Review

A review on the development of urease inhibitors as antimicrobial agents against pathogenic bacteria

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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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Introduction

Urease, an enzyme that strictly depends on nickel ions (Ni^{2+}) [1], is a type of hydrodase that accelerates the rate of urea hydrolysis to ammonia (NH₃) and carbamic acid, which disproportionate into ammonia and carbon dioxide (CO₂), 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 NH₃ 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 NH₃ 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, septicaemia 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 phosphorimidated 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].
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 \textit{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 \textit{C. ensiformis} urease.

More recently, Mustafa and co-workers identified several novel urease inhibitors \([49]\). The authors designed and synthesized \(N^1\)-toloyl, \(N^2\)-substituted urea derivatives and evaluated them using in vitro enzyme-inhibition assays that included \textit{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 tolly 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, Usato et al., based on the urease inhibiting capacity of hydroxurea, synthesized \(N^1\)-hydroxy-\(N^2\)-substituted derivatives and evaluated their inhibitory activity towards urease using hydroxurea 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 coworkers have synthesized a variety of substituted thioureas and screened their urease inhibitory activity \([52]\). 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 \([51]\).

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\[
\begin{align*}
R^1 &= IC_{50} 8.43 \mu M \\
R^2 &= IC_{50} 2,4-[\text{Cl}]\text{C}_{6}H_5 \\
R^3 &= IC_{50} 4-\text{NO}_2\text{C}_{6}H_5 \\
R^1 &= H, CH_3, C_6H_5, \text{C}_{6}H_5 \\
R^2 &= \text{CH}_3, \text{C}_{6}H_5, \text{C}_{6}H_5, \text{C}_{6}H_5, 4-\text{NO}_2\text{C}_{6}H_5, 2-\text{Pyridinyl}, \\
3-\text{Pyridinyl}, 4-\text{Pyridinyl}, \text{Morpholinyl}, (C_6H_5)_2\text{C}_{6}H_5. \\
\text{Standard inhibitor: thiourea (IC}_{50} 21.0 \mu M) \\
\text{Series: 14 compounds (IC}_{50} 1.25 to 55.6 \mu M) \\
\text{Best inhibitor: 1 (IC}_{50} 1.25 \mu M; R^1 = 4-\text{NO}_2\text{C}_{6}H_5, R^2 = H, R^3 = 4-\text{NO}_2\text{C}_{6}H_5) \\
\text{*Values of IC}_{50} determined with urease from Canavalia ensiformis} \\
\text{Scheme 2. Chemical structures of } N^1,N^2\text{-di- and tri-substituted urea derivatives reported as potential urease inhibitors.}
\end{align*}
\]

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 \textit{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\(_3\)-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 \textit{C. ensiformis} urease was evidenced in a direct comparison between extent of inhibition achieved by the monosubstituted \(N^1\)-benzoylthiourea 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 hydroxurea (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\(_2\), \(m\)– 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- thichlorophenyl 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 \textit{para}-ethyl benzoate \([58]\) or \textit{para}-sulfanilamide \([57]\) at \(N^2\) nitrogen of benzoylthioureas
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 N2-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 IC50 values of 0.21 and 0.13 μM, respectively. Notably, compounds derived from 3-chloro and 2,4-dichloro benzoic acid also showed comparable IC50 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 Ki values of 0.21 and 0.13 μM, respectively. Notably, compounds derived from 3-chloro and 2,4-dichloro benzoic acid also showed comparable IC50 values [58].

In 2017, Saeed and co-workers reported the synthesis of two series of N-acetyl thioureas derived from myristic (Series B, Scheme 9) [59] and palmitic acids (Series A, Scheme 9) [60]. The inhibitory effects of substituted phenyl rings at the remaining nitrogen atom on C. ensiformis urease were evaluated. All tested compounds presented very low IC50 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.

Scheme 4. Chemical structures of N1-hydroxy-N2-substituted derivatives described as urease inhibitors.

Scheme 5. Chemical structures of N1-aryl-N2-(aryl or alkyl)-substituted thiourea derivatives that possesses antiureolytic activity.

Scheme 6. Chemical structures of symmetrical (bis)-thiourea which possesses antiurease activity.
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 IC₅₀ values for C. ensiformis urease. Compounds bearing 3-NO₂ and 4-N(CH₃)₂ as substituents showed very good inhibitory activity (compounds 31 and 32, respectively).

R = 4-Cl, 2-Cl, 2,4-NO₂, 2-CN-4-NO₂, 3-Cl, 4-CH₃, 4-COOH, acetyl, 2,3-Cl, 4-NO₂, 4-SO₂H, naphthyl, mesityl

Standard inhibitor: thiourea (IC₅₀ = 4.72 and 18.19 μM)

Series A: 10 compounds (IC₅₀ = 0.01 to 0.07 μM)

Series B: 11 compounds (IC₅₀ = 0.03 to 0.09 μM)

Best inhibitors: 25 (A) (Kᵢ = 0.014 μM; R = 4-Cl); 26 (B) (Kᵢ = 0.021 μM; R = 3-Cl)

*Values of IC₅₀ and Kᵢ determined with urease from Canavalia ensiformis

Scheme 9. Chemical structures of N-acyl thioureas-urease inhibitors derived from myristic or palmitic acids.

R = purine, 4-COOH-C₆H₄, 3-NO₂-C₆H₄, 2-NO₂-C₆H₄.

Standard inhibitor: thiourea (IC₅₀ = 21.0 μM)

Series: 10 compounds (IC₅₀ = 26.3 to 31.1 μM)

Best inhibitors: 27 (IC₅₀ = 26.3 μM; R = 4-COOH-C₆H₄); 28 (IC₅₀ = 26.7 μM; R = 3-NO₂-C₆H₄); 29 (IC₅₀ = 28.4 μM; R = purine); 30 (IC₅₀ = 31.1 μM; R = 2-NO₂-C₆H₄)

*Values of IC₅₀ determined with urease from Canavalia ensiformis

Scheme 10. Chemical structures of symmetrical isophthalyl-bis-(thioureas) urease inhibitors.

R = 3-NO₂, 4-N(CH₃)₂

Standard inhibitor: Thiourea (Kᵢ = 19.6 ± 0.45 μM)

Series: 18 compounds (Kᵢ = 0.090 ± 0.06 to 0.910 ± 0.11 μM)

Best inhibitors: 31 (Kᵢ = 0.090 ± 0.06 μM; R = 3-NO₂); 32 (Kᵢ = 0.122 ± 0.11 μM; R = 4-N(CH₃)₂)

*Values of Kᵢ 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 N4-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 N4 position exerted a positive effect on enzyme activity, as compounds showed more potent urease inhibition (IC50 = 20.6 μM) than the standard thiourea (IC50 = 21.0 μM) [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).

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 IC50 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
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 α-chymotrypsin, cholinesterases, phosphodiesterase and β-glucuronidase. Dimerization also reduces the IC50 of the derivative by 50%, from 94 μM to 44 μM. 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 amidazole-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 IC50 values as low as 0.12 μM.

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 IC50 = 156 ± 10 μM [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 α313–α346 residues, opening the enzyme’s active site. The most active compound, 57 (Series D;
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 antiucreolytic 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 ± 0.04 mM and a $K_i$ for the urease-urea-compound 67 of 3.17 ± 0.69 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 $IC_{50}$ of approximately 18 µg/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 IC50 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 N2-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-
Scheme 19. Chemical structures of urease inhibitors based on hybrids of salicilates and metronidazole or secnidazole.

\[
\begin{align*}
R^1 &= H, \text{CH}_3, \text{NO}_2, \text{I, Br, Cl} \\
R^2 &= H, \text{Cl, CF}_3, \text{NH}_2, \text{OCH}_3, \text{CH}_3 \\
R^3 &= H, \text{Br, I, Cl, CH}_3, \text{OCH}_3, \text{SO}_2\text{H}, \text{NO}_2 \\
\end{align*}
\]

- Standard inhibitor: acetylamidoxamic acid (IC\text{\textsubscript{50}} = 17 \mu M, IP = 91.2\% [72]; 16 \mu M [73])
- Series C: 9 compounds (IC\text{\textsubscript{50}} = 51 to 1.7 \mu M)
- Series A: 8 compounds (IP = 38.3\% to 95.3\%)
- Series B: 6 compounds (IP = 38.3\% to 56.8\%)
- Series D: 8 compounds (IC\text{\textsubscript{50}} = 37 to 1.0 \mu M)
- Series E: 5 compounds (IC\text{\textsubscript{50}} = 29 to 2 \mu M)
- Series F: 8 compounds (IC\text{\textsubscript{50}} = 45 to 19 \mu M)

Best inhibitors: 57 (D) (IC\text{\textsubscript{50}} = 1.0 \pm 0.4 \mu M; R^1 = H; R^2 = Cl; R^3 = H; R^4 = H); 58 (A) (IC\text{\textsubscript{50}} = 26 \mu M; R^1 = H; R^2 = H; R^3 = Br; R^4 = H); 59 (A) (IC\text{\textsubscript{50}} = 12 \mu M; R^1 = H; R^2 = H; R^3 = Cl; R^4 = H); 60 (C) (IC\text{\textsubscript{50}} = 1.7 \pm 0.5 \mu M; R^1 = H; R^2 = H; R^3 = H; R^4 = H); 61 (E) (IC\text{\textsubscript{50}} = 2.0 \pm 1 \mu M; R^1 = H; R^2 = H; R^3 = \text{CH}_3; R^4 = H)

*IP = inhibition percentage at 1mM

*Values of IC\text{\textsubscript{50}} determined with urease from Helicobacter pylori

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

\[
\text{R = H, CH}_3, \text{C}_3\text{H}_5, \text{CH}_3\text{C}_2\text{H}_5, \text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_3, \text{C}(\text{CH}_3)_3, \text{C}_2\text{H}_5\text{(CH}_2\text{)}_3\text{CH}_3, \text{CH}_3(\text{CH}_2)_2\text{CH}_3, \text{CH}_3(\text{CH}_2)_2\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_3, \text{CH}_2(\text{CH}_2)_2\text{CH}_3, \text{CH}_2(\text{CH}_2)_2\text{CH}(\text{CH}_3)\text{CH}_3, \text{CH}_3(\text{CH}_2)_2\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_3, \text{CH}_3(\text{CH}_2)_2\text{CH}_3, \text{CH}_2(\text{CH}_2)_2\text{CH}_3, \text{CH}_2(\text{CH}_2)_2\text{CH}(\text{CH}_3)\text{CH}_3}
\]

- Standard inhibitor: thiourea (IC\text{\textsubscript{50}} = 21.01 \mu M), pH = 5.0
- Series: 14 compounds (IC\text{\textsubscript{50}} = 180.99 to 0.29 \mu M)

Best inhibitors: 62 (IC\text{\textsubscript{50}} = 0.29 \pm 0.012 \mu M; R = \text{CH}_3(\text{CH}_2)_2\text{CH}_3); 63 (IC\text{\textsubscript{50}} = 0.40 \pm 0.003 \mu M; R = \text{CH}_3(\text{CH}_2)_2\text{CH}_3); 64 (IC\text{\textsubscript{50}} = 0.34 \pm 0.60 \mu M; R = \text{CH}_2(\text{CH}_2)_2\text{CH}_2(\text{CH}_2)_2\text{CH}_3)

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

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

\[ R = \text{phenyl}, 4'-\text{NO}_2C_6H_5, 4'-\text{CN}C_6H_5, 4'-\text{N(H}_2)C_6H_5, 4'-\text{SCH}_2C_6H_5, 4'-\text{OCH}_3C_6H_5, 3'-\text{OCH}_2C_6H_5, 4'-\text{F}C_6H_5, 4'-\text{OH}C_6H_5, 3'-\text{OH}C_6H_5, 2'-\text{OH}C_6H_5, 2'-\text{COOH}C_6H_5, 3,5'-\text{OCH}_2O-C_6H_5, 2'-\text{thiophenyl}, 2'-\text{pyrrolyl}, 2'-\text{furyl}, 4'-\text{pyridinyl}, 3'-\text{pyridinyl}, \text{cyclohexyl.} \]

Standard inhibitor: thiourea (26% inhibition)

Series A: 18 compounds

Best inhibitors: 67 (K = 1.02 ± 0.04 mM; R = 3'-pyridinyl); 68 (R = 4'-pyridinyl); 69 (R = 2'-COOH-C_6H_5)

*Values of % inhibition and K, determined with urease from Canavalia ensiformis

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

\[ R = \text{phenyl, 3,5-CF}_3C_6H_5, 4'-\text{Cl}C_6H_5, 4'-\text{CH}_3C_6H_5, 3,5'-\text{CH}_3C_6H_5, 3,4'-\text{Cl}C_6H_5, 4'-\text{OCH}_3C_6H_5, 5'-\text{methyl-thiophen-2-yl}. \]

Standard inhibitor: thiourea (IC\text{50} = 23.1 \mu g/ml)

Series: 8 compounds (IC\text{50} = 19.2 to 16.5 \mu g/ml)

Best inhibitor: 70 (IC\text{50} = 16.5 \mu g/ml; R = 4'-CH_2CH_2)

*Values of IC\text{50} determined with urease from Helicobacter pylori

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

\[ R = \text{CH}_3, (\text{CH}_3)_2\text{CH}, (\text{CH}_3)_2\text{CHCH}_2, (\text{CH}_3)\text{CHCH}_2\text{CH}_2\text{OH}, \text{CH}_2\text{C}_6\text{H}_5, \text{CH}_2\text{CH}_2\text{SCH}_2, 5'-(\text{imidazol-4-yl})\text{methyl}, 5'-(\text{indol-3-yl})\text{methyl} \]

Standard inhibitor: thiourea (IC\text{50} = 21.0 ± 0.1 \mu M)

Series: 9 compounds (IC\text{50} = 22.0 to 43.8 \mu M)

Best inhibitor: 70 (IC\text{50} = 22.0 ± 0.5 \mu M; R = (\text{CH}_3)_2\text{CH})

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

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

\[ R = \text{phenyl, 2-Cl}C_6H_5, 3-\text{Cl}C_6H_5, 2-\text{Br}C_6H_5, 3-\text{Br}C_6H_5, 4-\text{Br}C_6H_5, 2-\text{F}C_6H_5, 3-\text{F}C_6H_5, 4-\text{F}C_6H_5 \]

Standard inhibitor: thiourea (IC\text{50} = 21.0 ± 0.1 \mu M)

Series: 10 compounds (IC\text{50} = 15.0 to 30.5 \mu M)

Best inhibitors: 72 (IC\text{50} = 7.8 ± 0.2 \mu M; R = 2-Br); 73 (IC\text{50} = 12.4 ± 0.2 \mu M; R = 3-Br)

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

Scheme 25. Chemical structures of urease inhibitor bearing the 1,2,4-triazole-3-thione moiety.

74 (IC\text{50} = 26.54 ± 2.01 \mu g/ml) 75 (IC\text{50} = 77.09 ± 8.66 \mu g/ml)

Standard inhibitor: thiourea (IC\text{50} = 98.18 ± 2.13 \mu g/ml)

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

Scheme 26. Chemical structures of 1,2,4-triazole derivatives – potent substances described as urease inhibitor.
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-arylaminooxadiazoles (Scheme 29) for urease-inhibiting activity [83]. The influence of the nature of the 2-arylaminosubstituent 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-Oxadiazole-2-thione analogues containing methoxylated aromatic groups (compounds 89 and 90, Scheme 31) also showed high antidiastase activity [85]. Although halogenated derivatives generally showed the lowest activity, the para-chlorobenzyl analogue was the most active tested inhibitor (IC_{50} 1.15 ± 0.2 μM).

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). 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-

![Scheme 28. Chemical structures of triazole derivatives described as potential urease inhibitors.](image)

![Scheme 29. Chemical structures of oxadiazoles described as potential urease inhibitors.](image)

![Scheme 30. Chemical structures of aminophenyl benzylalcohols which possesses antidiastase properties.](image)

![Scheme 31. Chemical structures of 1,3,4-oxadiazoles-2-thione analogues described as potent inhibitors of urease enzyme.](image)
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 IC₅₀ 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 IC₅₀ 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 (IC₅₀ from 0.037 to 0.084 μM) [89]. Remarkably, the most active inhibitor was the chiral compound containing a (S)-2-hydroxy-1-methylthaneamine substituent (compound 100; Scheme 35), but its enantiomer displayed the lowest inhibition. A kinetic study of compound 100 was performed and exhibited a mixed type inhibitory behavior with a Kᵢ = 0.01 μM. Saify et al. reported 7-azoindole derivatives (Scheme 36), whose IC₅₀ 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 IC₅₀ of 2.19 ± 0.37 μM, whereas the second best inhibitor, compound 104, only had an IC₅₀ of 133.31 ± 0.46 μM.

Selenium compounds are also reported to possess antiureolytic activity, such as ebselen (105, Scheme 37, IC₅₀ = 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 (IC₅₀ of 25.4 μM to inactive). The activity returned to the previous order of magnitude for the corresponding methyl ester counterpart [IC₅₀ 3.3 μM (106) to 4.07 μM (107); Scheme 37]. Compounds containing phe-
nyl group, including ebsalen (105; Scheme 37), and methyl ester derivatives were the most active inhibitors, with IC$_{50}$ 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

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

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

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

**Scheme 39.** Chemical structures of hybrid compounds.

**Scheme 40.** Chemical structures of pyridinone-based urease inhibitors.

**Scheme 41.** Chemical structures of dihydropyrimidine-based urease inhibitors.
Scheme 39. Chemical structures of urease inhibitors derived from linezolid core.

**R** = OCH$_3$CH$_3$, NHNH$_2$, NH(N)(CH(4-Br-C$_6$H$_4$), NH(N)(CH)$_3$C$_6$H$_5$, (NH)$_2$CSNHCO$_2$H$_2$

Series: 7 compounds (IC$_{50}$ = 2.37 ± 0.19 to 13.23 ± 2.25 μM)

Best inhibitor: 116 (IC$_{50}$ = 2.37 ± 0.19 μM; R = )

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

(Scheme 41) coordinates with the *bis*-nickel center via its 4-methoxy group at phenyl ring of semithiocarbazide; 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-dihydropyrimidin-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 xanthones 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).
Scheme 41. Chemical structures of urease inhibitors based on dihydropyrimidines.

\[ R^1 = H, \ OMe, \ O(CH_2)_3CH_3 \]
\[ R^2 = Br, \ OH, \ NO_2 \]
\[ R^3 = H, \ OCH_3, \ Br \]

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

**Series C:** 2 compounds \( (IC_{50} = 0.23 \text{ and } 5.2 \ \mu M) \)

**Series A:** 4 compounds \( (IC_{50} = 33.71 \text{ to } 111 \ \mu M) \)

**Series D:** 2 compounds \( (IC_{50} = 10.87 \text{ and } 12.3 \ \mu M) \)

**Series B:** 4 compounds \( (IC_{50} = 0.58 \text{ to } 1.05 \ \mu M) \)

**Series E:** 3 compounds \( (IC_{50} = 10.54 \text{ to } 16.7 \ \mu M) \)

**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.
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 cyanoacetamide 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,
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 ± 0.20 to 123.2 ± 0.37 μM [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.

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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 antimalarial 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

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

Scheme 44. Chemical structures of xanthenes analogues that possesses urease inhibitory properties.
inhibitor-\textit{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, phosphoramidates 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 phosphoramidates as urease inhibitors is restricted due to their susceptibility to hydrolysis. In an attempt

\begin{align*}
\text{Standard inhibitor: Thiourea (IC}_{50} = 21 \pm 0.11 \\ \mu\text{M})}
\end{align*}

\begin{align*}
\text{Best inhibitors: 123 (A) (IC}_{50} = 13.0 \pm 1.2 \mu\text{M}; R^1 = R^2 = R^3 = H; R^4 = F)}
\end{align*}

\begin{align*}
\text{Series B: 23 compounds (IC}_{50} = 9.18 \mu\text{M to no activity) [130]}
R^1 = H, OH, Cl
R^2 = H, Cl, OH, OCH$_3$, OCH$_2$CH$_3$, NO$_2$
R^3 = H, Cl, Br, OH, SCH$_3$, OCH$_3$, N(CH$_3$)$_2$
R^4 = H, CH$_2$CH$_3$, OCH$_3$
\end{align*}

\begin{align*}
\text{Standard inhibitor: Thiourea (IC}_{50} = 21 \pm 0.11 \\ \mu\text{M})}
\end{align*}

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

\begin{align*}
\text{*Values of IC}_{50} \text{ determined with urease from Bacillus pasteurii}
\end{align*}

\textbf{Scheme 45.} Chemical structures of urease inhibitors based on barbituric acid moiety.

\textbf{Scheme 46.} Chemical structures of urease inhibitors based on thiobarbituric acid moiety.
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 conditions.

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

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

\[
R^1 = 3,4-OCH_2C_6H_3, 3,4,5-OCH_3C_6H_4, 3-OCH_3-4-OH-C_6H_4, \text{furanyl, thieryl} \\
R^2 = H, CH_3 \\
X = O, S \\
\text{Standard inhibitor: thiourea (IC}_{50} = 27.5 \mu g/mL} \\
\text{Series A: 15 compounds (IC}_{50} = 17.34 \mu g/mL to no activity)} \\
\text{Series B: 5 compounds (IC}_{50} = \text{no activity)} \\
\text{Best inhibitors: 127 (A) (IC}_{50} = 17.34 \pm 0.01 \mu g/mL; R^1 = \text{furanyl; R}^2 = \text{CH}_3; X = O); 128 (A) (IC}_{50} = 36.75 \pm 0.05 \mu g/mL; R^1 = \text{thieryl; R}^2 = H; X = S) \\
\]

\[
R^4 = H, NO_2, Cl \\
R^5 = H, OH, Cl \\
\text{Standard inhibitor: Thiourea (IC}_{50} = 21 \pm 0.011 \mu M) \\
\text{Series A: 36 compounds (IC}_{50} = 8.53 \mu M to ND (2015))} \\
\text{Series B: 14 compounds (IC}_{50} = 3.76 \mu M to ND (2011))} \\
\]

\[
R^1 = H, COOH, CF_3 Cl, OH, SH \\
R^2 = H, COOH, OH, CF_3 Cl \\
R^3 = H, COOH, OH, NO_2, CF_3 Cl, I \\
\text{Standard inhibitor: Thiourea (IC}_{50} = 21 \pm 0.11 \mu M) \\
\text{Best inhibitors: 129 (A) (IC}_{50} = 8.53 \pm 0.027 \mu M; X = S; R^1 = COOH; R^2 = R^3 = R^4 = H; R^5 = NO_2); 130 (A) (IC}_{50} = 8.93 \pm 0.027 \mu M; X = S; R^1 = R^2 = R^4 = R^5 = H; R^3 = COOH) \\
\]

\[
R^6 = H, CNHNH_2, 4,6-(CH_3)_2-pyrimidyl, 4-CH_3-lisoxazyl, thiazolyl \\
\text{Standard inhibitor: Thiourea (IC}_{50} = 21 \pm 0.11 \mu M) \\
\text{Best inhibitor: 131 (B) (IC}_{50} = 3.76 \pm 0.027 \mu M; X = S; R^1 = H) \\
\]
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 enzyme active site [173]. The IC\textsubscript{50} and K\textsubscript{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

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

---

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

Scheme 50. Chemical structures of urease inhibitors based on bis-barbituric acid derivatives.
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 ($K_i = 0.62 \mu M$) 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 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 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 carbonyl moiety (Ala366).

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

$$R^1 = H, 4-NO_2, 3,5-CH_3, 2-NO_2-4-CH_3, 3-OCH_3$$
$$4\text{-cyclohexyl}, 2,5-OCH_3, 4-OCH_2H_5, 2-NO_2, 2-CF_3$$
$$2-OCH_3, 2-OCH_2-4-NO_2$$

$$R^2 = H, 6-OCH_2CH_3, 6-F, 4-OCH_3, CH_3$$

$$X = O, NH$$

$$Y = O, S$$

Best inhibitors: 143 (B) ($IC_{50} = 2 \text{ nM}$; $R^1 = H; X = NH; Y = O$); 144 (A) ($IC_{50} = 3 \text{ nM}$; $R^1 = 2-\text{NO}_2$; $X = NH; Y = O$); 145 (A) ($IC_{50} = 3.5 \text{ nM}$; $R^1 = 2-\text{NO}_2-4-\text{CH}_3; X = NH; Y = O$); 146 (B) ($IC_{50} = 5 \text{ nM}$; $R^1 = 6-OCH_2CH_3; X = NH; Y = O$); 147 (B) ($IC_{50} = 5 \text{ nM}$; $R^2 = 6-F; X = NH; Y = O$); 148 (B) ($IC_{50} = 10 \text{ nM}$; $R^1 = 4-OCH_3; X = NH; Y = O$)

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

Scheme 54. Chemical structures of urease inhibitors based on phosphoroamidic acid.

$$X = O, S$$

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

Best inhibitors: 150 (B) ($IC_{50}$ 3.8 ± 0.4 $\mu$M; $R^2 = R^3 = CH_3$); 151 (B) ($IC_{50}$ ±1.7$\mu$M; $R^2 = R^3 = CH_3; R^3 = OH$); 152 (B) ($IC_{50}$ 60±0.3 $\mu$M; $R_2 = H$; $R^2 = R^3 = CH_3$)

Standard inhibitor:

Acetohydroxyamic acid, $K_i$ (S. pasteurii, 0.108 $\mu$M to no activity); (P. mirabilis, 0.202 $\mu$M to NA)

Best inhibitor: 153 (C)

($K_i$ S. pasteurii = 0.108 ± 0.0116 $\mu$M and $K_i$

P. mirabilis = 0.202 ± 0.05 $\mu$M; $R^2 = R^3 = CH_3; R^3 = CH_3$)
molecular docking studies [176]. Based on the IC50 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 β-aryl-β-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 IC50 = 6 μM (the IC50 of the standard hydroxyurea = 100 μM). The improved antiurease activity of the compound was associated with π–π interactions between the sulfur atom in Met366 residue and phenyl ring at R2, closer interactions with His 274 and 138 residues by changing Cl to F at R2; hydrogen bonding between the NH2 group and Asn 168, the presence of electron-donating and -withdrawing substituents on rings at R2 and R3, respectively, and the presence of an electron-donating substituent in R1.

Batool et al. reported the antibacterial, antiurease and NO scavenging activities of a small series of substituted (prop-2-ynyl) 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.

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

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

Scheme 57. Chemical structures of urease inhibitors based on β-aryl-β-mercapto ketone derivatives.

Scheme 58. Chemical structures of urease inhibitors bearing a (prop-2-ynyl) benzene scaffold.

Standard inhibitor: Thiourea (IC50 = 21.10 ± 0.310 μM)

Series A: 1 compound – 158 (A) (IC50 = 32.51 ± 0.045 μM)

Series B: 4 compounds (IC50 = 19.07 to 36.71 μM)

Series C: 4 compounds (IC50 = 91.21 μM to NA)

Best inhibitor: 159 (B) (IC50 = 19.07 ± 0.045 μM; R1 = 2-OH; R2 = 3,4-OCH3)

*Values of IC50 determined with urease from Canavalia ensiformis

Scheme 59. Chemical structures of urease inhibitors bearing a 1,5-benzothiazepine moiety.
Scheme 58. Chemical structures of urease inhibitors based on substituted \((\text{prop-2-ynyloxy})\text{benzene derivatives.}\)

\[
R^1 = \text{H, CH}_x \text{H, Cl, Br} \\
R^2 = \text{H, CH}_x \text{H} \\
R^3 = \text{H, CH}_x \text{F, Cl, Br, NO}_2 \\
Y = \text{NH, O}
\]

**Standard inhibitor:** Thiourea \((\text{IC}_{50} = 23.3 \ \mu\text{g/mL})\)

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

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

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

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

\[
R^4 = \text{CH}_y \text{H} \\
R^5 = \text{H, OCH}_x \text{OH, OCH}_x \text{CH}_x \text{H}_y \text{F, Cl, Br, CF}_y \text{N(CH}_x \text{H}_y)_2 \\
R^6 = \text{H, F, Cl} \\
R^7 = \text{H, OH, OCH}_x \text{CH}_x \text{H}_y \text{Cl}
\]

**Standard inhibitor:** acetoxyhydroxamic acid \((\text{IC}_{50} = 27.6 \pm 2.5 \text{ and } 23.8 \pm 1.5 \ \mu\text{M})\)

**Series:** 44 compounds \((\text{IC}_{50} = 0.083 \text{ to } 405 \ \mu\text{M})\)

**Best inhibitors:** **162** \((K_i = 0.014 \pm 0.003 \ \mu\text{M}; R^1 = \text{H}; R^2 = \text{Cl}; R^3 = R^4 = R^6 = \text{H})\); 163 \((K_i = 0.045 \pm 0.007 \ \mu\text{M}; R^1 = \text{H}; R^2 = R^3 = R^5 = \text{Cl}; R^6 = R^7 = \text{H})\); 164 \((K_i = 1.11 \ \mu\text{M}; R^1 = \text{H}; R^2 = R^3 = R^5 = \text{H}; R^1 = \text{Cl}; R^6 = \text{OH})\); 165 \((K_i = 0.13 \ \mu\text{M}; R^1 = \text{H}; R^2 = R^3 = R^6 = \text{H}; R^3 = \text{Cl}; R^6 = \text{OCH}_x \text{CH}_x \text{H}_y)\)

*Values of IC\(_{50}\) and K\(_i\) determined with urease from Helicobacter pylori*

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

\[
A \\
\begin{array}{c}
\text{OH} \\
\text{nCOOH}
\end{array}
\]

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

\[
B \\
\begin{array}{c}
\text{OH} \\
\text{mCOOH}
\end{array}
\]

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

\[
n = 1, 2, 4, 5, 6, 7, 8, 9, 10, 11 \\
m = 1, 4, 5, 7, 10
\]

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

**Best inhibitors:** **167** \((B)\) \((\text{IC}_{50} = 0.07 \pm 0.001 \ \mu\text{g/mL; m = 5); 168\} (B) \((\text{IC}_{50} = 0.083 \pm 0.002 \ \mu\text{g/mL; m = 7)\})\)}
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 IC₅₀ 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, consistent with the findings reported by Sokmen, who varied the position of the hydroxyl group in a 14C chain.

Since α-β-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 α-β-unsaturated carbonyl unit is the major substituent responsible for the inhibitory activity, possibly because of the interaction between the cysteine sulphydryl moiety and unsaturated carbonyl β-carbon unit; electron-donators attached to the aromatic system were also responsible for increasing the inhibitory activity.

Due to the structural relationship between α-hydroxyketones, acetoxyhydroxamic acid and hydroxyurea, in 2004, Tanaka and co-workers synthesized several potentially novel urease inhibitors based on α-hydroxyketones (Scheme 62) [196]. SAR studies revealed that the hydrophobic character of the R substituent in the RCOCH₂OH moiety may play an important role in the inhibitory activity. The interaction between α-hydroxyketone and the sulphydryl 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 (IC₅₀) of 4.08 ± 2.54 µM, a value that was much higher than the standard thiourea [197].

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Scheme 64. Chemical structures of urease inhibitors based on 1,2-arylethanes and analogues.
Li and Xiao et al. reported results for their systematic studies of the urease inhibitory activity of 1,2-arylethanols. 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 acetylhydroxamic 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 allostic 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 allostic 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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References

[1] Dixon NE, Gazzola C, Watters JJ, Blakeley RL, Zerner B. Jack Bean Urease (EC 3.5.1.5) [letter]. A metalloenzyme. A simple biological role for nickel? J Am Chem Soc 1975;97(14):4131–3.
[2] Krajewska B, Ureases I. Functional, catalytic and kinetic properties: a review. J Mol Catal B Enzym 2009;59(1):9–21.
[3] Follmer C. Ureases as a target for the treatment of gastric and urinary infections. J Clin Pathol 2010;63:424–30.
[4] Maroney MJ, Ciurli S. Nonredox nickel enzymes. Chem Rev 2014;114:4206–28.
[5] Takeuchi T. On the occurrence of urease in higher plants. J Coll Agric Tokyo Imp Univ 1909;1:1–14.
[6] Modolo LV, de Souza AX, Horta LP, Araujo DP, de Fátima A. An overview on the potential of natural products as ureases inhibitors: A review. J Adv Res 2015;6:35–44.
[7] Summer JB. The isolation and crystallization of the enzyme urease. J Biol Chem 1926;69:435–41.
[8] Sirko A, Brodzik R. Plant ureases: Roles and regulation. Acta Biochim Pol 2000;47:1189–95.
[9] Jabri E, Carr MB, Hausinger RP, Karplus PA. The crystal structure of urease from Klebsiella aerogenes. Science 1995;268:998–1004.
Saeed A, Khan MS, Rafique H, Shahid M, Iqbal J. Design, synthesis, molecular characterization, antibacterial and urease inhibition studies of some novel arylthiophene-2-carbaldehydes via suzuki-miyaura reaction and its role in virulence. Contrib Microbiol Immunol 1999;13:262–3.

Dhar MS, Virdi JS. Strategies used by Yersinia enterocolitica to evade killing by the host: Thinking beyond yops. Microbes Infect 2014;16(2):87–95.

Koning-Ward TL, Vries N, Hartland EL, Rohns-Brown KM. The urease complex gene of Yersinia enterocolitica and its role in virulence. Contrib Microbiol Immunol 1995;13:262–3.

de Fátima A, Pereira OP, Olímpio CRSCD, Oliveira BGF, Franco LL, da Silva PHC, Schaal B. Bases and their metal complexes as urease inhibitors – a brief review. J Adv Res 2018;13:113–26.

Perveen S, Khan KM, Lodhi MA, Choudhary MI, Atta-ur-Rahman, Voelter W. Urease and α-chymotrypsin inhibition effects of selected urea derivatives. Lett Drug Des Discov 2009;6:401–5.

Mustafa S, Perveen S, Khan KM. Synthesis, enzyme inhibition and anticancer investigations of unsymmetrical 1,3-disubstituted thioureas. J Serb Chem Soc 2016;81:1–10.

Usato S, Hashimoto Y, Nishino M, Nagaoka Y, Kuwajima H. N-substituted hydroxamides as urease inhibitors. Chem Pharm Bull 2002;50(9):1280–2.

Rajic Z, Korkovic I, Butula I, Zorc B, Hadjipavlou-Litina D, Pontiki E, et al. Synthesis and biological evaluation of O-methyl and O-ethyl NSAID derivatives and their metal complexes. J Serb Chem Soc 2005;70:1707–17.

Khan KM, Naz F, Taha M, Khan A, Perveen S, Choudhary MI, et al. Synthesis and in vitro urease inhibitory activity of N,N’-disubstituted thioureas. Eur J Med Chem 2014;74:314–23.

Taha M, Ismail NH, Ibrahim S, Wadood A, Rahim F, Riaz M. Synthesis of potent urease inhibitors based on disulfide scaffold and their molecular docking studies. Biorg Med Chem 2015;23:7211–8.

Broto TO, Souza AX, Mota YCC, Morais VSS, Souza LT, de Fátima A, et al. Design, synthesis and biological evaluation of benzothiazole analogs as urease inhibitors of aspergillus niger. Molecules 2010;15:1289–302.

Rauf MK, Zaib S, Talib A, Ebihara M, Badshah A, Bolte M, et al. Solution-phase synthesis of potent 1-acyl-3-arylthioureas: synthesis, kinetic mechanism and molecular docking studies. Drug Res 2017;67:1–10.

Jamil M, Zubair M, Rasool N, Altaf AA, Rizwan K, Hafeez S, et al. Synthesis, characterization, antibacterial and urease inhibition studies of some novel symmetrically N,N’-disubstituted isophthalaldehydes. Asian J Chem 2013;25(10):5328–32.

Aslam MAS, Mahmood S, Shahid M, Saeed A, Iqbal J. Synthesis, biological assay in vitro and molecular docking studies of some new Schiff base derivatives as potential urease inhibitors. Eur J Med Chem 2011;46:5473–9.

Pervez H, Iqbal MS, Tahir MY, Nasim F, Choudhary MI, Khan KM. In vitro cytotoxic, antibacterial, antifungal and urease inhibitory activities of some new N,N’-disubstituted siatin-3-thiosemicarbazones. J Enzyme Inhib Med Chem 2008;23(6):848–54.

Pervez H, Chohan ZR, Ramzan M, Nasim F, Khan KM. Synthesis and biological evaluation of some new N,N’-disubstituted isatin-thiosemicarbazones. J Enzyme Inhib Med Chem 2009;24(2):437–46.

Sharma A, Suhar R, Gowda DC. Urease/thiolase of benzo[d]isothiazole analog conjugated glutamic acid: synthesis and biological evaluation. Arch Pharm Chem Life Sci 2013;346:359–66.

Brito TO, Souza AX, Mota YCC, Morais VSS, Souza LT, de Fátima A, et al. Design, synthesis and biological evaluation of benzothiazole analogs as antiurease agent: Synthesis and molecular docking studies. Eur J Med Chem 2018;2008;23(6):848–54.

Ali S, Rasool N, Ullah M, Nasir FH, Yaqoob A, Zubair M, et al. Design and synthesis of arylhydrozephe-2-carbalehyde via suzuki-miyaura reaction and their biological evaluation. Molecules 2013;18:1471–25.
Macegoniuk K, Dzielen A, Mucha A, Berlicki L. Bis(aminomethyl)phosphinic acids. Bioorg Med Chem Lett 2009;19:3747–51.

Hideo T, Teruomi J, inventors; Sankyo Co, assignee. Benzopyrano[2,3-b]pyridine-3-carboxamide and its preparation. Japan patent JP 200004145; 2000 Jan 27.

Donghi M, Attenni B, Gardelli C, Marco AD, Fiore F, Giuliano C, et al. Synthesis and urease inhibition studies of barbituric and thiobarbituric acid derivatives as prospective new therapeutic agents. J Med Chem 2005;48:38–47.

Vassiliou S, Grabowiecka A, Kosikowska P, Yiotakis A, Kafarski P, Berlicki L. Synthesis and anti-inflammatory properties of bis (2-hydroxy-1-phenyl)-2,2'-bioxanthene derivatives and its preparation. Japan patent JP 56005480; 1981 Jul 27.

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[179] Kumar SK, Hager E, Pettit C, Gurulingappa H, Davidson NE, Khan SR. Design, synthesis, and evaluation of novel boronic-chalcone derivatives as antitumor agents. J Med Chem 2003;46(14):2813–8.

[180] Nielsen SF, Christensen SB, Cruciani G, Khazazi A, Lijefors T. Antileishmanial chalcones: statistical design, synthesis, and three-dimensional quantitative structure-activity relationship analysis. J Med Chem 1998;41:4819–32.

[181] Lopez SN, Castelli MV, Zaczino SA, Dominguez JN, Lobo G, Chris-Charriss J, et al. In vitro antifungal evaluation and structure-activity relationships of a new series of chalcone derivatives and synthetic analogues, with inhibitory properties against polymers of the fungal cell wall. Bioorg Med Chem 2001;9:1999–2013.

[182] Murakami S, Muramatsu M, Aihara H, Otomo S. Inhibition of gastric H+K+-ATPase by the antiinflammatory agent sulfofon. Biochem Pharmacol 1991;42(7):1447–51.

[183] Deshpande AM, Argade NP, Natu AA, Eckman J. Synthesis and screening of a combinatorial library of naphthalene substituted chalcones: inhibitors of leukotriene B4. Bioorg Med Chem 1999;7(6):1237–40.

[184] Khathib S, Nerya O, Shmoel M, Tamir S, Vaya J. Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. Bioorg Med Chem 2005;13(2):433–41.

[185] Severi F, Benvenuti S, Costantino L, Vampa G, Melegari M, Antolini L. Synthesis and activity of a new series of chalcones as aldose reductase inhibitors. Eur J Med Chem 1999;33:859–66.

[186] Ahari-Mostafavi MM, Shiraf A, Mirzaei M, Amanlou M. Novel and versatile methodology for synthesis of β-aryl-β-mercapto ketone derivatives as potential urease inhibitors. J Iran Chem Soc 2014;11:1113–9.

[187] Batool T, Rasool N, Gull Y, Noreen M, Nasim FUH, Yaoabo A, et al. A convenient method for the synthesis of (Prop-2-Vinloxy benzene derivatives via reaction with propargyl bromide, their optimization, scope and biological evaluation. PLoS ONE 2014;9:1–19.

[188] Xiao ZP, Peng ZY, Dong JJ, Deng RC, Wang XD, Ouyang H, et al. Synthesis, molecular docking and kinetic properties of β-hydroxy-β-phenylpropionyl hydroxamic acids as Helicobacter pylori urease inhibitors. Eur J Med Chem 2013;81:212–21.

[189] Shi WK, Deng RC, Wang PF, Yue QQ, Liu Q, Ding KL, et al. 3-Arylpropionyl hydroxamic acid derivatives as Helicobacter pylori urease inhibitors: Synthesis, molecular docking and biological evaluation. Bioorg Med Chem 2016;24:4519–27.

[190] Sokmen BB, Hasdemir B, Yusufoglu A, Yanardag R. Some monohydroxy tetradecanoin acid isomers as novel urease and elastase inhibitors and as new antioxidants. Appl Biochem Biotechnol 2014;172:1358–64.

[191] Tanaka T, Kawase M, Tanu S. Urease inhibitory activity of simple alpha, beta-unsaturated ketones. Life Sci 2005;73(23):2985–90.

[192] Zhao G, Yu T, Wang R, Wang X, Jing Y. Synthesis and structure-activity relationship of ethylenic acid analogues on glutathione-S-transferase P1–1 activity inhibition. Bioorg Med Chem 2005;13(12):4056–62.

[193] Zhao G, Liu C, Wang R, Song D, Wang X, Lou H, et al. The synthesis of alpha, beta-unsaturated carbonyl derivatives with the ability to inhibit both glutathione S-transferase P1–1 activity and the proliferation of leukemia cells. Bioorg Med Chem 2007;15(7):2701–7.

[194] Janser I, Vortolomei CM, Meka RK, Walsh CA, Janser RJF. Ethylenic acid as a lead structure for the development of potent urease inhibitors. C R Chim 2013;16:660–4.

[195] Tanaka T, Kawase M, Tani S. A-Hydroxyketones as inhibitors of urease. Bioorg Med Chem 2004;12:501–5.

[196] Sirajuddin M, Ali S, Zaib S, Iqbal T, Hadda TB. Design, structural and spectroscopic elucidation and in vitro antitumour, anticancer, antileishmanial, urease inhibition activities and interaction with SS-DNA of newly synthesized amide based carboxylic acid. Inorganica Chim Acta 2015;427:178–87.

[197] Li HQ, Xiao ZP, Yin-Luo, Yan T, Lv PC, Zhu HL. Amines and oximes derived from deoxybenzonios as Helicobacter pylori urease inhibitors. Eur J Med Chem 2009;44:2246–51.

[198] Xiao ZP, Ma TW, Fu WC, Peng XC, Zhang AH, Zhu HL. The synthesis, structure and activity evaluation of pyrogallol and catechol derivatives as Helicobacter pylori urease inhibitors. Eur J Med Chem 2010;45:5064–70.

[199] Xiao ZP, Shi WK, Wang PF, Wei W, Zeng XT, Zhang JR, et al. Synthesis and evaluation of N-alkyl-1,2-diaxylidethane as Helicobacter pylori urease inhibitors. Bioorg Med Chem 2015;23:4508–13.

[200] Aziz-ur-Rehman, Abbasi MA, Rasool S, Ashraf M, Ejaz SA, Hassan R, et al. Synthesis, spectral characterization and enzyme inhibition studies of different chlorinated sulphonamides. Pak J Pharm Sci 2014;27(6):1739–45.
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