $IC_{50}$  and  $K_i$  determined with urease from *Canavalia ensiformis*

**Scheme 42.** Chemical structures of urease inhibitors based on dihydropyrimidine platform.

### Barbituric acid derivatives

Khan et al. synthesized and screened antiurease activity of several barbituric acid derivatives in two different studies evaluating the influence of the group attached to N in the barbituric acid moiety and the substituents at the phenyl ring (Scheme 45) [130,131]. However, the best results were obtained for the series containing the endocyclic NH group. Among these compounds, the addition of substituents at the para position of the phenyl ring increased the inhibitory capacity.

The role played by the substitution of thiobarbituric acid derivatives on the phenyl ring was also verified (Scheme 46) [132]. As shown in the results of the SAR study, substituents that bind the nickel center, such as OH, sulfur atoms or even the pyridyl moiety instead of the phenyl group, increased the inhibitory activity. Additionally, the steric hindrance of large groups of some compounds was probably responsible for decreasing the inhibition of the enzyme.

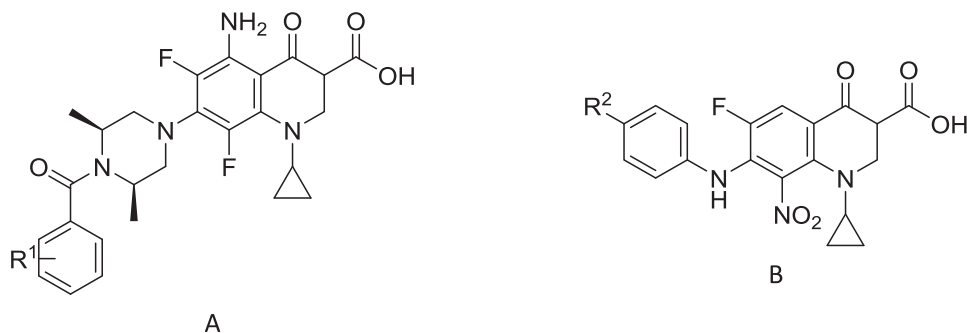
Motivated by broad spectrum of biological and pharmaceutical applications of cyano acetamide derivatives [135–143], in 2015, Qureshi et al. screened several compounds based on this moiety (Scheme 47) to continue determine their urease inhibitory activity [144] and continue previous studies of urease inhibitors [133,134]. According to the authors, the high urease inhibitory activity potentially resulted from the extra interaction of the furan and thiophene ring with the urease nickel center.

The antiurease activities of both barbituric acid and thiobarbituric acid derivatives substituted with aniline [145] and several sulfonamides [146] (Scheme 48) were studied by Rauf et al. In both cases, thiobarbituric acid derivatives showed greater inhibition than their corresponding oxygenated analogues. The SAR studies also revealed an increase in urease inhibition by compounds containing a carboxyl group at the aniline moiety, probably due to hydrogen bonding with the nickel center. The authors use the same explanation to rationalize the finding that SH and OH also showed better results than other substituents (Scheme 48).

Pyrano pyrimidine dione derivatives (Scheme 49) showed high inhibitory values, which were associated with the presence of hydrophobic substituents at the phenyl ring [147]. According to the authors, the reduction of the partial charge on nitrogen atoms in the compounds with a phenyl ring bearing electron-withdrawing groups was responsible for their relatively low inhibitory activity. These conclusions could explain the high inhibitory capacity of compound **132** (Scheme 49).

Barakat et al. screened several bis-barbituric acid derivatives (Scheme 50) for urease inhibition. The best compounds had similar  $IC_{50}$  values to the positive control thiourea [148,149]. According to molecular docking studies, several interactions potentially explain the high activity of compounds **133** and **134** (Scheme 50), such as hydrogen bonding between the carbonyl of the barbituric acid moiety and KCX219 and Arg338; hydrogen bonding between the amine group adjacent to the carbonyl moiety and KCX219,





Series A: 3 compounds ( $IC_{50} = 393.9$  to  $421.76 \mu M$ ) [120]

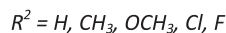


Standard inhibitor: thiourea ( $IC_{50} = 21 \pm 0.011 \mu M$ )

Best inhibitor: **120 (A)** ( $IC_{50} = 393.9 \pm 0.99 \mu M$ ;  $R^1 = OH$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

Series B: 5 compounds ( $IC_{50} = NA$  to  $151 \mu M$ ) [121]

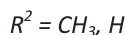
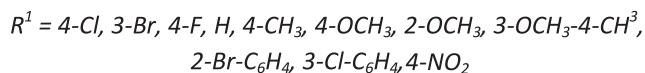
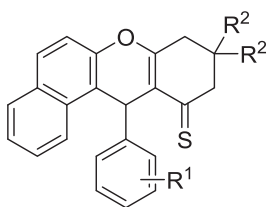


Standard inhibitor: acetohydroxamic acid ( $IC_{50} = 27 \mu M$ )

Best inhibitor: **121 (B)** ( $IC_{50} = 151 \mu M$ ;  $R^2 = OCH_3$ )

\*Values of  $IC_{50}$  determined with urease from *Helicobacter pylori*

**Scheme 43.** Chemical structures of quinolones described as urease inhibitors.



Series: 14 compounds (up to 47% inhibition after 9h)

Best inhibitor: **122** (47% inhibition after 9h;  $R^1 = H$ ;  $R^2 = H$ )

**Scheme 44.** Chemical structures of xanthenes analogues that possesses urease inhibitory properties.

Ala169, Gly279 and Asp362; hydrophilic interactions between the aldehyde group of A2 and His323. Moreover, all compounds studied showed similar conformations at the active site of the urease enzyme, interacting with the nickel center and amino acid residues. In addition to the substituted phenyl ring derivatives, the authors also synthesized naphthyl substituents, which exhibited  $IC_{50}$  values ranging from  $22.7 \pm 0.20$  to  $123.2 \pm 0.37 \mu M$  [148,149].

Rahim et al. reported the synthesis and the results of urease inhibition assays for bis-thiobarbituric derivatives (Scheme 51) [150]. The most effective compounds possessed a substituted phenyl ring, whereas indole and naphthyl derivatives, among others, showed moderate activity against urease. SAR studies revealed that electron-donating groups and substituents capable of making hydrogen bonds, such as OH, exhibited better  $IC_{50}$  values.

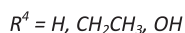
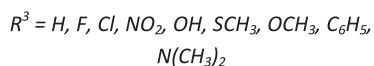
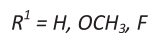
#### Organophosphorus compounds

Phosphoramidates represent one of the most active classes of compounds that function as urease inhibitors [151]. This class of compounds also presents other biological activities, such as anti-malarial activity [152], insecticide activity [153,154], antiHCV activity [155], antiHIV activity [156,157], and inhibitors of reverse transcriptase [158] and hepatitis C virus [159]. Due the clinical relevance of this class of compounds, Oliveira and co-workers [160] synthesized 25 new phosphoramidates (Scheme 52) and screened their inhibitory activity towards urease isolated from Jack bean. The most active compounds (138 to 142; Scheme 52) showed the properties expected for drug-like compounds, qualifying them to have good pharmacokinetics and drug bioavailability. Considering the correlation between electronic properties and biological activity [161,162], a correlation between the urease inhibitory activity of positive controls hydroxyurea/thiourea and compounds 139, 140 and 142 was predicted.

Dominguez and co-workers performed molecular modeling studies based on data obtained from the structures of the



Series A: 15 compounds ( $IC_{50} = 13.0 \mu\text{M}$  to no activity) [131]

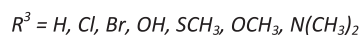
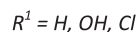


Standard inhibitor: Thiourea ( $21.1 \pm 0.3 \mu\text{M}$ )

Best inhibitor: **123 (A)** ( $IC_{50} = 13.0 \pm 1.2 \mu\text{M}$ ;  $R^1 = R^2 = R^4 = H$ ;  $R^3 = F$ )

\*Values of  $IC_{50}$  determined with urease from *Bacillus pasteurii*

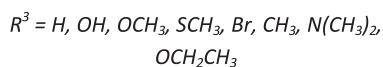
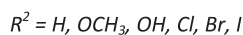
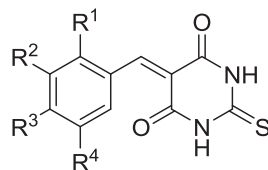
Series B: 23 compounds ( $IC_{50} = 91.83 \mu\text{M}$  to no activity) [130]



Standard inhibitor: Thiourea ( $IC_{50} = 21 \pm 0.11 \mu\text{M}$ )

Best inhibitor: **124 (B)** ( $IC_{50} = 91.83 \pm 0.59 \mu\text{M}$ ;  $R^1 = R^4 = H$ ;  $R^2 = R^3 = OH$ )

**Scheme 45.** Chemical structures of urease inhibitors based on barbituric acid moiety.



Standard inhibitor: Thiourea ( $IC_{50} = 21 \pm 0.11 \mu\text{M}$ )

Series: 19 compounds ( $IC_{50} = 1.61$  to  $58.5 \mu\text{M}$ )

Best inhibitors: **125** ( $IC_{50} = 1.61 \pm 0.18 \mu\text{M}$ ;  $R^1 = R^4 = H$ ;  $R^2 = R^3 = OH$ ); **126** ( $IC_{50} = 6.7 \pm 0.27 \mu\text{M}$ ;  $R^1 = R^2 = R^4 = H$ ;  $R^3 = CH_3$ )

\*Values of  $IC_{50}$  determined with urease from *Bacillus pasteurii*

**Scheme 46.** Chemical structures of urease inhibitors based on thiobarbituric acid moiety.

inhibitor-*Bacillus pasteurii* urease complexes to design and synthesize 40 phosphor(di)amide derivatives (Scheme 53). The data obtained from the docking study allowed the authors to propose a model to explain the interactions between the studied compounds and the active site of the enzyme. The most active compounds (**143** to **148**; Scheme 53) present a flat aromatic or heteromatic ring atomic structure containing substituents ( $NO_2$ ) that are able to establish hydrogen bonds with free  $NH_2$  terminal

groups or similar groups present in surrounding residues in the active site of the enzyme. Moreover, phosphordiamides have a greater inhibitory activity than phosphoramides. Steric hindrance, as observed for *N*-alkyl-substituted diamidophosphate derivatives, reduces the ureolytic activity once the inhibitor interacts with Ni atoms in the active site [163,164].

The application of phosphordiamidates as urease inhibitors is restricted due to their susceptibility to hydrolysis. In an attempt



$R^1 = 3,4\text{-OCH}_3\text{-C}_6\text{H}_3, 3,4,5\text{-OCH}_3\text{-C}_6\text{H}_2, 3\text{-OCH}_3\text{-}4\text{-OH-C}_6\text{H}_3, \text{furanlyl, thienyl}$

$R^2 = \text{H, CH}_3$

$X = \text{O, S}$

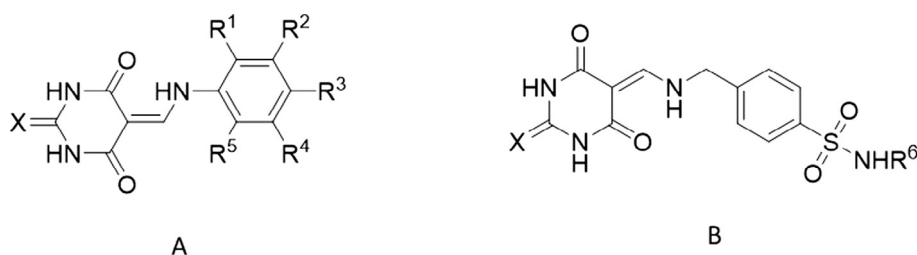
Standard inhibitor: thiourea ( $IC_{50} = 27.5 \mu\text{g/mL}$ )

Series A: 15 compounds ( $IC_{50} = 17.34 \mu\text{g/mL}$  to no activity)

Series B: 5 compounds ( $IC_{50} = \text{no activity}$ )

Best inhibitors: **127 (A)** ( $IC_{50} = 17.34 \pm 0.01 \mu\text{g/mL}$ ;  $R^1 = \text{furanlyl}$ ;  $R^2 = \text{CH}_3$ ;  $X = \text{O}$ ); **128 (A)** ( $IC_{50} = 36.75 \pm 0.05 \mu\text{g/mL}$ ;  $R^1 = \text{thienyl}$ ;  $R^2 = \text{H}$ ;  $X = \text{S}$ )

**Scheme 47.** Chemical structures of cyano acetamide barbituric-like substances that shown antiurease properties.



Series A: 36 compounds ( $IC_{50} = 8.53 \mu\text{M}$  to ND) (2015)

$X = \text{O, S}$

$R^1 = \text{H, COOH, CF}_3, \text{Cl, OH, SH}$

$R^2 = \text{H, COOH, OH, CF}_3, \text{Cl}$

$R^3 = \text{H, COOH, OH, NO}_2, \text{CF}_3, \text{Cl, I}$

$R^4 = \text{H, NO}_2, \text{Cl}$

$R^5 = \text{H, OH, Cl}$

Standard inhibitor: Thiourea ( $IC_{50} = 21 \pm 0.011 \mu\text{M}$ )

Best inhibitors: **129 (A)** ( $IC_{50} = 8.53 \pm 0.027 \mu\text{M}$ ;  $X = \text{S}$ ;  $R^1 = \text{COOH}$ ;  $R^2 = R^3 = R^5 = \text{H}$ ;  $R^4 = \text{NO}_2$ ); **130 (A)** ( $IC_{50} = 8.93 \pm 0.027 \mu\text{M}$ ;  $X = \text{S}$ ;  $R^1 = R^2 = R^4 = R^5 = \text{H}$ ;  $R^3 = \text{COOH}$ )

Series B: 14 compounds ( $IC_{50} = 3.76 \mu\text{M}$  to ND) (2011)

$X = \text{O, S}$

$R^6 = \text{H, CNHNH}_2, 4,6\text{-(CH}_3)_2\text{-pyrimidyl, 4-CH}_3\text{-lisoxazolyl, thiazolyl}$

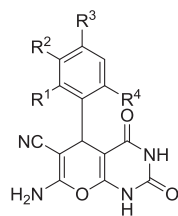
Standard inhibitor: Thiourea ( $IC_{50} = 21 \pm 0.11 \mu\text{M}$ )

Best inhibitor: **131 (B)** ( $IC_{50} = 3.76 \pm 0.027 \mu\text{M}$ ;  $X = \text{S}$ ;  $R^1 = \text{H}$ )

**Scheme 48.** Chemical structures of urease inhibitors based on (thio)barbituric aniline-substituted derivatives.

to mitigate this problem, Berlicki and co-workers [165–168] adopted a structurally related analogue to phosphorodiamidic acid as a scaffold to design and synthesize phosphonic and phosphinic acid derivatives and evaluate their functions as urease inhibitors

(Scheme 54). These classes of compounds are potent inhibitors of enzymatic hydrolysis of the amide bond [169,170]. Since these compounds do not contain the unstable P-N bonds (phosphoramides), they are expected to be stable under physiological



Standard inhibitor: Thiourea ( $IC_{50} = 21.0 \mu M$ )

$R^1 = H, Cl$

Series A: 12 compounds ( $IC_{50} = 19.45$  to  $279.14 \mu M$ )

$R^2 = H, NO_2, CH_3, OCH_3$

$R^3 = H, Cl, Br, CH_3, OCH_3, NO_2, OH$

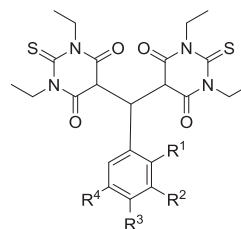
Best inhibitor = **132** ( $IC_{50} = 19.45 \mu M$ ;  
 $R^1 = R^3 = Cl$ ;  $R^2 = R^4 = H$ )

$R^4 = H, Cl$

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 49.** Chemical structures of urease inhibitors based on pyrano pyrimidine platforms.

conditions. Moreover, this class of compounds exhibits similar inhibitory properties to phosphoramides, as the computed free energy of binding is very similar [171,172]. Most of the studied compounds constitute a group of competitive, reversible inhibitors of bacterial ureases [151]. Higher inhibitory efficiency in controlling urease activity was observed for P-methyl thiophosphinic acids than for P-methyl phosphinic acids, most probably due to the stronger interaction of a sulfur atom with nickel ions in the



Standard inhibitor: Thiourea ( $IC_{50} = 21.10 \pm 0.012 \mu M$ )

$R^1 = H, OH$

$R^2 = H, OCH_3, OH, C(CH_3)_3$

$R^3 = H, OH, OCH_3, OCH_2C_6H_5, N(CH_3)_2$

$R^4 = H, OCH_3, C(CH_3)_3$

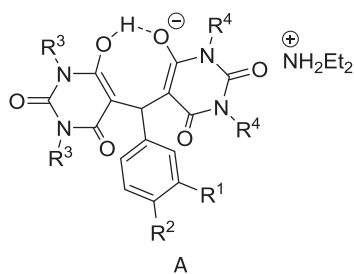
Series: 10 compounds ( $IC_{50} = 7.45$  to  $74.24 \mu M$ )

Best inhibitors: **135** ( $IC_{50} = 7.45 \pm 0.12 \mu M$ ;  
 $R^1 = R^3 = H$ ;  $R^2 = R^4 = OCH_3$ ); **136** ( $IC_{50} = 8.61 \pm 0.45 \mu M$ ;  $R^1 = R^2 = R^4 = H$ ;  $R^3 = N(CH_3)_2$ ); **137** ( $IC_{50} = 18.17 \pm 1.03 \mu M$ ;  $R^1 = R^3 = R^4 = H$ ;  
 $R^2 = OH$ )

\*Values of  $IC_{50}$  determined with urease from *Bacillus pasteurii*

**Scheme 51.** Chemical structures of urease inhibitors based on bis-thiobarbituric acid derivatives.

enzyme active site [173]. The  $IC_{50}$  and  $K_i$  values decreased to the micromolar range with the structural extension of the N-terminal group in compound **148** (Scheme 54). Compared to the compound **148**-urease complex, computer-aided studies of the compound **149**-urease complex have shown that hydrophobic bulky substituents docked well in the enzyme and two additional hydrogen

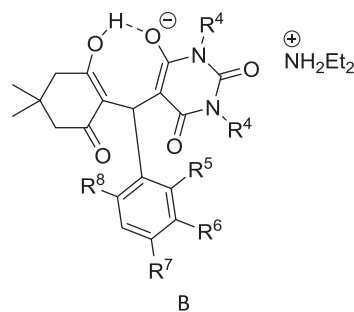


$R^1 = H, CH_3, Br$

$R^2 = H, CH_3, OH, OCH_3, CHO, NO_2, Cl$

$R^3 = H, CH_3$

$R^4 = H, CH_3$



$R^5 = H, NO_2, Cl$

$R^6 = H, Br, OH$

$R^7 = H, CH_3, OCH_3, Cl, Br, N(CH_3)_2, OH, CHO$

$R^8 = H, Cl$

Standard inhibitor: Thiourea ( $IC_{50} = 21.2 \pm 1.3 \mu M$ )

Series A: 14 compounds ( $IC_{50} = 17.2$  to  $54.2 \mu M$ )

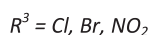
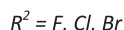
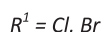
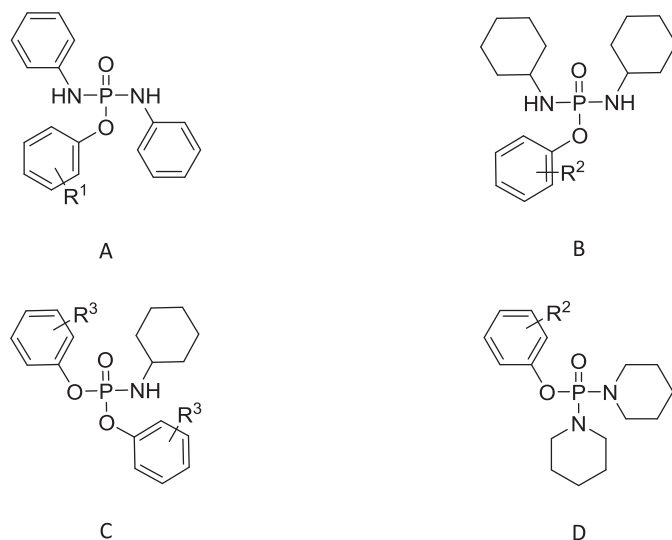
Series B: 19 compounds ( $IC_{50} = 23.7$  to  $142.1 \mu M$ )

Best inhibitors: **133 (A)** ( $IC_{50} = 17.6 \pm 0.23 \mu M$ ;  $R^1 = R^3 = R^4 = H$ ;  $R^2 = CH_3$ );

**134 (A)** ( $IC_{50} = 17.2 \pm 0.44 \mu M$ ;  $R^1 = R^4 = H$ ;  $R^2 = CHO$ ;  $R^3 = CH_3$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 50.** Chemical structures of urease inhibitors based on bis-barbituric acid derivatives.



Standard inhibitors: Hydroxyurea (80.02 ± 6.03% of inhibition)/ Thiourea (22.62 ± 13.78% of inhibition)

Series A: 2 compounds (up to 50.25% of inhibition)

Series B: 4 compounds (up to 36.00% of inhibition)

Series C: 4 compounds (up to 72.05% of inhibition)

Series D: 4 compounds (up to 62.05% of inhibition)

Best inhibitors: **138 (A)** (29.00 ± 7.30% inhibition;  $R^1 = 4\text{-Cl}$ ); **139 (B)** (36.00 ± 4.80% inhibition;  $R^2 = 3\text{-Cl}$ ); **140 (C)** (69.42 ± 4.0% inhibition;  $R^3 = 4\text{-Cl}$ ); **141 (C)** (72.05 ± 6.15% inhibition;  $R^3 = 4\text{-Br}$ ); **142 (D)** (62.05 ± 2.27% inhibition;  $R^2 = 4\text{-Cl}$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 52.** Chemical structures of urease inhibitors based on phosphoramidates.

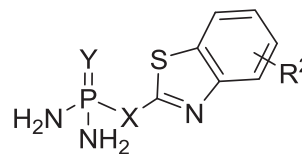
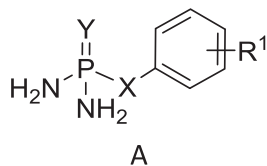
bonds were formed (carbonyl oxygen atom-Arg339 and NH of the amide group-Ala366) [151]. In a subsequent work [166], the authors proposed a mode of binding between compound **151** ( $K_i = 0.62 \mu\text{M}$ ) and the *B. pasteurii* enzyme in which the phosphinic acid group forms two hydrogen bonds with the enzyme active residues (His222 and Asp363) and interacts with the two nickel ions. Furthermore, one hydrogen bond is broken between bulk water and its amine group to form one hydrogen bond with the carbonyl moiety (Ala366). In the compound **148**-urease complex, the energy balance is less favorable during the complexation process because three hydrogen bonds with water molecules must be broken to form one hydrogen bond with its amine group. After synthesizing and evaluating the substituted aminomethane-*P*-hydroxymethylphosphinic acids (Scheme 54), the authors observed that approximately half of the studied compounds did not show inhibitory activity against *B. pasteurii* urease. Mono *N*-substituted derivatives were more active than their *N,N*-disubstituted counterparts [167]. On the other hand, the majority of the *bis*(aminomethyl)-phosphinic acid derivatives showed significant inhibitory potency in a low micromolar range.

Based on the structure of the complex between the *Sporosarcina pasteurii* urease enzyme and citrate [174], Ntatsopoulos and co-workers synthesized a group of organophosphorus derivatives containing a phosphonate/carboxylate system (Scheme 55) and evaluated their inhibitory effect on *S. pasteurii* urease [175]. In con-

trast to citrate, the authors postulated that both phosphonic acidic groups are fixed on a hydrophobic, partially rigid core that mimic the 1,2-dicarboxylate portion of citrate in its affinity for urease. According to the computer-based studies, the *p*-methyl group of the aromatic ring of the most active inhibitor (compound **154**; Scheme 55) of urease is conveniently accommodated in the hydrophobic cleft of the urease active site. The polar region of compound **154** is involved in strong interactions in the enzyme active site once the carboxylate group forms two hydrogen bonds mediated by a salt bridge in a region opposite to the guanidine moiety of Arg339. Furthermore, the oxygen atom of the phosphoryl group forms a bridge between both metallic centers and interacts with a nitrogen atom of the imidazole group (His222) through a hydrogen bond. The authors hypothesized that the  $\alpha,\beta$ -unsaturated system formed between the free carboxylate group and the aromatic ring present in compound **154** presents the size and rigidity that comply with the steric and electronic demands of the enzyme active site. Thus, both the loss of compound rigidity and separation of the acidic groups from three to four bonds in distance decreases the  $K_i$  value approximately 150- and 110-fold, respectively.

#### Miscellaneous

Derivatives of 1,5-benzothiazepines (Scheme 56) were synthesized, screened for urease inhibitory activity and examined using



$R^1 = H, 4\text{-NO}_2, 3,5\text{-CH}_3, 2\text{-NO}_2\text{-4-CH}_3, 3\text{-OCH}_3, 4\text{-cyclohexyl}, 2,5\text{-OCH}_3, 4\text{-OC}_6\text{H}_5, 2\text{-NO}_2, 2\text{-CF}_3, 2\text{-OCF}_3, 2\text{-OCH}_3\text{-4-NO}_2$

$R^2 = H, 6\text{-OCH}_2\text{CH}_3, 6\text{-F}, 4\text{-OCH}_3, \text{CH}_3$

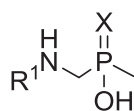
$X = O, \text{NH}$

$Y = O, S$

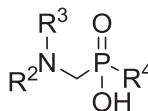
Best inhibitors: **143 (B)** ( $IC_{50} = 2 \text{ nM}$ ;  $R^2 = H$ ;  $X = \text{NH}$ ;  $Y = O$ ); **144 (A)** ( $IC_{50} = 3 \text{ nM}$ ;  $R^1 = 2\text{-NO}_2$ ;  $X = \text{NH}$ ;  $Y = O$ ); **145 (A)** ( $IC_{50} = 3.5 \text{ nM}$ ;  $R^1 = 2\text{-NO}_2\text{-4-CH}_3$ ;  $X = \text{NH}$ ;  $Y = O$ ); **146 (B)** ( $IC_{50} = 5 \text{ nM}$ ;  $R^1 = 6\text{-OCH}_2\text{CH}_3$ ;  $X = \text{NH}$ ;  $Y = O$ ); **147 (B)** ( $IC_{50} = 5 \text{ nM}$ ;  $R^2 = 6\text{-F}$ ;  $X = \text{NH}$ ;  $Y = O$ ); **148 (B)** ( $IC_{50} = 10 \text{ nM}$ ;  $R^1 = 4\text{-OCH}_3$ ;  $X = \text{NH}$ ;  $Y = O$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

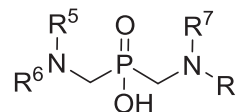
**Scheme 53.** Chemical structures of phosphor(di)amides-based urease inhibitors.



A



B



C

$R^1 = H, \text{CH}_3, \text{C(O)CH}_2\text{NH}_2, \text{C(O)OCH}_2\text{C}_6\text{H}_5, \text{C(O)CH}_2\text{NHCOOCH}_2\text{C}_6\text{H}_5, \text{C(O)CH(NH}_2\text{)CH}_2\text{OH}, \text{C(O)CH(NH}_2\text{)CH}_2\text{OCH}_2\text{C}_6\text{H}_5, \text{C(O)CH(NH}_2\text{)CH}_2\text{C}_6\text{H}_5$

$X = O, S$

Series A: 10 compounds; ( $IC_{50}$  *Bacillus pasteurii* =  $1.8 \mu\text{M}$  to NA); ( $IC_{50}$  *Proteus vulgaris* =  $3.1 \mu\text{M}$  to no activity)

Best inhibitors: **148 (A)** ( $IC_{50}$   $1.8 \pm 0.1$  and  $3.1 \pm 0.3 \mu\text{M}$ ;  $R^1 = \text{C(O)CH}_2\text{NHC(O)OCH}_2\text{C}_6\text{H}_5$ ;  $X = S$ ); **149 (A)** ( $IC_{50}$   $112 \pm 0.6$  and  $158 \pm 1.3 \mu\text{M}$ );  $R^1 = \text{C(O)OCH}_2\text{C}_6\text{H}_5$ ;  $X = S$ )

$R^2 = H, \text{CH}_3$

$R^3 = H, \text{CH}_3, \text{COCH}_3, \text{CH}_2\text{C}_6\text{H}_5$

$R^4 = \text{OH}, \text{CH}_3$

Series B: 10 compounds; ( $IC_{50}$

*B. pasteurii* =  $3.8 \mu\text{M}$  to no activity)

Best inhibitors: **150 (B)** ( $IC_{50}$   $3.8 \pm 0.4 \mu\text{M}$ ;  $R^2 = R^3 = R^4 = \text{CH}_3$ ); **151 (B)** ( $IC_{50}$   $49 \pm 1.7 \mu\text{M}$ ;  $R^2 = R^3 = \text{CH}_3$ ;  $R^4 = \text{OH}$ ); **152 (B)** ( $IC_{50}$   $60 \pm 0.3 \mu\text{M}$ ;  $R_2 = H$ ;  $R^3 = R^4 = \text{CH}_3$ )

$R^5 = H, \text{CH}_3$

$R^6 = H, \text{CH}_3, \text{CH}_2\text{C}_6\text{H}_5$

$R^7 = H, \text{CH}_3, \text{C}_2\text{H}_5, [\text{CH}_2]_5$

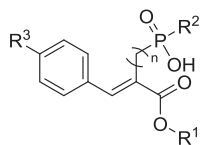
$R^8 = H, \text{CH}_3, \text{C}_2\text{H}_5, [\text{CH}_2]_{(2-7)}\text{CH}_3, \text{C}_6\text{H}_{11}, \text{CH}_2\text{C}_6\text{H}_5$ ;

Standard inhibitor: Acetohydroxamic acid,  $K_i = (\text{Sporosarcina pasteurii}, 3.3 \pm 0.4 \mu\text{M})$ ; (*Proteus mirabilis*,  $57 \pm 0.4 \mu\text{M}$ )

Series C: 16 compounds  $K_i = (\text{S. pasteurii}, 0.108 \mu\text{M}$  to no activity); (*P. mirabilis*,  $0.202 \mu\text{M}$  to NA)

Best inhibitor: **153 (C)** ( $K_i$  *S. pasteurii* =  $0.108 \pm 0.0116 \mu\text{M}$  and  $K_i$  *P. mirabilis* =  $0.202 \pm 0.057 \mu\text{M}$ ;  $R^5 = R^6 = R^7 = H$ ;  $R^8 = [\text{CH}_2]_5\text{CH}_3$ )

**Scheme 54.** Chemical structures of urease inhibitors based on phosphorodiamidic acid.



$$R^1 = H, CH_3$$

$$R^2 = H, OH, CH_3, CH_2OH, CH_2NH_2$$

$$R^3 = H, CH_3, F, Cl, NHC(O)CH_3, OCH_3$$

$$n = 1, 2.$$

Series: 31 compounds ( $K_i = 0.594 \mu M$  to no activity)

Best inhibitors: **154** ( $K_i = 0.594 \pm 0.028 \mu M$ ;  $R^1 = H$ ;  $R^2 = OH$ ;  $R^3 = CH_3$ ;  $n = 1$ );

**155** ( $K_i = 3.05 \pm 0.16 \mu M$ ;  $R^1 = CH_3$ ;  $R^2 = OH$ ;  $R^3 = H$ ;  $n = 2$ );

**156** ( $K_i = 13.1 \pm 2.3 \mu M$ ;  $R^1 = R^3 = H$ ;  $R^2 = OH$ ;  $n = 1$ );

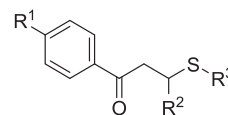
**157** ( $K_i = 66.7 \pm 6.6 \mu M$ ;  $R^1 = R^3 = H$ ;  $R^2 = OH$ ;  $n = 2$ )

\*Values of  $K_i$  determined with urease from *Bacillus pasteurii*

**Scheme 55.** Chemical structures of organophosphorus urease inhibitors containing a phosphonate/carboxylate scaffold.

molecular docking studies [176]. Based on the  $IC_{50}$  values and the results of molecular docking studies, the authors concluded that strong interactions with the active center of urease, such as coordination of the 2-hydroxyl group with the nickel atoms, hydrogen bonds between the same group and His139 and Ala170, and hydrophobic contacts with other amino acids residues, were the main contributors to the increased inhibitory activity of compound **158** (Scheme 56).

Due to the various biological activities of chalcone derivatives [177–185], Ahari-Mostafavi and co-workers synthesized several  $\beta$ -aryl- $\beta$ -mercapto ketone derivatives (Scheme 57) and evaluated their inhibitory potential against urease [186]. Compound **160** (Scheme 57) inhibited the enzyme with the greatest efficiency, with an  $IC_{50} = 6 \mu M$  (the  $IC_{50}$  of the standard hydroxyurea =  $100 \mu M$ ). The improved antiurease activity of the compound was



$$R^1 = H, OH, 4-OCH_3-C_6H_4, CH_3$$

$$R^2 = H, 4-CH_3-C_6H_4, 4-OCH_3-C_6H_4, 2-NO_2-C_6H_4, 4-Cl-C_6H_4, C_6H_5, 4-CN-C_6H_4, 4-F-C_6H_4$$

$$R^3 = 4-Cl-C_6H_4, 2-NH_3-C_6H_4, 4-NH_3-C_6H_4, C_6H_5, furan, CH_3-furan, CH_2C_6H_5, naphthyl, (CH_2)_7CH_3$$

Standard inhibitor: Hydroxyurea ( $IC_{50} = 100 \mu M$ )

Series: 22 compounds ( $IC_{50} = 6$  to  $181 \mu M$ )

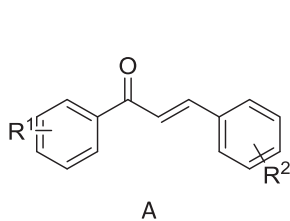
Best inhibitor: **160** ( $IC_{50} = 6 \mu M$ ;  $R^1 = H$ ;  $R^2 = 2-NO_2-C_6H_4$ ;  $R^3 = 2-NH_3-C_6H_4$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

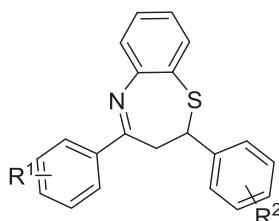
**Scheme 57.** Chemical structures of urease inhibitors based on  $\beta$ -aryl- $\beta$ -mercapto ketone derivatives.

associated with  $\pi - \pi$  interactions between the sulfur atom in Met366 residue and phenyl ring at  $R^2$ , closer interactions with His 274 and 138 residues by changing Cl to F at  $R_2$ , hydrogen bonding between the  $NH_2$  group and Asn 168, the presence of electron-donating and -withdrawing substituents on rings at  $R^2$  and  $R^3$ , respectively, and the presence of an electron-donating substituent in  $R^1$ .

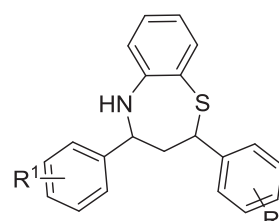
Batool et al. reported the antibacterial, antiurease and NO scavenging activities of a small series of substituted (prop-2-ynoxy) benzene derivatives (Scheme 58) [187]. According to the results from the urease inhibitory assay and SAR studies, compounds with electron-donating groups carried by the phenyl ring exhibited increased activity, which was probably caused by the stronger interaction between the ring and the active site of the enzyme. Since strong binding to the nickel center is important for urease inhibition, the higher chelation properties might explain increased activity of phenolic compounds.



A



B



C

$$R^1 = OH, H$$

$$R^2 = OCH_2O, H, CH_3, NO_2, F$$

Standard inhibitor: Thiourea ( $IC_{50} = 21.10 \pm 0.310 \mu M$ )

Series A: 1 compound – **158 (A)** ( $IC_{50} = 32.51 \pm 0.045 \mu M$ )

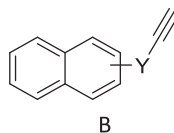
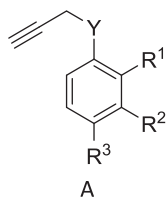
Series B: 4 compounds ( $IC_{50} = 19.07$  to  $36.71 \mu M$ )

Series C: 4 compounds ( $IC_{50} = 91.21 \mu M$  to NA)

Best inhibitor: **159 (B)** ( $IC_{50} = 19.07 \pm 0.045 \mu M$ ;  $R^1 = 2-OH$ ;  $R^2 = 3,4-OCH_2O$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 56.** Chemical structures of urease inhibitors bearing a 1,5-benzothiazepine moiety.



Standard inhibitor: Thiourea ( $IC_{50} = 23.3 \mu\text{g/mL}$ )

$R^1 = \text{H, CH}_3, \text{Cl, Br}$

Series A: 10 compounds ( $IC_{50} = 60.2 \mu\text{g/mL}$  to no activity)

$R^2 = \text{H, CH}_3$

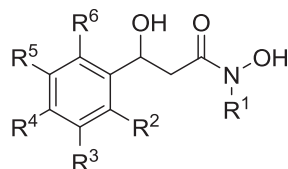
Series B: 3 compounds ( $IC_{50} = 68 \mu\text{g/mL}$  to no activity)

$R^3 = \text{H, CH}_3, \text{F, Cl, Br, NO}_2$

$Y = \text{NH, O}$

Best inhibitor: **161 (A)** ( $IC_{50} = 60.2 \mu\text{g/mL}$ ;  $R^1 = \text{Cl}$ ;  $R^2 = \text{H}$ ;  $R^3 = \text{Br}$ ;  $Y = \text{O}$ )

**Scheme 58.** Chemical structures of urease inhibitors based on substituted (prop-2-ynoxy)benzene derivatives.



$R^1 = \text{CH}_3, \text{H}$

$R^4 = \text{H, OCH}_3, \text{OCH}_2\text{C}_6\text{H}_5, \text{F, Cl, Br, CF}_3, \text{N}(\text{CH}_3)_2$

$R^2 = \text{H, OCH}_3, \text{OH, F, Cl}$

$R^5 = \text{H, F, Cl}$

$R^3 = \text{H, OH, OCH}_3, \text{OCH}_2\text{C}_6\text{H}_5, \text{F, Cl, Br, CF}_3$

$R^6 = \text{H, OH, OCH}_2\text{C}_6\text{H}_5, \text{Cl}$

Standard inhibitor: acetohydroxamic acid ( $IC_{50} = 27.6 \pm 2.5$  and  $23.8 \pm 1.5 \mu\text{M}$ )

Series: 44 compounds ( $IC_{50} = 0.083$  to  $405 \mu\text{M}$ )

Best inhibitors: **162** ( $K_i = 0.014 \pm 0.003 \mu\text{M}$ ;  $R^1 = \text{H}$ ;  $R^3 = \text{Cl}$ ;  $R^2 = R^4 = R^5 = R^6 = \text{H}$ );

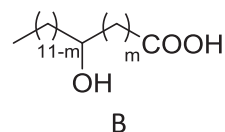
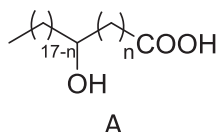
**163** ( $K_i = 0.045 \pm 0.007 \mu\text{M}$ ;  $R^1 = \text{H}$ ;  $R^2 = R^3 = \text{Cl}$ ;  $R^4 = R^5 = R^6 = \text{H}$ );

**164** ( $K_i = 1.11 \mu\text{M}$ ;  $R^1 = \text{H}$ ;  $R^2 = R^4 = R^5 = \text{H}$ ;  $R^3 = \text{Cl}$ ;  $R^6 = \text{OH}$ );

**165** ( $K_i = 0.13 \mu\text{M}$ ;  $R^1 = \text{H}$ ;  $R^2 = R^4 = R^5 = \text{H}$ ;  $R^3 = \text{Cl}$ ;  $R^6 = \text{OCH}_2\text{C}_6\text{H}_5$ )

\*Values of  $IC_{50}$  and  $K_i$  determined with urease from *Helicobacter pylori*

**Scheme 59.** Chemical structures of urease inhibitors design from hydroxamic acids.



Series A: 10 compounds ( $IC_{50} = 0.012$  to  $50.9 \text{ ng/mL}$ ) [**190**]

Series B: 5 compounds ( $IC_{50} = 0.07$  to  $0.101 \mu\text{g/mL}$ ) [**191**]

$n = 1, 2, 4, 5, 6, 7, 8, 9, 10, 11$

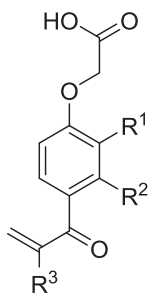
$m = 1, 4, 5, 7, 10$

Best inhibitor: **166 (A)** ( $IC_{50} = 0.012 \pm 0.0092 \text{ ng/mL}$ ;  $n = 6$ )

Best inhibitors: **167 (B)** ( $IC_{50} = 0.07 \pm 0.001 \mu\text{g/mL}$ ;  $m = 5$ ); **168 (B)** ( $IC_{50} = 0.083 \pm 0.002 \mu\text{g/mL}$ ;  $m = 7$ )

**Scheme 60.** Chemical structures of urease inhibitors based on hydroxyl fatty acids.





Standard inhibitors: hydroxyurea ( $IC_{50} = 0.22$  mM)  
and acetohydroxamic acid ( $IC_{50} = 0.05$  mM)

$R^1 = H, Cl, OCH_3$  Series: 8 compounds ( $IC_{50} = 0.05$  mM  
to no activity)

$R^2 = H, Cl, OCH_3$

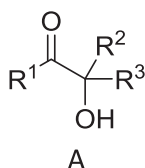
Best inhibitor: **169** ( $IC_{50} = 0.05$  mM;  
 $R^1 = OCH_3$ ;  $R^2 = H$ ;  $R^3 = CH_3$ )

\*Values of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 61.** Chemical structures of  $\alpha,\beta$ -unsaturated ketones which showed antiurease activity.

Because of the well-known chelating properties and antiurease potential of hydroxamic acids, in 2013 and 2016, Xiao et al. and Shi et al., respectively, synthesized a large series of derivatives with different groups at positions 2, 3 and 4 of the benzene ring (Scheme 59). Both studies reported remarkable results for urease inhibitory activity, including very low  $IC_{50}$  values for some of the tested title compounds [188,189]. As shown in the SAR studies, the hydrophobic behavior of the substituent is important to increase the inhibitory activity, although bulky hydrophobic groups reduce the potency.

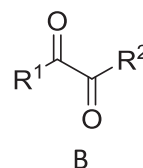
Onar and collaborators evaluated the influence of the position of the hydroxyl group in a series of 20C chain fatty acids on the urease inhibitory activity (Scheme 60) [190,191]. The addition of a hydroxyl group in the middle region yielded better results,



$R^1 = CH_3, C_6H_5, 2\text{-furyl}, 2\text{-thenyl}, C_5H_{11},$   
 $(CH_2)_3NHC(O)C_6H_5, (CH_2)_4NHC(O)C_6H_5$

$R^2 = H, CH_3, CF_3, C_6H_5, OH, OCH_3,$   
 $2\text{-furyl}, 2\text{-thenyl}$

$R^3 = H, CH_3$



Standard inhibitor: hydroxyurea ( $IC_{50} = 0.10$  mM);  
acetohydroxamic acid ( $IC_{50} = 0.005$  mM)

Series A: 13 compounds ( $IC_{50} = 0.18$  mM to no activity)

Series B: 7 compounds (no activity)

Best inhibitor: **170 (A)** ( $IC_{50} = 0.18$  mM;  
 $R^1 = 2\text{-thenyl}$ ;  $R^2 = 2\text{-thenyl}$ ;  $R^3 = H$ )

\*Values of  $IC_{50}$  determined with urease from  
*Canavalia ensiformis*

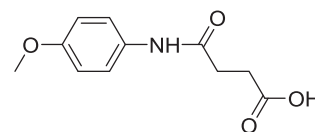
**Scheme 62.** Chemical structures of urease inhibitors based on  $\alpha$ -hydroxyketones.

consistent with the findings reported by Sokmen, who varied the position of the hydroxyl group in a 14C chain.

Since  $\alpha$ - $\beta$ -unsaturated ketones were reported to inhibit *C. ensiformis* urease activity [192] and ethacrynic acid exhibits other pharmacological properties [193,194], Janser et al. synthesized hybrid analogues (Scheme 61) and screened their abilities to inhibit urease [195]. Based on the results of these studies, the authors concluded that the  $\alpha$ - $\beta$ -unsaturated carbonyl unit is the major substituent responsible for the inhibitory activity, possibly because of the interaction between the cysteine sulfhydryl moiety and unsaturated carbonyl  $\beta$ -carbon unit; electron-donators attached to the aromatic system were also responsible for increasing the inhibitory activity.

Due to the structural relationship between  $\alpha$ -hydroxyketones, acetohydroxamic acid and hydroxyurea, in 2004, Tanaka and co-workers synthesized several potentially novel urease inhibitors based on  $\alpha$ -hydroxyketones (Scheme 62) [196]. SAR studies revealed that the hydrophobic character of the R substituent in the  $RCOCH_2OH$  moiety may play an important role in the inhibitory activity. The interaction between  $\alpha$ -hydroxyketone and the sulfhydryl group from cysteine, for example, also seems to contribute to urease inhibition.

N-[(4-Methoxyphenyl)]-4-oxo-4-[oxy]butanamide (compound **171**, Scheme 63) showed urease inhibitory activity ( $IC_{50}$ ) of  $4.08 \pm 2.54$   $\mu$ M, a value that was much higher than the standard thiourea [197].



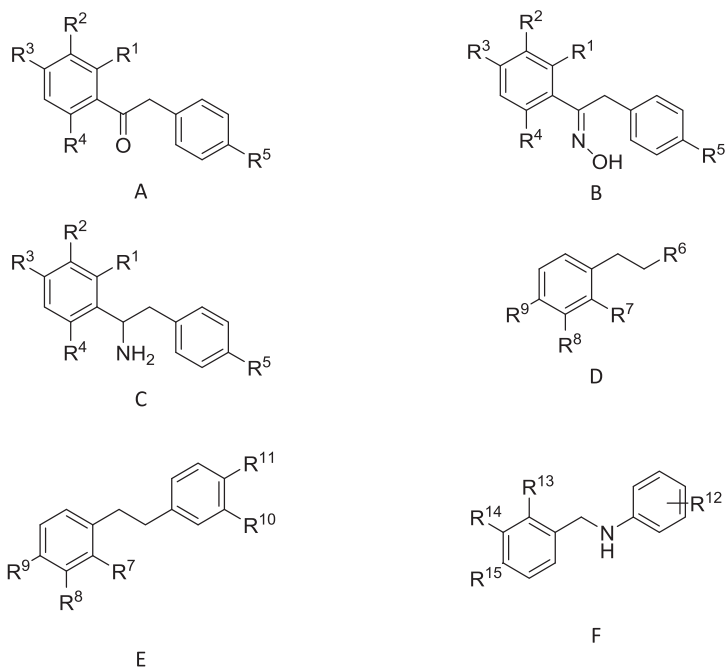
**171**

Standard inhibitor: thiourea ( $IC_{50} = 22.3 \pm 1.2$   $\mu$ M)

$IC_{50} = 4.08 \pm 2.54$   $\mu$ M

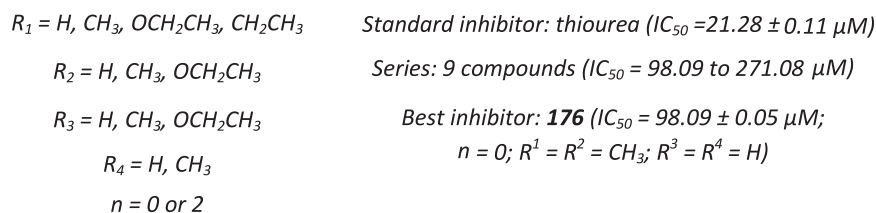
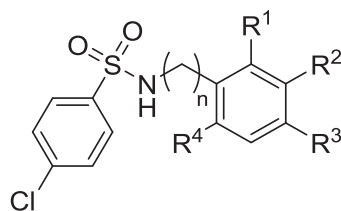
\*Value of  $IC_{50}$  determined with urease from *Canavalia ensiformis*

**Scheme 63.** Chemical structures of urease inhibitors based on N-[(4-methoxyphenyl)]-4-oxo-4-[oxy]butanamide.



[198]	[199]	[200]
$R^1 = H, OH, OCH_3$	$R^6 = H, CH_2CH_3, (CH_2)_3CH_3,$ $(CH_2)_5CH_3, (CH_2)_7CH_3,$ $(CH_2)_9CH_3, (CH_2)_{11}CH_3$	$R^{12} = OCH_3, NO_2, Cl$
$R^2 = H, OH, OCH_3$		$R^{13} = H, NO_2$
$R^3 = OH, OCH_3$	$R^7 = H, OH$	$R^{14} = H, OH, OCH_3, 3,4-OCH_2O,$ $NO_2$
$R^4 = H, OH, OCH_3$	$R^8 = H, OH, Cl$	$R^{15} = H, OH, OCH_3, 3,4-OCH_2O,$ $N(CH_3)_2, Cl, NO_2$
$R^5 = OH, OCH_3, NO_2, F$	$R^9 = H, OH, Cl$	
Standard inhibitor: Acetohydroxamic acid (% inhibition = 94.3%; $IC_{50} =$ $0.017 \pm 0.002$ mM)	$R^{10} = Cl, Br, OCH_3, OCH_2CH_3$ $R^{11} = OH, OCH_3, OCH_2CH_3, F,$ $Cl, Br$	Standard inhibitor: Acetohydroxamic acid ( $IC_{50} =$ $18.4 \pm 1.3$ $\mu$ M)
Series A: 10 compounds (% inhibition = inactive to 51%) Series B: 10 compounds (% inhibition = inactive to 73%)	Standard inhibitor: Acetohydroxamic acid ( $IC_{50} =$ $17.2 \pm 0.9$ $\mu$ M)	Series F: 21 compounds ( $IC_{50} =$ $0.62$ to $>500$ $\mu$ M) Best inhibitor: <b>175 (F)</b> ( $IC_{50} =$ $0.62 \pm 0.04$ $\mu$ M; $R^{12} = 4-NO_2;$ $R^{13} = H; R^{14} = R^{15} = OH$ )
Series C: 10 compounds (% inhibition = inactive to 97.3%)	Series D: 11 compounds ( $IC_{50}$ $= 4.9$ to $250$ $\mu$ M)	* All values of $IC_{50}$ and % inhibition determined with urease from <i>Helicobacter</i> <i>pylori</i>
Best inhibitors: <b>172 (C)</b> (% inhibition = 97.3%; $IC_{50} =$ $0.011 \pm 0.0012$ mM; $R^1 = R^2 =$ $R^3 = R^5 = OH; R^4 = H$ ); <b>173 (C)</b> (% inhibition = 92.5%; $IC_{50} =$ $0.047 \pm 0.005$ mM; $R^2 = R^3 =$ $R^5 = OH; R^1 = R^4 = H$ )	Series E: 18 compounds ( $IC_{50}$ $= 1.5$ to $277$ $\mu$ M) Best inhibitor: <b>174 (E)</b> ( $IC_{50} =$ $1.5 \pm 0.2$ $\mu$ M; $R^8 = R^9 = R^{11} =$ $OH; R^7 = R^{10} = H$ )	

**Scheme 64.** Chemical structures of urease inhibitors based on 1,2-arylethanes and analogues.



**Scheme 65.** Chemical structures of urease inhibitors based on sulfonamides.

Li and Xiao et al. reported results for their systematic studies of the urease inhibitory activity of 1,2-arylethanes. The first results, which were published in 2009 [198], showed that compounds **172** and **173** were most potent inhibitors among the amine and oxime derivatives (Scheme 64). The authors suggested that hydroxyl substituents at benzene ring increased the inhibitory activity due to a direct interaction with the nickel center, whereas the amine group is responsible for interactions with residues from His a323 of *H. pylori* urease. In a subsequent work (2010) [199], compound **174** inhibited urease more effectively than all other synthesized compounds (Scheme 64). SAR studies and molecular docking helped to relate the high activity of compound **174** to the presence of the hydroxyl groups at the benzene rings, which provides extra hydrogen bonds with Asn168 and Glu222 in the active site of urease compared to acetohydroxamic acid. Their most recent study on this class of molecules examined benzylanilines (Scheme 64), of which the most potent compound was **175**. SAR studies and molecular docking showed that the presence of an electron-donating group in the benzyl moiety increases the activity, but only 3,4-hydroxyl substituents provides extra hydrogen bonds; meanwhile, the nitro group at *p*- and *m*- positions of the aniline moiety enhances the inhibitory potential [200].

Aziz-ur-Rehman and coworkers [201] evaluated a small series of chlorinated sulfonamide derivatives (Scheme 65) using a urease inhibition assay. According to the SAR studies, alkyl groups at *ortho* and *meta* positions of the aniline moiety were responsible for the higher antiurease activity of compound **176** than the other compounds [202].

## Conclusions and future perspectives

The amount of screening data that is currently available represents irrefutable progress in the development of drugs for the treatment of infections associated with ureolytic bacteria. Several very highly active organic substances have been identified as promising lead compounds from model screening assays involving *Canavalia ensiformis* urease. Nevertheless, despite the high degree of homology among bacteria and vegetal ureases, important differences can result in a partial or complete decrease of inhibitory activity when probed against enzymes from different species. Since the urease allosteric site is considerably less conserved than the catalytic site, inhibitors acting by participating in interactions with the former site are more susceptible to a potential lack of association among their inhibitory profile for different ureases. Therefore, studies involving native or recombinant microbial enzymes is one of the further and necessary stages of the development of therapies

for diseases caused by ureolytic pathogens. Advances in this field will also require analyses of structure–activity relationships of organic compounds based on bioassays using different sources of ureases, particularly studies comparing the mechanisms of action of these compounds for different ureases. These approaches will lead to a better understanding and identification of druggable allosteric sites, which may allow researchers to identify selective sites for ureases derived from different sources.

## Conflict of interest

The authors have declared no conflict of interest.

## Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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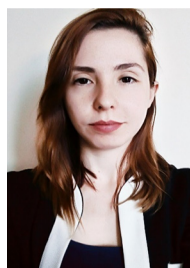
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