Istanbul J Pharm 51 (1): 85-91
DOI: 10.26650/IstanbulJPharm.2021.844783
Original Article

In vitro urease and trypsin inhibitory activities of some sulfur compounds

Eda Dağsuyu[1], Refiye Yanardağ[1]

[1]Istanbul University-Cerrahpasa, Faculty of Engineering, Department of Chemistry, Istanbul, Turkey

ORCID IDs of the authors: E.D. 0000-0003-0395-1058; R.Y. 0000-0003-4185-4363

Cite this article as: Dagsuyu, E., & Yanardag, R. (2021). *In vitro* urease and trypsin inhibitory activities of some sulfur compounds. *İstanbul Journal of Pharmacy, 51*(1), 85-91.

ABSTRACT

Background and Aims: Organosulfur compounds modulate the activities of plurality of metabolic enzymes, especially those that activate (cytochrome P_{450s}) or detoxify (glutathione-S-transferases) carcinogens. They also inhibit the formation of DNA adducts in different target tissues. The aim of the present study was to investigate the effect of some sulfur compounds on urease and trypsin activities *in vitro*.

Methods: In the present study, the inhibitory effect of sulfur compounds on the activities urease and trypsin were determined according to the method of Hanif et al. (2012) and Ribeiro et al. (2010), respectively.

Results: In comparison to the reference standard thiourea (IC_{50}= 53.81±0.68 µg/mL), S-allyl-L-cysteine (IC_{50}= 0.88±0.01 µg/mL) and D, L-methionine (IC_{50}= 0.91±0.02 µg/mL) had the highest urease inhibitor activity, corresponding to the lowest IC_{50} values among the sulfur compounds. Among the sulfur compounds used in this study, D,L-methionine ($IC_{5}0$= 0.13±0.01 mg/mL) exhibited the lowest IC_{50} value for trypsin inhibitor, though its activity was less than that of tannic acid which was used as a standard (IC_{50}= 0.06±0.01 mg/mL).

Conclusion: The present outcome suggests that sulfur compounds are potential inhibitors of urease and trypsin activities, and may find importance in medicine and agriculture.

Keywords: Enzyme, inhibitors, sulfur compounds, urease, trypsin

INTRODUCTION

Urease (urea amidohydrolase, EC 3.5.1.5) is an enzyme that catalyzes the hydrolysis of urea to ammonia and carbon dioxide or carbamate. This enzyme contains nickel ion in its active center (Saeed et al., 2017). Ureases are found in soil, higher plants, algae, fungi, bacteria and in invertebrates (Krajewska, 2009). The enzymes are important in the pathogenesis of many clinical conditions that are harmful to humans, animals, and crops. Increasing urease activity in agriculture causes important environmental and economic challenges. The ammonia released by the enzyme damages plants and soil (Amtul et al., 2006). Urease is a virulence factor found in a variety of pathogenic bacteria. *Helicobacter pylori* urease, one of the most studied bacterial ureases, causes gastritis, peptic ulceration and gastric cancer (Cox, Mukherjee, Cole, Casadevall, & Perfect 2000). Additionally, urease causes kidney stones formation and contributes to the pathogenesis of urolithiasis, hepatic coma, urinary catheter, encrustation, pyelonephritis, ammonia and hepatic encephalopathy, as well as reactive arthritis (Mobley, Island, & Hausinger, 1995; Ragsdale, 2009). Urease inhibitors are therapeutically important anti-ulcer drugs and are used to subdue microbial virulence (Onoda, Takido, Magaribuchi, & Tamaki, 1990). Since urease plays a key role in medicine and agriculture, its inhibitors are paramount for reducing the increased activity of the enzyme. Several classes of compounds have been shown to be urease inhibitors. Hydroxamate complex (Cheng, Zhang, You, Wang & Hai-Hua, 2014), homoserine lactone derivatives (Czerwonka et al., 2014), quinolones, oxadiazoles derivatives (Akhtar, Khan, Iqbal, Jones, & Hameed, 2014), oxindole derivatives (Taha et al., 2015), thiobarbituric acid derivatives

Address for Correspondence:
Refiye YANARDAĞ, e-mail: yanardag@istanbul.edu.tr

Submitted: 21.12.2020
Revision Requested: 14.01.2021
Last Revision Received: 14.01.2021
Accepted: 15.01.2021

(Khan et al., 2014a), pyrogallol and catechol (Xiao, Ma & Zhu, 2010), 1,2,4-triazole and 1,3,4-thiadiazole derivatives (Khan et al., 2010), 3,4,5-trihydroxybenzohydrazone (Taha et al., 2019), benzophenone semicarbazones/thiosemicarbazones (Arshia et al., 2016), and thioureas (Khan et al., 2014b) have already been investigated. However, current inhibitors are inefficient and may have toxic effects. For this reason, the search for new and effective inhibitors continues.

Trypsin (EC 3.4.21.4) is a digestive enzyme produced by pancreatic acinar cells and belongs to a class of enzymes called serine proteases. Serine proteases are involved in processes such as food digestion, blood coagulation, fibrinolysis, blood pressure control, protein maturation, and immune response. These enzymes also play important roles in a wide variety of important pathological processes such as atherosclerosis, inflammation and cancer (Borg, 2004). The pancreatic serine endoprotease activity of trypsin is involved in protein digestion, specifically by cleaving peptidic bonds on the C-terminal group of lysine or arginine (Kang, Kana, Yeung & Liu, 2006). It acts in the duodenum by hydrolyzing peptide bonds and breaking down proteins into smaller peptides, after which the peptide products are hydrolyzed to amino acids via the action of other proteases (Rawlings & Barrett, 1994; Byrne, et al., 2002).

Trypsin inhibitors are used in the treatment of pancreatitis shock and disseminated intravascular coagulation (Inoue & Takano, 2010). Plants have been good sources of trypsin inhibitors (VanderJagt, et al., 2000). For example, high concentrations of trypsin inhibitors are found in Fabaceae seeds as well as other plant tissues (Mosolov & Valueva, 2005; Ruan, Chen, Shao, Wu, & Han, 2011). More so, different inhibitors have been synthesized for trypsin and trypsin-like enzymes (Mares-Guia & Shaw, 1965; Markwardt, Landman, & Walsmann, 1968; Evans, Olson, & Shore, 1982; Toyota, Chinen, Sekizaki, Itoh, & Tanizawa, 1996; Liu, Jiang, Luo, Yan, & Shen, 1998; Venkatesin & Sundanam, 1998; Toyota, et al., 2001). Unfortunately, the synthesized compounds have side effects, therefore prompting continual studies in search for new and safer antitrypsin compounds/inhibitors. The aim of this study was to examine the inhibitory potential of some sulfur compounds on urease and trypsin activities.

MATERIALS AND METHODS

In vitro urease inhibitory activity

Urease inhibitory activity was assayed spectrophotometrically by the method of Hanif et al. (2012). In brief, phosphate buffer (0.1 M, pH 7.50, and containing 0.1 M urea), test compound and enzyme (5 Unit/mL) were incubated for 10 minutes at 37°C. Solutions of phenol reagent and alkali reagent were thereafter added to each well. Then, the reaction mixture was incubated for 10 minutes at 37°C again. Absorbance levels were read at 625 nm. Thiourea was used as standard. Percentage inhibition was calculated using the following formula:

$$\text{Urease inhibitory activity (\%)} = \left[\left(\frac{A-B}{A}\right) \times 100\right]$$

A is the enzyme activity without inhibitor.

B is the activity in presence of inhibitor.

In vitro trypsin inhibitory activity

Trypsin inhibitory activity was determined by the method of Ribeiro et al., which depends on the hydrolysis of N-α-benzoyl-DL-arginine-p-nitroanilide (BAPNA) by trypsin (Ribeiro, Cunha, & Sales, 2010). The test reaction mixture comprised of a sample at varying concentrations, trypsin solution (0.3 mg/mL from bovine pancreas), 2.5 mM HCl and 50 mM potassium phosphate buffer (pH 7.50). The reaction mixture was incubated at 37ºC for 10 minutes, followed by the addition of BAPNA (2.5 mM) solution to the reaction mixture and further incubation for 15 minutes at 37ºC. The reaction was stopped by adding 30% acetic acid solution. The absorbance of the samples was measured at 410 nm and tannic acid was used as positive control. The percentage of trypsin inhibitory activity was determined according to the following equation:

$$\text{Trypsin inhibitory activity (\%)} = \left[\left(\frac{A-B}{A}\right) \times 100\right]$$

A is the enzyme activity without inhibitor.

B is the activity in presence of inhibitor.

The IC_{50} was determined as the concentration of sulfur compounds required to inhibit urease and trypsin activity by 50%. Percentage enzyme inhibition activities of the inhibitors were used to calculate half maximum inhibitions (IC_{50}) for individual enzymes, via regression analysis data. The lower the IC_{50} values, the higher the inhibition activity.

RESULTS AND DISCUSSION

The inhibitory effects of some sulfur compounds on urease and their respective (IC_{50}) values are shown in Table 1.

The urease inhibitory activity of sulfur compounds were investigated *in vitro*, in comparison with thiourea. In order to quantify the urease inhibitory activity, the half maximal inhibitory concentration (IC_{50}) values were calculated as shown in Table 1. All the sulfur compounds used in the present study inhibited urease activity (Table 1), in a dose-dependent manner. Low IC_{50} values indicate that the enzyme is highly inhibited. Among the sulfur compounds used, S-allyl-L-cysteine (IC_{50}= 0.88±0.01 µg/mL) and D, L-methionine (IC_{50}= 0.91±0.02 µg/mL) demonstrated higher urease inhibitory activity than that of the reference standard thiourea (IC_{50}= 53.81±0.68 µg/mL). N-acetyl-L-cysteine (IC_{50}= 65.55±0.89 µg/mL), S-benzyl-L-cystein (IC_{50}= 93.17±3.54 µg/mL) and L-cystine (IC_{50}= 120.96±7.55 µg/mL) also showed promising inhibitions against urease enzyme activity. The urease inhibitory activities of the sulfur compounds used in the present study decreased in the order of: S-allyl-L-cysteine > D,L-methionine > thiourea > N-acetyl-L-cysteine > S-benzyl-L-cysteine > L-cystine > D,L-homocysteine > L-cysteine hydrochloride > S-methyl-L-cysteine > taurine > L-alliin > vitamin U > S-phenyl-L-cysteine (Table 1).

Table 1. Inhibitory activities of sulfur compounds on urease.

Compounds/Standard	Concentration (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)*
N-Acetyl-L-cysteine	500	97.42±1.55	65.55±0.89
	100	76.29±1.03	
	10	35.05±3.09	
	1	23.71±0.01	
	0.1	14.95±0.52	
L-Alliin	5000	96.56±1.15	562.16±7.08
	2500	83.21±0.76	
	1000	74.43±1.15	
	500	45.04±0.01	
	250	32.82±3.05	
S-Allyl-L-cysteine	1000	97.29±0.96	0.88±0.01
	500	74.77±0.46	
	1	57.02±0.88	
	0.01	45.87±0.92	
	0.001	31.31±3.78	
S-Benzyl-L-cysteine	5000	87.07±1.36	93.17±3.54
	2500	75.85±1.70	
	500	62.93±0.34	
	100	53.74±2.04	
	10	41.16±0.34	
L-Cysteine hydrochloride	1000	95.45±1.14	290.22±28.57
	500	69.89±0.57	
	100	46.02±5.11	
	10	34.09±0.01	
	1	15.34±2.84	
L-Cystine	100	42.16±1.96	120.96±7.55
	50	30.88±2.45	
	25	25.49±1.96	
	10	19.12±2.45	
	1	10.29±1.47	
D,L-Homocysteine	500	92.13±0.46	226.82±6.78
	250	68.52±1.85	
	100	21.30±0.93	
	10	12.96±1.85	
	1	6.94 ±1.39	
D,L-Methionine	1000	98.60±0.01	0.91±0.02
	500	84.27±0.35	
	100	67.83±0.01	
	1	55.24±1.40	
	0.001	30.07±3.50	
S-Methyl-L-cysteine	500	62.00±1.00	339.19±22.37
	1	38.00±1.00	
	0.1	26.50±4.50	
	0.01	19.00±3.00	
	0.001	14.00±4.00	
S-Phenyl-L-cysteine	5000	70.86±0.01	3263.57±46.26
	2500	39.07±1.32	
	500	24.17±0.33	
	250	16.56±0.66	
	100	3.97±0.66	
Taurine	2500	90.08±0.83	509.30±21.40
	1000	69.42±0.83	
	500	49.17±2.07	
	100	30.17±0.41	
	10	15.70±1.65	

Table 1. Continue.

Compounds/Standard	Concentration (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)*
Thiourea **	250	84.12±0.59	53.81±0.68
	100	54.71±4.12	
	50	46.47±0.59	
	10	38.24±0.59	
	1	22.94±1.76	
Vitamin U	10000	97.75±0.01	899.82±27.27
	5000	70.79±2.25	
	1000	55.62±1.69	
	10	36.52±1.69	
	0.1	21.35±0.01	

* Mean ± SD of triplicate values; ** It means standard.

Urease has the potential to be used in anti-ulcer drugs (Krajewska, 2009). Sulfur containing compounds such as thiourea, thiosemicarbazone, and thiocarbonyl compounds can form chelate with transition metal ions. Some thiosemicarbazone and thiourea derivatives have been reported to be efficient urease inhibitors (Pervez, Chohan, Ramzan, Nasim & Khan, 2009; Arshia et al., 2016; Pervez et al., 2018; Islam, et al., 2019; Li et al., 2020; Shehzad et al., 2020). Thiosemicarbazone derivatives strongly inhibit the enzyme. This is because the sulfur atom in the structure of the compound binds two nickel ions of the enzyme's active site, thereby hindering its activity. Many thiourea derivatives are known to be urease inhibitors. This is also due to their ability to chelate nickel ions at the enzyme's active sites (Kanwal et al., 2019; Kumar & Kayastha, 2010; Li et al., 2018). Islam et al. (2019) demonstrated that thiosemicarbazone derivatives highly inhibited the urease enzyme. The strong urease inhibition effect of methionine and S-allyl-L-cysteine as seen in the present study may be linked to the ability of the compounds sulphur ion to easily and strongly bind to the nickel ion of the urease enzyme active site and thus inhibiting the enzyme.

The percentage inhibitory effect and half maximal inhibitory concentration (IC_{50}) values effects of some sulfur compounds on trypsin activity are presented in Table 2.

The *in vitro* trypsin inhibitory effect of sulfur compounds, in comparison with tannic acid, are presented in Table 2. Lower (IC_{50}) values indicate greater trypsin inhibitory activity. All the tested compounds exhibited trypsin inhibition activities, except S-benzyl-L-cysteine and S-phenyl-L-cysteine. D,L-methionine (IC_{50}= 0.13±0.01 mg/mL) exhibited the highest trypsin inhibition activity among the sulfur compounds used in the present study. The trypsin inhibitory activities of the sulfur compounds decreased in the order of: tannic acid > D,L-methionine > taurine > S-allyl-L-cysteine > N-acetyl-L-cysteine > L-alliin > L-cysteine hydrochloride > D,L-homocysteine > vitamin U > S-methyl-L-cysteine > L-cystine. D,L-methionine (IC_{50}= 0.13±0.01 mg/mL), taurine (IC_{50}= 0.55±0.08 mg/mL), S-allyl-L-cysteine (IC_{50}= 1.27±0.21 mg/mL), N-acetyl-L-cysteine (IC_{50}= 2.60±0.17 mg/mL) and L-alliin (IC_{50}= 7.45±0.22 mg/mL) showed better inhibitions against trypsin activity.

Excessive activity of trypsin is strongly implicated in many diseases such as acute pancreatitis, inflammation and tumour formation. The balanced activity of trypsin is necessary for different physiological functions. The abnormal activity of proteolytic enzymes causes disorders such as pulmonary emphysema, arthritis, muscle dysentery, pancreatitis and cancer (Shahwar, Raza, Rehman, Abbasi & Rahman, 2012). Managing

Table 2. Inhibitory activities of sulfur compounds on trypsin.

Compounds/Standard	Concentration (mg/mL)	Inhibition (%)*	IC_{50} (mg/mL)*
N-Acetyl-L-cysteine	5	91.26±2.20	2.60±0.17
	2.5	38.25±2.36	
	1	33.06±3.65	
	0.1	22.95±1.29	
	0.01	4.10±0.45	
L-Alliin	5	37.66±0.60	7.45±0.22
	3.75	21.63±1.18	
	2.5	13.14±3.34	
	1	6.89±1.13	
	0.1	2.73±0.98	
S-Allyl-L-cysteine	0.25	16.58±1.43	1.27±0.21
	0.1	13.11±0.45	
	0.05	10.38±1.55	
	0.01	9.47±1.29	
	0.00001	7.83±1.36	

Table 2. Continue.

Compounds/Standard	Concentration (mg/mL)	Inhibition (%)*	IC_{50} (mg/mL)*
S-Benzyl-L-cysteine	5 2.5 1 0.1 0.01	N.D.	N.D.
L-Cysteine hydrochloride	25 10 1 0.0001 0.000001	98.78±1.44 72.26±1.04 41.16±3.74 26.52±2.35 3.96±0.50	8.00±0.66
L-Cystine	0.1 0.09 0.08 0.07 0.06	30.33±4.83 15.85±2.11 9.56±1.34 4.10±1.34 1.37±0.45	155.51±14.13
D,L-Homocysteine	5 4 1 0.25 0.01	26.50±1.05 20.85±2.69 14.70±2.72 10.94±1.28 6.67±0.73	12.32±1.00
D,L-Methionine	0.1 0.01 0.005 0.0025 0.0001	40.00±1.95 30.81±1.08 15.86±1.56 11.35±2.16 7.21±2.67	0.13±0.01
S-Methyl-L-cysteine	50 1 0.5 0.25 0.01	49.74±1.21 37.69±4.78 19.74±2.93 10.77±1.67 1.54±0.24	51.37±1.93
S-Phenyl-L-cysteine	5 1 0.5 0.1 0.01	N.D.	N.D.
Tannic acid**	0.1 0.075 0.05 0.025 0.01	85.50±0.71 51.50±0.71 47.83±0.62 41.17±1.55 7.67±0.24	0.06±0.01
Taurine	1 0.01 0.0001 0.00001 0.0000001	69.82±2.49 43.60±5.77 34.15±1.75 31.71±1.72 5.49±1.75	0.55±0.08
Vitamin U	50 25 10 1 0.01	99.36±0.23 78.21±0.60 39.10±2.67 29.81±3.49 12.66±0.48	16.07±1.11

* Mean ± SD of triplicate values; ** It means standard; N.D. means not detected.

or treating such diseases with protease inhibitors obtained from natural sources provides an important goal in pharmaceutical research (Frecer, Maliar, & Miertus, 2000; Maliar, Jedinak, Kadrabova, & Sturdik, 2004; Tossi, Bonin, & Anthceva, 2000). Studies on the inhibitory effects of plant polyphenols and other phytochemicals on trypsin activity have been ongoing (Rohn, Rawel, & Kroll, 2002; Klomklao, Benjakul, Kishimura & Chaijan, 2011; Shahwar et al., 2012). The various inhibitors bind to the amino acids (serine, histidine and aspartate) at the active center of the enzyme, therefore causing inhibition of the enzyme. Among the sulfur compounds examined in the present study, methionine, taurine and S-ally-L-cysteine, which are the

sulfur amino acids, were the most active inhibitors of trypsin. These substances make the enzyme inactive by binding to the serine amino acid in the active site of the enzyme.

Methionine, a precursor of succinyl-CoA, homocysteine, cysteine, creatine, and carnitine, is an essential sulfur-containing amino acid. It is also known to inhibit oxidative damage in various tissues. It prevents DNA damage, cancer, cardiovascular diseases, neuropsychiatric disorders and neurodegenerative diseases. In addition, it plays an important role in detoxification, since it removes sulfur containing sections by chelation (Patra, Swarup, & Dwivedi, 2001; Martinez et al., 2017). Therefore, its aforementioned medical significance coupled with its strong trypsin inhibition may go a long way in managing and subsiding degenerative diseases if properly exploited.

CONCLUSIONS

The results of the present study indicate that sulfur-containing compounds are effective inhibitors of urease and trypsin activities. The highest inhibitory effect was exhibited by methionine. Therefore, methionine can be suggested to have an important role in enzyme inhibition, as well as in preventing diseases that may arise due to hyper activities of these enzymes.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.D., R.Y.; Data Acquisition- E.D., R.Y.; Data Analysis/Interpretation- E.D., R.Y.; Drafting Manuscript- E.D., R.Y.; Critical Revision of Manuscript-R.Y.; Final Approval and Accountability- E.D., R.Y.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: Authors declared no financial support.

REFERENCES

- Akhtar, T., Khan, M. A., Iqbal, J., Jones, P. G. & Hameed, S. (2014). A facile one-pot synthesis of 2-arylamino-5-aryloxylalkyl-1,3,4-oxadiazoles and their urease inhibition studies. *Chemical Biology & Drug Design*, *84*, 92-98. https://doi.org/10.1111/cbdd.12297
- Amtul, Z., Kausar, N., Follmer, C., Rozmahel, R. F., Atta-Ur-Rahman, Kazmi, S. A. ... Choudhary, M. I. (2006). Cysteine based novel non-competitive inhibitors of urease(s)—Distinctive inhibition susceptibility of microbial and plant ureases. *Bioorganic & Medicinal Chemistry*, *14*, 6737-6744. https://doi.org/10.1016/j.bmc.2006.05.078
- Arshia, A., Khan, A., Khan, K. M. Saad, S. M., Siddiqui, S. J., Perveen, S., & Choudhary, M. I. (2016). Synthesis and urease inhibitory activities of benzophenone semicarbazones/thiosemicarbazones. *Medicinal Chemistry Research*, *25*, 2666–2679. https://doi.org/10.1007/s00044-016-1673-0
- Borg T. K. (2004). It's the matrix! ECM, proteases, and cancer. *The American Journal of Pathology*, *164*, 1141–1142. https://doi.org/10.1016/S0002-9440(10)63201-4
- Byrne, M. F. Mitchell, R. M., Stiffler, H., Jowell, P. S., Branch, M. S., Pappas, T. N... Baillie J. (2002). *Canadian Journal of Gastroenterology and Hepatology*, *16*, 849-854.
- Cheng, X. S., Zhang, J. C., You, Z. L., Wang, X., & Hai-Hua Li. (2014). Synthesis, structures, and Helicobacter Pylori urease inhibition of hydroxamate-coordinated oxovanadium complexes with benzohydrazone ligands. *Transition Metal Chemistry*, *39*, 291–297. https://doi.org/10.1007/s11243-014-9802-4
- Cox, G. M., Mukherjee, J., Cole, G. T., Casadevall, A., & Perfect, J. R. (2000). Urease as a virulence factor in experimental cryptococcosis. *Infection and Immunity*, *68*, 443–448. https://doi.org/10.1128/iai.68.2.443-448.2000
- Czerwonka, G., Arabski, M., Wąsik, S., Jabłońska-Wawrzycka, A., Rogala, P., & Kaca, W. (2014). Morphological changes in Proteus mirabilis O18 biofilm under the influence of a urease inhibitor and a homoserine lactone derivative. *Archives of Microbiology*, *196*, 169-177. doi: 10.1007/s00203-014-0952-8
- Evans, S. A., Olson, S. T., & Shore, J. (1982). p-Aminobenzamidine as a fluorescent probe for the active site of serine proteases. *Journal of Biological Chemistry*, *257*, 3014–3017.
- Frecer, V., Maliar, T. & Miertus, S. (2000). Protease inhibitors as anticancer drugs: Role of molecular modelling and combinatorial chemistry in drug design. *International Journal of Medicine, Biology and the Environment*, *28*, 161-173.
- Hanif, M., Shoaib, K., Saleem, M., Hasan Rama, N., Zaib, S., & Iqbal, J. (2012). Synthesis, urease inhibition, antioxidant, antibacterial, and molecular docking studies of 1,3,4-oxadiazole derivatives. *International Scholarly Research Notices: Pharmacology*, 928901. https://doi.org/10.5402/2012/928901
- Inoue, K. I., Takano, H. (2010). Urinary trypsin inhibitor as a therapeutic option for endotoxin-related inflammatory disorders. *Expert Opinion on Investigational Drugs*, *19*, 513–520. https://doi.org/10.1517/13543781003649533
- Islam, M., Khan, A., Shehzad, M. T., Hameed, A., Ahmed, N., Halim, S. A. … Al-Harrasi, A. (2019). Synthesis and characterization of new thiosemicarbazones, as potent urease inhibitors: In vitro and in silico studies. *Bioorganic Chemistry, 87*, 155–162. https://doi.org/10.1016/j.bioorg.2019.03.008
- Kang, K., Kana, C., Yeung, A., & Liu, D. (2006). The immobilization of trypsin on soap-free P(MMA-EA-AA) latex particles. *Materials Science and Engineering: C*, *26*, 664–669. https://doi.org/10.1016/j.msec.2005.07.020
- Kanwal,-., Khan, M., Arshia,-., Khan, K. M., Parveen, S., Shaikh, M., Fatima, N., Choudhary, M. I. (2019). Syntheses, in vitro urease inhibitory activities of urea and thiourea derivatives of tryptamine, their molecular docking and cytotoxic studies, *Bioorganic Chemistry*, *83*, 595–610. https://doi.org/10.1016/j.bioorg.2018.10.070
- Khan, I., Ali, S., Hameed, S., Rama, N. H., Hussain, M. T., Wadood, A. … Choudhary, M. I. (2010). Synthesis, antioxidant activities and urease inhibition of some new 1,2,4-triazole and 1,3,4-thiadiazole derivatives. *European Journal of Medicinal Chemistry*, *45*, 5200-5207. https://doi.org/10.1016/j.ejmech.2010.08.034.
- Khan, M. K., Rahim, F., Khan, A., Shabeer, M., Hussain, S., Rehman, W. ... Choudhary, M.I. (2014a). Synthesis and structure–activity relationship of thiobarbituric acid derivatives as potent inhibitors of urease, *Bioorganic & Medicinal Chemistry*, *22*, 4119–4123. https://doi.org/10.1016/j.bmc.2014.05.057
- Khan, K. M., Naz, F., Taha, M., Khan, A., Perveen, S., Choudhary, M. I., & Voelter, W. (2014b). Synthesis and in vitro urease inhibitory activity of N,N′-disubstituted thioureas, *European Journal of Medicinal Chemistry*, *74*, 314–323. https://doi.org/10.1016/j.ejmech.2014.01.001.
- Klomklao, S., Benjakul, S., Kishimura, H., & Chaijan, M. (2011). Extraction, purification and properties of trypsin inhibitor from Thai mung bean (*Vigna radiata* (L.) R. Wilczek), *Food Chemistry*, *129*, 1348–1354. https://doi.org/10.1016/j.foodchem.2011.05.029
- Kumar, S., & Kayastha, A.M. (2010). Soybean (Glycine max) urease: significance of sulfhydryl groups in urea catalysis. *Plant Physiology and Biochemistry*, 48, 746-750. doi: 10.1016/j.plaphy.2010.05.007
- Krajewska, B. (2009). Ureases I. Functional, catalytic and kinetic properties: A review. *Journal of Molecular Catalysis B: Enzymatic*, *59*, 9–21. https://doi.org/10.1016/j.molcatb.2009.01.003

- Li, C., Huang, P., Wong, K., Xu, Y., Tan, L., Chen, H. … Xie, J. (2018). Coptisine-induced inhibition of Helicobacter pylori: elucidation of specific mechanisms by probing urease active site and its maturation process. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *33*, 1362–1375. https://doi.org/10.1080/14756366.2018.1501044
- Li, W. Y., Ni, W. W., Ye, Y. X., Fang, H. L., Pan, X. M., He, J. L. … Zhu, H. L. (2020). N-monoarylacetothioureas as potent urease inhibitors: synthesis, SAR, and biological evaluation. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *35*, 404–413. doi: 10.1080/14756366.2019.1706503
- Liu, J. Q., Jiang, M. S., Luo, G. M., Yan, G. L., & Shen, J. C. (1998). Conversion of trypsin into a selenium containing enzyme by using chemical mutation. *Biotechnology Letters*, *20*, 693–696. https://doi.org/10.1023/A:1005378709179
- Maliar, T., Jedinak, A., Kadrabova, J., & Sturdik, E. (2004). Structural aspects of flavonoids as trypsin inhibitors. *European Journal of Medicinal Chemistry*, 39, 241–248. doi: 10.1016/j.ejmech.2003.12.003
- Mares-Guia, M. & Shaw, E. J. (1965). Studies on the active center of trypsin. The binding of amidines and guanidines as models of the substrate side chain. *Journal of Biological Chemistry*, *240*, 1579–1585.
- Markwardt, F., Landman, H., & Walsmann, P. (1968). Comparative studies on the inhibition of trypsin, plasmin and thrombin by derivatives of benzylamine and benzamidine. *European Journal of Biochemistry*, 6, 502–506. doi: 10.1111/j.1432-1033.1968.tb00473.x
- Martínez, Y., Li, X., Liu, G., Bin, P., Yan, W., Más, D. … Yin, Y. (2017). The role of methionine on metabolism, oxidative stress, and diseases. *Amino Acids*, 49, 2091–2098. doi: 10.1007/s00726-017-2494-2
- Mobley, H. L., Island, M. D., & Hausinger, R. P. (1995). Molecular biology of microbial ureases. *Microbiological reviews*, *59*, 451–480.
- Mosolov, V. V. & Valueva, T. A. (2005). Proteinase inhibitors and their function in plants: a review. *Applied Biochemistry and Microbiology*, *1*, 227–246. https://doi.org/10.1007/s10438-005-0040-6
- Onoda, Y., Takido, M., Magaribuchi, T., & Tamaki, H. (1990). Effects of 12-sulfodehydroabietic acid monosodium salt (ta-2711), a new anti-ulcer agent, on gastric mucosal lesions induced by necrotizing agents and gastric mucosal defensive factors in rats, *The Japanese Journal of Pharmacology*, *52*, 631–638. https://doi.org/10.1254/jjp.52.631
- Patra, R. C., Swarup, D., Dwivedi, S. K. (2001). Antioxidant effects of α tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. *Toxicology*, *162*, 81–88. doi: 10.1016/s0300-483x(01)00345-6
- Pervez, H., Chohan, Z. H., Ramzan, M., Nasim, F. H, & Khan, K. M. (2009). Synthesis and biological evaluation of some new N4-substituted isatin-3-thiosemicarbazones. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*, 437–446. https://doi.org/10.1080/14756360802188420
- Pervez, H., Khan, N., Iqbal, J., Zaib, S., Yaqub, M., Tahir, M. N., & Naseer, M. M. (2018). Synthesis, crystal structure, molecular docking studies and bio-evaluation of some N4-benzyl-substituted isatin- 3-thiosemicarbazones as urease and glycation inhibitors. *Heterocyclic Communications*, *24*, 51–58. doi: https://doi.org/10.1515/hc-2017-0148
- Ragsdale S. W. (2009). Nickel-based enzyme systems. *Journal of Biological Chemis*try, *284*, 18571–18575. doi: 10.1074/jbc.R900020200
- Rawlings, N. D. & Barrett, A. J. (1994). Families of serine peptidases, *Methods in Enzymology*, *24*, 19–61. doi: 10.1016/0076-6879(94)44004-2
- Ribeiro, J. K., Cunha, D. D., Fook, J. M., & Sales, M. P. (2010). New properties of the soybean trypsin inhibitor: Inhibition of human neutrophil elastase and its effect on acute pulmonary injury. *European Journal of Pharmacology*, *644*, 238–244. doi: 10.1016/j.ejphar.2010.06.067
- Rohn, S., Rawel, H. M., Kroll, J. (2002). Inhibitory effects of plant phenols on the activity of selected enzymes, *Journal of Agricultural and Food Chemistry*, *50*, 3566-3571. doi: 10.1021/jf011714b
- Ruan, J. J., Chen, H., Shao, J. R., Wu, Q. & Han, X. Y. (2011). An antifungal peptide from Fagopurum tataricum seeds. *Peptides*, *32*, 1151-1158. https://doi.org/10.1016/j.peptides.2011.03.015
- Saeed, A., Ur-Rehman, S., Channar, P., Larik, F., Abbas, Q., Hassan, M. ... Seo, S. Y. (2017). Jack bean urease inhibitors, and antioxidant activity based on palmitic acid derived 1-acyl-3- arylthioureas: synthesis, kinetic mechanism and molecular docking studies. *Drug Research*, *67*, 596–605. doi: 10.1055/s-0043-113832
- Shahwar, D., Raza, M. A., Rehman, S. U., Abbasi, M. A., & Rahman, A. U. (2012). An investigation of phenolic compounds from plant sources as trypsin inhibitors, *Natural Product Research: Formerly Natural Product Letters*, 26, 1087–1093.
- Shehzad, M. T., Khan, A., Islam, M., Hameed, A., Khiat, M., Halim, S. A. … Shafiq, Z. (2020). Synthesis and urease inhibitory activity of 1,4-benzodioxane-based thiosemicarbazones: Biochemical and computational approach. *Journal of Molecular Structure*, *1209*, 127922. https://doi.org/10.1016/j.molstruc.2020.127922
- Taha, M., Ismail, N. H., Baharudin, M. S., Lalani, S., Mehboob, S., Khan, K. M. ... Choudhary, I. (2015). Synthesis crystal structure of 2-methoxybenzoylhydrazones and evaluation of their α-glucosidase and urease inhibition potential. *Medicinal Chemistry Research*, *24*, 1310–1324. https://doi.org/10.1007/s00044-014-1213-8
- Taha, M., Shah, S. A. A., Khan, A., Arshad, F., Ismail, N. H., Afifi, M., & Choudhary, M. I. (2019). Synthesis of 3,4,5-trihydroxybenzohydrazone and evaluation of their urease inhibition potential, *Arabian Journal of Chemistry*, *12*, 2973–2982. https://doi.org/10.1016/j.arabjc.2015.06.036
- Tossi, A., Bonin, I. & Anthceva, N. (2000). Aspartic protease inhibitors. An integrated approach for the design and synthesis of diaminodiol-based peptidomimetics. *European Journal of Biochemistry*, 267, 1715–1722. https://doi.org/10.1046/j.1432-1327.2000.01164.x
- Toyota, E., Chinen, C., Sekizaki, H., Itoh, K., & Tanizawa, K. (1996). Application of spontaneous Schiff base copper chelates formation process to the design of a trypsin inhibitor. *Chemical and Pharmaceutical Bulletin*, *44*, 1104–1106. https://doi.org/10.1248/cpb.44.1104
- Toyota, E., Ng, K. K., Sekizaki, H., Itoh, K., Tanizawa, K., & James, M. N. G. (2001). X-ray crystallographic analyses of complexes between bovine b-trypsin and schiff base copper(II) or iron(III) chelates. *Journal of Molecular Biology*, *305*, 471–479. doi: 10.1006/jmbi.2000.4303
- VanderJagt, D., Freiberger, C., Vu, H. T., Mounkaila, G., Glew R. S. & Glew R. H. (2000). The trypsin inhibitor content of 61 wild edible plant foods of Niger. *Plant Foods for Human Nutrition*, *55*, 335–346. https://doi.org/10.1023/A:1008136100545
- Venkatesin, R. & Sundanam, R. V. (1998). Modulation of stability properties of bovine trypsin after in vitro structural changes with a variety of chemical modifiers. *Protein Engineering, 11*, 691–698. doi: 10.1093/protein/11.8.691
- Xiao, Z., Ma, T. W., Fu, W. C., Peng, X. C., Zhang, A. H., Zhu, H. L. (2010). The synthesis, structure and activity evaluation of pyrogallol and catechol derivatives as *Helicobacter pylori* urease inhibitors. *European Journal of Medicinal Chemistry*, *45*, 5064–5070. https://doi.org/10.1016/j.ejmech.2010.08.01