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Synthesis and urease inhibition studies of ureas and thioureas derived from amino acids conjugated heterocycle

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ABSTRACT

A series of amino acids-2,3-dichlorophenyl piperazine conjugates derived ureido and thioureido derivatives were synthesized and confirmed the structure by physical and spectroscopical studies. Their urease inhibitory property was evaluated against jack bean urease *in vitro*. Preliminary results showed that most of the derivatives have significant antiurease activity with the IC_{50} values < 10 µM. Most importantly, compounds bearing fluoro group at the para position on the phenyl ring exhibited exclusive urease inhibition activity with IC_{50} < 5 µM as against the standard inhibitor, thiourea (21.0 µM). This work provided a series of derivatives based on amino acids-heterocycle conjugates with highly potent antiurease activity.

Keywords: Amino acids, Piperazine, Conjugation, Urea/thiourea, Urease inhibitors.

1. INTRODUCTION

In humans, *Helicobacter pylori* is highly associated with a number of diseases of the upper gastrointestinal tract including chronic superficial gastritis, duodenal gastric ulcers, gastric adenocarcinoma and non-Hodgkin's lymphomas of the stomach. Urease produced by *Helicobacter pylori* is widely distributed in nature and is found in a variety of plants, algae, fungi and bacteria. This enzyme is directly involved in the formation of stones and contributes to the pathogenesis of urolithiasis, pylenephritis, ammonia and hepatic encephalopathy, hepatic coma and urinary catheter encrustation [1]. Urease (urea amidohydrolase, E.C. 3.5.1.5) is an enzyme that catalyzes the hydrolysis of urea to ammonia and carbamate, which is the final step of nitrogen metabolism in living organisms. Carbamate rapidly and spontaneously decomposes, yielding a second molecule of ammonia [2]. These reactions may cause significant increase in pH and are responsible for negative effects of urease activity in human health and agriculture. Urease is responsible for urinary tract and gastrointestinal infections by releasing ammonia [3]. Furthermore high concentration of ammonia disturbs mucosal permeability, in particular hydrogen ions passage through mucosal surface and cause formation of peptic ulcers [4]. In this respect, increasing attention has been focused on the search for compounds that will inhibit urease activity for the possible development of highly needed therapy for urease mediated bacterial infections [5].

Piperazine analogues, a versatile group of heterocycles are known chemotherapeutic agents. The pharmaceutical importance of these compounds lies in the fact that they are important pharmacophores that can be found in many marketed drugs such as Crixivan, Ranolazine, Fluphenazine and many drugs under development. Piperazine derivatives have found wide clinical applications in the therapy of functional diseases and exhibit insecticidal, antidiabetic, antimicrobial, acetyl cholinesterase inhibitors, antimalarial, dopamine transporter, D_2/D_4 antagonist, MC_4 receptor and HIV-protease inhibitor [6-13]. On the other hand, amino acids/peptides conjugated heterocycles and their derivatives constitute an important class of compounds in the field of medicinal chemistry because of their broad spectrum of biological activities like antiglycation, antidiabetic, antimicrobial, anti-inflammatory and analgesic [14-18].

Several classes of compounds are known to exhibit significant inhibitory activities against urease enzyme. Among them, urea and thiourea compounds are the best recognized urease inhibitors [19, 20]. These are the functional moieties that are commonly found in natural products and often display a wide range of biological activities. Many of urea and thiourea derivatives have found applications as HIV-1 protease inhibitors, oral antidiabetic agents, antimicrobial, algaecides, antiglycating agents, anti-inflammatory and

inhibitory activities against NO· production in lipopolysaccharide-activated macrophages [21].

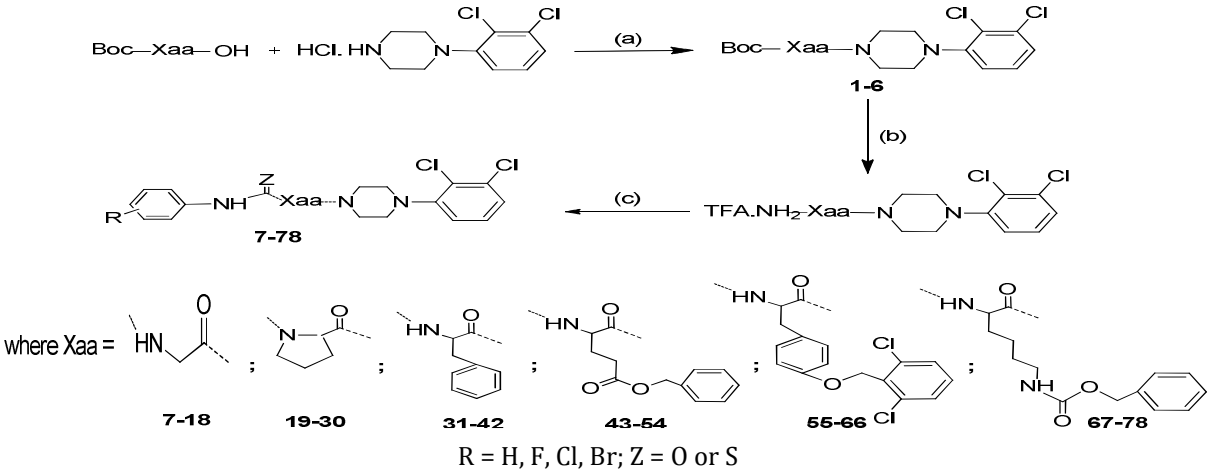
As part of the on-going programme in developing novel bioactive molecules [22-26], we have synthesized a series of glycine and proline conjugated heterocycle and tested for their antiglycation activity. The results were promising and motivated by this fact, we have also tested a few derivatives of these conjugates for their antiurease potential (unpublished). Interestingly, these compounds exhibited significant results and hence we herein report urease inhibitory activities of a small library of urea and thiourea derivatives of amino acids conjugated to 2,3-dichlorophenyl piperazine.

2. MATERIALS AND METHODS

Amino acids used were of *L*-configuration unless otherwise mentioned. EDCI, HOBt and TFA were purchased from Advanced Chem. Tech. (Louisville, Kentucky, USA). NMM and phenyl isocyanates/isothiocyanates were purchased from Sigma Chemical Co. (St. Louis, MO). Melting point was determined on a Superfit melting point apparatus (India) and are uncorrected. TLC was performed on pre-coated silica gel plates (Kieselgel 60 F254, E. Merck, Germany) with the solvent system comprising chloroform/methanol/acetic acid in the ratio 98:2:3 (R_f^a) and 95:5:3 (R_f^b) and the compounds on TLC were detected by iodine vapors. Solvents used were of reagent grade. IR spectra of the compounds were recorded on Jasco Spectrometer (USA). ^{1}H NMR spectra were obtained on VARIAN 400 MHz instrument (USA) using DMSO-d_6 and the chemical shifts are reported as parts per million (δ ppm) using TMS as an internal standard. Mass spectra were obtained on Bruker (model HP-1100) (USA) electrospray mass spectrometer. Elemental analysis was performed by using VARIO EL III Elementar (Germany). Jack bean urease enzyme was purchased from Research Organics, Cleveland while other chemicals were purchased from Sigma Aldrich.

2.1 General procedure for the conjugation of Boc-Xaa-OH [where Xaa = Gly, Pro, Phe, Glu(OBzl), Tyr(2,6-Cl$_2$-Bzl) and Lys(Z)] to 1-(2,3-dichlorophenyl)piperazine (1-6)

1-(2,3-Dichlorophenyl)piperazine. HCl was synthesized as previously reported method [27]. To Boc-Xaa-OH (0.01 mol) dissolved in acetonitrile (10 mL/g of compound) and cooled to 0 °C was added NMM (1.10 mL, 0.01 mol). To this EDCI (1.917 g, 0.01 mol) was added and stirred while maintaining the temperature at 0 °C. After stirring the reaction mixture for 10 min at this temperature, HOBt (1.531 g, 0.01 mol) in DMF (15 mL) was added slowly. The reaction mixture was stirred for an additional 10 min and a pre-cooled solution of 2,3-dichlorophenyl piperazine.HCl (2.68 g, 0.01 mol) and NMM (1.10 mL, 0.01 mol) in DMF (25 mL) was added slowly. After 20 min, pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred over night at room temperature. Acetonitrile was removed under reduced pressure and the residual DMF was poured into about 500 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitated compound was extracted into chloroform and washed sequentially with 5% $NaHCO_3$ solution (3 × 200 mL), water (3 × 200 mL), 0.1N cold HCl (3 × 200 mL) followed by brine. The organic layer was dried over anhydrous Na_2SO_4, solvent was removed under reduced pressure, triturated with ether, filtered and dried to get the conjugates 1-6.



R = H, F, Cl, Br; Z = O or S

Scheme - 1: Synthesis of ureido and thioureido derivatives of amino acids conjugated PZN

(**Reagents and conditions:** (a) EDCI, HOBt, NMM, 0 °C, overnight at rt, (b) TFA, 45 min, rt, (c) Phenyl isocyanate/ isothiocyanate, NMM, 0 °C; 8h at rt)

2.2. General procedure for the synthesis of ureido and thioureido derivatives (7-78)

Each time, Boc-Xaa-PZN (0.150g) [where Xaa = Gly, Pro, Phe, Glu(OBzl), Tyr(2,6-Cl_2-Bzl) and Lys(Z)] was stirred with 1.5 mL of TFA for 45 min at room temperature. After completion of the reaction (monitored by TLC), TFA was removed under vacuum, triturated with dry ether, filtered, washed with ether and dried to obtain TFA.H-Xaa-PZN. Further, TFA.H-Xaa-PZN (0.001 mol) was dissolved in DMF (10 mL/g of compound), cooled to 0 °C and NMM (0.10 mL, 0.001 mol) was added. To this solution respective substituted phenyl isocyanates and isothiocyanates (0.0012 mol) was added drop-wise while maintaining the temperature at 0 °C. The reaction mixture was stirred for 8h slowly warming to room temperature. DMF was evaporated under high vacuum and the residue was poured into about 50 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitate was extracted into chloroform and washed sequentially with 5% $NaHCO_3$ solution (2 × 30 mL), water (2 × 30 mL), 0.1N citric acid (2 × 30 mL) followed by brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, triturated with hexane, filtered and dried under vacuum.

2.3. Pharmacology [28]

Reaction mixtures comprising 25 µL of jack bean urease enzyme (10mg/mL concentration) solution and 55 µL of buffers containing 100 mM urea were incubated with 5 µL of the test compounds (0.5-500 mM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenols method as described by Weatherburn [29]. Breifly, 45 µL each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min using a microplate reader (Multimode plate reader, VARIOSKAN, Germany). All the reactions were performed in triplicate in a final volume of 200 µL. The entire assays were performed at pH 6.8. Percentage inhibition was calculated from the formula:

$$\% \text{ Inhibition} = 100 - \frac{\text{OD testwell}}{\text{OD control}} \text{ x } 100$$

3. RESULTS AND DISCUSSION

The synthetic route for the proposed compounds *viz*., urea and thiourea derivatives of the conjugates of Gly, Pro, Phe, Glu(OBzl), Tyr(2,6-Cl_2-Bzl) and Lys(Z) is outlined in Scheme 1. In this respect, we have synthesized six series of disubstituted urea and thiourea derivatives by reacting substituted phenyl isocyanates/isothiocyanates with amino acids conjugated 2,3-dichlorophenyl piperazine. Yields of the compounds were found to be >80% and were characterized by IR, ^{1}H NMR, mass and elemental analysis. IR spectra of the ureas and thioureas exhibited peaks at ν ~1620 cm^{-1} and ~2040 cm^{-1} for C=O and C=S respectively. ^{1}H NMR spectra showed singlet for -NH at δ ~8.75 and multiplet for another –NH proton at δ ~8.31 for urea derivatives. On the other hand, δ singlet at ~9.70 (-NH) and multiplet at δ ~8.49 (NH) was observed for thiourea derivatives. Further, all the other peaks were exactly matching the structure. Also % of each element (C, H, N and S) of the synthesized compounds was confirmed by elemental analysis and the values are found to be within ± 0.4% of the calculated ones. The synthesized compounds were confirmed by mass spectra which are in accordance with their molecular formulae.

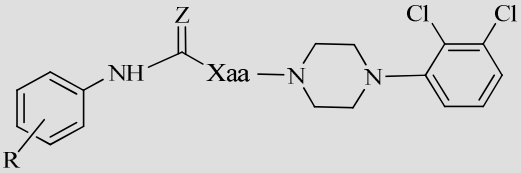
The *in vitro* antiurease activity of the target compounds was evaluated by examining their ability to inhibit the production of ammonia. The IC_{50} represented the concentration of the compound (µM) required to inhibit ammonia production by 50% and the results are summarized in Table 1. Thiourea was used as the reference compound with IC_{50} = 21.0 µM.

From activity profile, it could be seen that amino acids alone and their heterocyclic conjugates showed very less activity. Upon urea and thiourea derivatization, the compounds have exhibited very interesting activity. This indicated that urea and thiourea groups are essential in inhibition of the enzyme urease. Compounds containing phenyl ring (without substitution i.e., compounds 7, 8, 19, 20, 31, 32, 43, 44, 55, 56, 67 and 68 at the *N*-terminal were found to have moderate activity. When one of the hydrogens of the aryl ring was replaced by a substituent, there was a dramatic increase in the activity suggesting that presence of substituent on the ring is critical in enhancing the activity [30].

Among the substituents, presence of halogens is found worthy instead of methoxy group. [Earlier we have synthesized glycine and proline-heterocyclic conjugates containing methoxy substituents which were found to be less active - unpublished]. Among halogens, compounds with fluoro have profound activity than bromo and chloro analogues, which is ascribed for the high electronegativity of the former compared to latter. Further, it is observed that alteration of the position of the substituent is

also crucial. Compounds containing fluoro at the para position of the phenyl ring are the active inhibitors. Thus the introduction of appropriate substituent at 4th position of the title compounds could obviously improve the antiurease activity. Compounds containing thiourea have emerged as active moieties which once again confirm the earlier observation [31, 32].

Table - 1: Urease inhibition activities of urea and thiourea derivatives of amino acids-PZN (1-78)



where, Xaa = Gly = **7-18**, Pro = **19-30**, Phe = **31-42**, Glu(OBzl) = **43-54**, Tyr(2,6-Cl_2-Bzl) = **55-66**, Lys(Z) = **67-78**

		IC_{50} ± SEM* (µM)											
R	Z	**Entry**	Gly	**Entry**	Pro	**Entry**	Phe	**Entry**	Glu(OBzl)	**Entry**	Tyr(2,6Cl_2Bzl)	**Entry**	Lys(Z)
H	O	**7**	114.7 ± 4.2	**19**	140.0 ± 4.5	**31**	72.6 ± 3.4	**43**	84.0 ± 2.1	**55**	56.4 ± 2.0	**67**	98.0 ± 2.1
H	S	**8**	100.1 ± 3.2	**20**	126.3 ± 5.2	**32**	63.2 ± 2.8	**44**	60.0 ± 1.9	**56**	38.4 ± 2.1	**68**	82.6 ± 1.9
3F	O	**9**	15.4 ± 1.5	**21**	18.6 ± 1.9	**33**	8.6 ± 1.0	**45**	18.2 ± 1.5	**57**	9.5 ± 1.5	**69**	12.8 ± 1.5
4F	O	**10**	6.2 ± 1.9	**22**	5.2 ± 1.1	**34**	6.1 ± 0.9	**46**	6.4 ± 1.2	**58**	5.0 ± 0.8	**70**	5.6 ± 0.8
4F	S	**11**	2.6 ± 0.9	**23**	3.1 ± 0.8	**35**	4.2 ± 0.7	**47**	4.2 ± 0.9	**59**	2.9 ± 0.6	**71**	3.2 ± 0.9
3Cl	O	**12**	16.2 ± 1.4	**24**	18.6 ± 1.6	**36**	14.2 ± 1.2	**48**	12.1 ± 1.0	**60**	14.1 ± 1.0	**72**	32.0 ± 1.2
3Cl	S	**13**	12.6 ± 2.0	**25**	17.0 ± 1.8	**37**	13.1 ± 1.5	**49**	13.4 ± 1.0	**61**	12.2 ± 1.2	**73**	18.1 ± 0.9
4Cl	S	**14**	8.6 ± 1.1	**26**	9.2 ± 1.8	**38**	10.2 ± 1.1	**50**	10.8 ± 0.8	**62**	12.2 ± 1.2	**74**	17.6 ± 1.2
2Br	S	**15**	16.7 ± 2.3	**27**	15.1 ± 2.0	**39**	16.2 ± 1.4	**51**	15.4 ± 1.2	**63**	18.6 ± 1.0	**75**	20.1 ± 1.5
3Br	O	**16**	19.2 ± 1.8	**28**	20.2 ± 2.5	**40**	17.6 ± 1.8	**52**	16.2 ± 1.4	**64**	19.2 ± 1.3	**76**	28.2 ± 1.6
3Br	S	**17**	8.61 ± 1.3	**29**	17.6 ± 1.8	**41**	16.2 ± 1.5	**53**	18.0 ± 1.5	**65**	16.6 ± 1.4	**77**	18.5 ± 1.4
4Br	O	**18**	20.9 ± 2.0	**30**	19.8 ± 1.3	**42**	19.6 ± 1.6	**54**	14.1 ± 1.2	**66**	20.2 ± 1.0	**78**	38.6 ± 1.7
		1	140.1 ± 3.2	**2**	168.6 ± 3.5	**3**	190.2 ± 3.8	**4**	120.5 ± 2.8	**5**	102.1 ± 2.5	**6**	154.6 ± 2.0
Standard, thiourea 21.0 ± 0.01													

*Values are mean of three determinations, the ranges of which are <5% of the mean in all cases

4. CONCLUSION

In summary, a small library of over seventy urea and thiourea derivatives of six amino acids-heterocycle conjugates were synthesized and their urease inhibition activity was evaluated. The results revealed that compounds showed promising activity. Among them, molecules with fluoro at para position were highly significant with IC_{50} = 2.6-4.2 µM. The present work demonstrate that assembling the biological active unit of title compounds might be able to result in a class of lead compounds with potential antiurease activity.

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