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Synthesis and evaluation of urea/thiourea derivatives of dipeptide linked benzisoxazole as a new class of antiglycation and urease inhibitory agents

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ABSTRACT

Four dipeptides with varying hydrophobicity at *N*-terminus were synthesized and conjugated to [3-(4-piperidyl)-6-fluoro-1,2-benzisoxazole] followed by their conversion to urea/thiourea derivatives. The structure of newly synthesized compounds was established by analytical and spectral (IR, ^{1}H-NMR, MS) techniques and were evaluated for antiglycation and antiurease activities. Preliminary studies revealed that compounds with methoxy at *para* position and bromo at *ortho* position of the aryl moiety along with thiourea system significantly influenced the antiglycation and antiurease activities when compared to its bioisostere counterpart methoxy/bromo substituted aryl moiety along with urea system.

Keywords: Dipeptides, Urea/thiourea, Antiglycation, Urease inhibition

1. INTRODUCTION

The non-enzymatic glycation of proteins or the Maillard reaction is a process which links chronic hyperglycemia to a series of physiopathological alterations. The Maillard product undergo further rearrangement gives rise to a stable Amadori product. These products degrade into a variety of compounds which are more reactive than the sugars from which they are derived. These propagators again form yellow-brown, often fluorescent irreversible compounds, usually called Advanced Glycation End-Products (AGE's). Most of the AGE's are very unstable and reactive and are difficult to analyze. They accumulate on different proteins and cause damage [1]. These AGEs considered important in the development of chronic complications of diabetes and different diseases such as renal insufficiency and Alzheimer's disease [2]. The compounds such as aminoguanidine [3], aspirin [4], vitamin B_6, taurine, quercetin and ibuprofen [5-7] which are reported to be inhibitors of the glycation reaction have some deleterious side effects. Hence, the present work was intended to search for better agents which would enable to replace the existing ones suffering from serious limitations. BSA was selected as protein model because of its medical importance, low cost, ready availability, unusual ligand-binding and intrinsic fluorescence properties [8-9].

Urease (urea amidohydrolase EC 3.5.1.5) is a nickel-containing enzyme that catalyzes the hydrolysis of urea to ammonia and CO_2 or carbamate. The active site of the native enzyme binds to three water molecules and a hydroxide ion bridged between two nickel ions. In the course of enzymatic reaction, urea replaces these three water molecules and undergone further reaction due to this ammonia is released from the active site followed by the negatively charged carbamate. The latter decomposes rapidly and spontaneously and shown to be an important virulence determinant in the pathogenesis of many clinical conditions which are detrimental for human and animal health as well as for agriculture [10]. Moreover, it induces plant damage primarily by depriving plants of their essential nutrients and secondarily by ammonia toxicity, increasing the pH of the soil. Urease has been identified as an immunogenic modulator in several pathogen-induced inflammatory reactions [11]. Several classes of compounds show significant inhibitory activity against urease with hydroxamic acids being the best recognized inhibitors [12] and much of the early attention for the inhibition of the urease was focused on triazole and thiadiazole derivatives being the most active [13]. In addition to this, many heterocyclic entities such as piperidines [14] naphthoquinone [15], 4-methoxy benzaldehyde [16], benzimidazole derivatives [17] benzoquinone [18] have been used as *in vitro* antiurease agents. The exact knowledge about the region of the enzyme involved in the binding of substrates or inhibitors is a starting point in designing effective inhibitors.

Benzisoxazoles have been frequently found to display a variety of biological activities such as antihelmintic, anticonvulsant [19] antipsychotic [20], antihistaminic, anticancer [21], antiviral, antiproliferative, antioxidant, Alzheimer's disease [22] anticoagulant properties. This has attracted increasing interest to investigate the possible applications of benzisoxazole derivatives in other medicinal aspects. Our earlier reports have shown that conjugation of different amino acids to various biologically active scaffolds has fetched the remarkable results which are very promising and even enthusiastic [23-25]. Some simple cyclic dipeptides such as cyclo(Pro-Leu), cyclo(Pro-Val), cyclo(Pro-Phe), cyclo(Ala-Leu), cyclo(Pro-Tyr), cyclo(Pro-Trp), cyclo(His-Pro), cyclo(Leu-Gly), cyclo(Tyr-Arg) and cyclo(Asp-Pro) often occur together in a variety of bacterial and fungal cultures [26, 27]. The dipeptides anserine and carnosine[28] are tested as inhibitors of protein glycation and acetohydroxamic esters conjugated dipeptides[29] used as urease inhibitor. Moreover, the amino acid/peptide based drugs have low toxicity, ample bioavailability and permeability, modest potency and good metabolic and pharmacokinetic properties. Keeping all the above facts in mind, the current investigation involves the conjugation of four dipeptides with varying hydrophobicity to [3-(4-Piperidyl)-6-fluoro-1,2-benzisoxazole] and converted to urea/thiourea derivatives with intention to obtain novel, potent and selective inhibitors of glycation and urease.

2. MATERIALS AND METHODS

2.1. Chemistry

Boc-amino acids, HCl.Pro-OBzl, EDCI, HOBt and TFA were purchased from Advanced Chem. Tech. (Louisville, Kentucky, USA). NMM, substituted phenyl isocyanates/isothiocyanates, rutin, urease enzyme and other chemicals used for inhibition studies were purchased from Sigma Aldrich Co., USA. Silica gel for TLC was purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Progress of the reaction was monitored by thin layer chromatography with mobile phase chloroform/methanol/acetic acid in the ratio 98:2:3 (R_f^a) and 95:5:3 (R_f^b) and detection was made using iodine vapors. Melting point of the synthesized compounds was determined in open capillary on a Superfit melting point apparatus and is uncorrected. FT-IR spectra were recorded with nujol using a Jasco spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a VARIAN 400 MHz instrument using DMSO-d_6 and the chemical shifts are reported as parts per million (δ ppm) using TMS as internal standard. Mass spectra were recorded on an Agilent LC-MS.

2.2. PEPTIDE SYNTHESIS

2.2.1.General Procedure for the Synthesis of Boc-Xaa-Pro-OBzl: where Xaa = Tyr(2,6-Cl_2-Bzl) for I, Phe for II, Val for III and Lys(Z) for IV

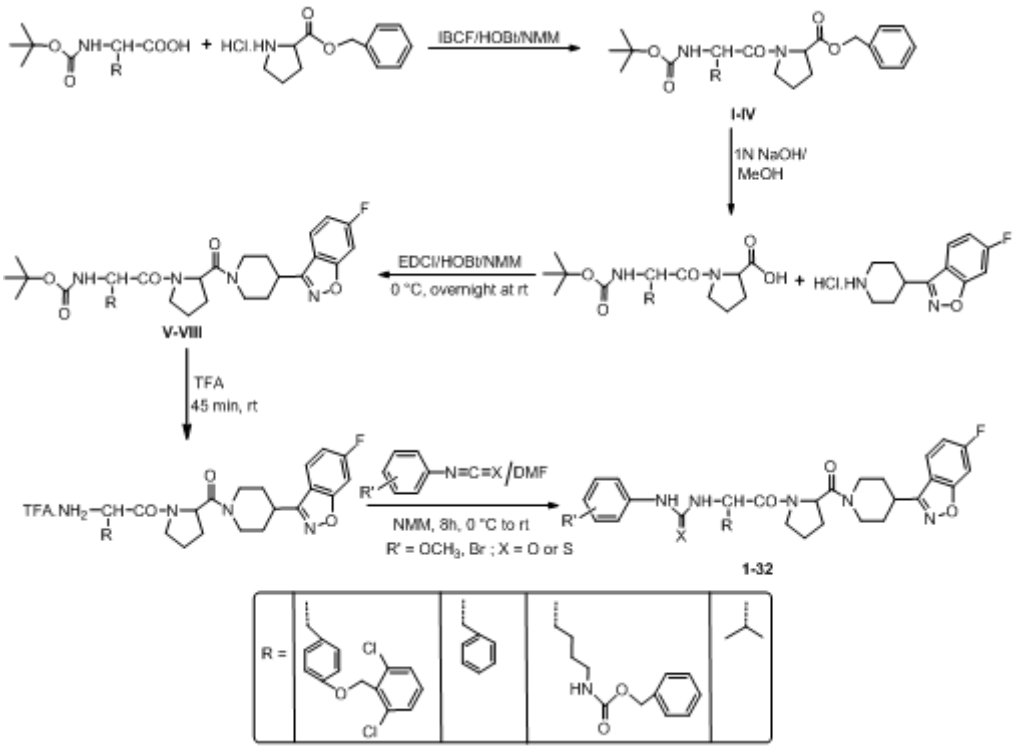
To Boc-Xaa (0.005 mol) dissolved in acetonitrile (10mL/g of amino acid) and cooled to 0 °C was added NMM (0.55 mL, 0.005 mol). The solution was cooled to -15 °C ± 1°C and IBCF (0.005 mol) was added under stirring while maintaining the temperature at -15 °C. After stirring the reaction mixture for 10 minutes at this temperature, a pre-cooled solution of HOBt (0.005 mol) in DMF (8 mL) was added. The reaction mixture was stirred for an additional 10 minutes and a pre-cooled solution of HCl.H-Pro-OBzl (0.005 mol) and NMM (0.05 mol) in DMF (13 mL) was added slowly. After 20 minutes, pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred overnight at room temperature. Acetonitrile was removed under reduced pressure and the residual DMF solution was poured into about 300 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 minutes. The precipitated peptide was filtered, washed with water, 1N HCl, water and dried. The crude peptide was recrystallized from ether and petroleum ether to obtain the desired products (I-IV).

2.2.2. General procedure for the saponification of benzyl ester of dipeptides:

To a solution of the compounds (**I-IV**) (0.004 mol) in methanol (10 mL/g of compound) was hydrolysed using cold solution of 1N NaOH for 1hr. After completion of the reaction monitored by TLC and the solvent was evaporated, cooled, neutralized with cold 1N HCl, extracted with chloroform, washed with 1N HCl followed by water and the solvent was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and triturated with ether, filtered and dried to obtain Boc-Xaa-Pro-OH.

2.2.3. General Procedure for the Coupling of Benzisoxazole Derivative with Boc-Xaa-Pro-OH where Xaa = Tyr(2,6-Cl_2-Bzl) for V, Phe for VI, Val for VII and Lys(Z) for VIII:

To Boc-Xaa-Pro-OH (0.004 mol) dissolved in DMF (10 ml/g of peptide) and cooled to °C was added NMM (0.44 ml, 0.004 mol). After stirring the reaction mixture for 10 minutes at this temperature, a pre-cooled solution of HOBt (0.005 mol) in DMF (8 mL) was added. EDCI (0.004 mol) was added under stirring while maintaining the temperature at 0 °C. The reaction mixture was stirred for an additional 10 min and pre-cooled solution of [3-(4-piperidinyl)-6-fluoro-1,2-benzisoxazole].HCl (0.004 mol) and NMM (0.005



Scheme -1: Synthesis of ureido and thioureido derivatives of peptide conjugated heterocycle

mol) in DMF (10 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. DMF was removed under reduced pressure and the residue was poured into about 200 ml ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitated product was taken into $CHCl_3$ and washed with 5% $KHCO_3$ solution (3 X 20 ml), water (2 X 20 ml), 0.1N cold HCl solution (3 X 20 ml) and finally brine solution (2 X 20 ml). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The products so obtained were recrystallized from ether/petroleum ether to obtain the desired conjugates (V-VIII).

2.2.4. General procedure for the synthesis of ureido and thioureido derivatives (1-32)

Each time, Boc-Xaa-Pro-Het (0.01g) (V-VIII) was stirred with 1.0 mL of TFA for 45 min at room temperature. After completion of the reaction monitored by TLC, the reaction mixture was concentrated in vacuum to get TFA.NH_2-Xaa-Pro-Het which was then triturated with dry ether, filtered, washed with ether and dried (Yield: 100%).

A solution of TFA.NH_2-Xaa-Het (0.001 mol) in DMF (10 mL/g of compound) was cooled to 0 °C and added NMM (0.10 mL, 0.001 mol). To this respective substituted phenyl isocyanates/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 20 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 15 minutes. The precipitated compound was extracted into ethyl acetate and washed with 5% $KHCO_3$ solution (2 x 10 mL), water (2 x 10 mL), 0.1N HCl (2 x 10 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.

2.2.5. BIOLOGY

2.2.5.1. Antiglycation assay

Evaluation of antiglycation activity of the synthesized compounds was examined by the method of Nakagawa et al., [30]. Sodium phosphate buffer (pH 7.4) was prepared by mixing Na_2HPO_4 and NaH_2PO_4 (67 mM) containing sodium azide (3 mM); phosphate buffer saline (PBS) was prepared by mixing NaCl (137 mM) + Na_2HPO_4 (8.1 mM) + KCl (2.68 mM) + KH_2PO_4 (1.47 mM) and pH 10 was adjusted with NaOH (0.25 mM), while BSA (10 mg/mL) and anhydrous glucose (50 mg/mL) solutions were prepared in sodium phosphate buffer. Eppendorf tubes (Tarsons, India) were used for incubation.

Bovine serum albumin (10 mg/mL) and glucose anhydrous (50 mg/mL) were prepared in sodium phosphate buffer (pH 7.4). DMSO used for dissolving the compounds was found to have no

effect on the reaction at <2% (v/v). Glycated control contains 20 µL BSA + 20 µL glucose + 20 µL sodium phosphate buffer, blank control contains 20 µL BSA and 40 µL sodium phosphate buffer, while the test contains 20 µL BSA+ 20 µL glucose + 20 µL compound ranging from 0.5-500 µg/mL concentration. All the incubation tubes containing the mixtures were sealed and incubated at 37 °C for 7 days. After incubation, 6 µL (100%) of TCA was added into each tube and centrifuged (15,000 rpm) for 4 min at 4 °C. After centrifugation, the pellets were rewashed with 60 µL (10%) of TCA. The supernatant containing glucose, inhibitor and interfering substance was removed and pellet containing advanced glycated end product-BSA were dissolved in 60 µL phosphate buffer solution (PBS) and transferred into 96-well ELISA plates (Tarsons, India). Evaluation of fluorescence spectrum (excitation 370 nm), and change in fluorescence intensity (excitation 370 nm to emission 440 nm), based on AGEs were monitored by using spectrofluorimeter (RF-1500, Shimadzu, Japan). % Inhibition was calculated using the formula:

$$\% \text{ Inhibition} = 1 - \frac{\text{Fluorescence of sample}}{\text{Fluorescence of glycated sample}} \text{ x } 100$$

2.2.5.2. Urease inhibition assay (*in vitro*)

Reaction mixtures comprising 25 µL of jack bean urease enzyme (10 mg/mL) solution and 55 µL of buffers containing 100 µM urea were incubated with 5 µL of the test compounds (0.5-500 µM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [31]. Briefly, 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 micro plate reader (RF-1500, Shimadzu, Japan). All the reactions were performed in triplicate in a final volume of 200 µL. Thiourea was used as the standard inhibitor of urease. The entire assays were performed at pH 6.8. Percentage inhibition was calculated from the formula,

$$\% \text{ I} = \left[100 - \left(\frac{\text{OD}_{\text{testwell}}}{\text{OD}_{\text{control}}}\right) \text{X } 100\right]$$

5. RESULT AND DISCUSSION

The peptide synthesis was performed by step-wise solution phase method using Boc chemistry. The synthesized peptides were conjugated with the heterocycle, [3-(4-piperidyl)-6-fluoro-1,2-benzisoxazole].HCl using EDCI/HOBt as coupling agent and NMM as base. Boc group of the conjugates was removed using TFA and reacted with various substituted phenyl isocyanates/isothiocyanates to obtain urea/thiourea derivatives respectively (Scheme). All the derivatives were obtained in high yields. The evidence for the formation of compounds was obtained from IR, ^{1}H NMR and mass datas. In IR spectra stretching frequencies appeared at ~1510 cm^{-1} (CS), 1615-1644 cm^{-1} (CO) and ~3300 cm^{-1} (NH) respectively. ^{1}H NMR spectra showed doublet for one proton (NH) at δ ~6.30, singlet for NH at δ ~7.80-8.01 for urea/thiourea derivatives. Further, all the other peaks are exactly matching the structure. The ^{1}H NMR, mass data were found to be in good agreement with the structures assigned (Table 2, 3).

Table – 2: Physical and mass data of the synthesized compounds *(I-VIII /1-32)*

Entry	R_f Values		Yield (%)	M.P. (°C)	Mol. For.	Theoretical mol. wt.	MS values
	R_f[a] 98:2:3	R_f[b] 95:5:3					
I	0.44	0.60	89	gum	$C_{33}H_{36}Cl_2N_2O_6$	627.55	628.6
II	0.53	0.75	92	97-100 (100)[35]	$C_{26}H_{32}N_2O_5$	452.54	453.7
III	0.50	0.61	94	82 (83)[36]	$C_{22}H_{32}N_2O_5$	404.50	405.4
IV	0.47	0.67	92	gum	$C_{31}H_{41}N_3O_7$	567.67	568.8
V	0.53	0.70	80	gum	$C_{38}H_{41}Cl_2FN_4O_6$	739.66	740.6
VI	0.52	0.69	85	gum	$C_{31}H_{37}FN_4O_5$	564.65	565.7

VII	0.47	0.64	84	gum	$C_{27}H_{37}FN_4O_5$	516.60	517.5
VIII	0.49	0.65	88	gum	$C_{36}H_{46}FN_5O_7$	679.78	680.7
1	0.51	0.62	80	--	$C_{41}H_{40}Cl_2FN_5O_6$	788.69	789.7
2	0.51	0.66	79	86-89	$C_{41}H_{40}Cl_2FN_5O_5S$	804.76	805.6
3	0.48	0.63	78	96-99	$C_{41}H_{40}Cl_2FN_5O_6$	788.69	789.6
4	0.47	0.61	81	82-85	$C_{41}H_{40}Cl_2FN_5O_5S$	804.76	805.7
5	0.57	0.69	83	101-105	$C_{40}H_{37}BrCl_2FN_5O_4S$	853.63	853.6 855.7
6	0.53	0.70	79	79-83	$C_{40}H_{37}BrCl_2FN_5O_5$	837.56	837.5 839.4
7	0.54	0.68	81	-	$C_{40}H_{37}BrCl_2FN_5O_4S$	853.63	853.5 855.6
8	0.55	0.67	80	152-155	$C_{40}H_{37}BrCl_2FN_5O_5$	837.56	837.6 839.5
9	0.50	0.63	82	60-64	$C_{34}H_{36}FN_5O_5$	613.68	614.7
10	0.53	0.66	84	64-66	$C_{34}H_{36}FN_5O_4S$	629.74	630.4
11	0.55	0.67	85	69-73	$C_{34}H_{36}FN_5O_5$	613.68	614.8
12	0.54	0.67	82	68-72	$C_{34}H_{36}FN_5O_4S$	629.74	630.2
13	0.55	0.63	86	70-73	$C_{33}H_{33}BrFN_5O_3S$	678.61	678.5 680.3
14	0.56	0.68	80	68	$C_{33}H_{33}BrFN_5O_4$	662.55	662.4 665.1
15	0.54	0.65	87	80-84	$C_{33}H_{33}BrFN_5O_3S$	678.61	678.8 680.4
16	0.50	0.62	79	98-101	$C_{33}H_{33}BrFN_5O_4$	662.55	662.2 665.2
17	0.55	0.68	80	64-68	$C_{30}H_{36}FN_5O_5$	565.64	566.7
18	0.54	0.67	81	-	$C_{30}H_{36}FN_5O_4S$	581.25	582.4
19	0.55	0.67	83	94-97	$C_{30}H_{36}FN_5O_5$	565.64	566.8
20	0.55	0.69	85	-	$C_{30}H_{36}FN_5O_4S$	581.25	581.2
21	0.53	0.64	84	67-71	$C_{29}H_{33}BrFN_5O_3S$	630.57	630.4 632.8
22	0.52	0.63	89	73-76	$C_{29}H_{33}BrFN_5O_4$	614.51	614.3 616.9
23	0.56	0.67	76	81-84	$C_{29}H_{33}BrFN_5O_3S$	630.57	629.9 632.7

24	0.54	0.66	82	69-72	$C_{29}H_{33}BrFN_5O_4$	614.51	614.5 616.2
25	0.49	0.58	81	58-60	$C_{39}H_{45}FN_6O_7$	728.81	729.7
26	0.48	0.56	83	62-67	$C_{39}H_{45}FN_6O_6S$	744.87	745.6
27	0.51	0.60	84	--	$C_{39}H_{45}FN_6O_7$	728.81	729.8
28	0.50	0.58	79	63-67	$C_{39}H_{45}FN_6O_6S$	744.87	745.7
29	0.48	0.59	84	70-74	$C_{38}H_{42}BrFN_6O_5S$	792.21	792.1 794.2
30	0.47	0.58	77	85-88	$C_{38}H_{42}BrFN_6O_6$	777.68	777.7 779.9
31	0.48	0.60	80	75-79	$C_{38}H_{42}BrFN_6O_5S$	792.21	792.3 794.3
32	0.46	0.57	83	92-96	$C_{38}H_{42}BrFN_6O_6$	777.68	777.2 780.2

Table – 3: IR and ^{1}H NMR data of the synthesized compounds

Entry	IR (Nujol, ν_{max}, cm^{-1})	Component ^{1}H NMR (DMSO-d_6, δ ppm)
V	1627 (C=O) 3309 (NH)	Boc = 1.43 (9H, s, Me_3) Tyr = 4.94-4.97 (1H, m, $^{\alpha}CH$), 3.69-3.72 (2H, d, $^{\beta}CH_2$), 7.09-7.47 (4H, m, Ar-H), 2,6-Cl_2-Bzl = 5.10 (2H, s, CH_2), 7.09-7.47 (3H, m, Ar-H), Pro = 4.64-4.74 (1H, t, -$^{\alpha}CH$), 1.33-1.47 (4H, m, -$^{\beta,\gamma}CH_2$), 3.56-3.66 (2H, t, $^{\delta}CH_2$); Heterocycle[b] = 1.02-1.09 (4H, m, -CH_2), 2.70-2.81 (1H, m, -CH), 3.08-3.26 (4H, t, -CH_2), 7.09-7.47 (3H, m, Ar-H)
VI	1615 (C=O) 3341 (NH)	Boc = 1.43 (9H, s, Me_3) Phe = 4.89-4.94 (1H, m, $^{\alpha}CH$), 3.69-3.75 (2H, d, $^{\beta}CH_2$), 7.18-7.61 (5H, m, Ar-H); Pro = 4.84-4.88 (1H, t, -$^{\alpha}CH$),1.81-2.05 (4H, m, -$^{\beta,\gamma}CH_2$), 3.40-3.46 (2H, t,-$^{\delta}CH_2$)
VII	1644 (C=O) 3354 (NH)	Boc = 1.43 (9H, s, Me_3) Val = 1.02-1.08 (6H, d, $(CH_3)_2$), 4.43 (1H, t, $^{\alpha}CH$), 1.86-1.99 (1H, m, $^{\beta}CH$); Pro = 4.04-4.09 (1H, t, -$^{\alpha}CH$), 1.08-1.29 (4H, m, -$^{\beta,\gamma}CH_2$), 3.39 (2H, t, -$^{\delta}CH_2$)
VIII	1638 (C=O) 3332 (NH)	Boc = 1.44 (9H, s, Me_3) Lys = 4.46-4.50 (1H, m, $^{\alpha}CH$), 1.97-2.09 (2H, m, $^{\beta}CH_2$), 1.87-1.94 (2H, m, $^{\gamma}CH_2$), 1.66-1.72 (2H, m, $^{\delta}CH_2$), 3.28-3.34 (2H, m, $^{€}CH_2$) Z = 7.56-7.67 (1H, t, NH), 5.20 (2H, s, CH_2), 7.10-7.81 (5H, m, Ar-H) Pro = 4.46 (1H, t, -$^{\alpha}CH$), 1.82-2.36 (4H, m, -$^{\beta,\gamma}CH_2$), 3.19-3.34 (2H, t, -$^{\delta}CH_2$)
1	1622 (C=O) 3349 (NH)	Urea = 3.62 (3H, s, -OCH_3), 6.24-6.36 (1H, d, -NH), 7.08-7.49 (4H, m, Ar-H), 7.90 (1H, s, NH) Tyr = 4.94-4.98 (1H, m, $^{\alpha}CH$), 3.70-3.75 (2H, d, $^{\beta}CH_2$), 7.08-7.49 (4H, m, Ar-H), 2,6-Cl_2-Bzl = 5.09 (2H, s, CH_2), 7.08-7.49 (3H, m, Ar-H), Pro = 4.62-4.79 (1H, t, -$^{\alpha}CH$), 1.24-1.39 (4H, m, -$^{\beta,\gamma}CH_2$), 3.66-3.71 (2H, t, -$^{\delta}CH_2$)
2	1507 (C=S) 1631 (C=O) 3358 (NH)	Thiourea = 3.56 (3H, s, -OCH_3), 6.27-6.36 (1H, d, -NH), 7.10-7.64 (4H, m, Ar-H), 7.80 (1H, s,NH) Tyr = 4.86-4.90 (1H, m, $^{\alpha}CH$), 3.67-3.73 (2H, d, $^{\beta}CH_2$), 7.10-7.64 (4H, m, Ar-H), 2,6-Cl_2-Bzl = 5.10 (2H, s, CH_2), 7.10-7.64 (3H, m, Ar-H), Pro = 4.54-4.69 (1H, t, -$^{\alpha}CH$), 1.24-1.39 (4H, m, -$^{\beta,\gamma}CH_2$), 3.66-3.77 (2H, t, -$^{\delta}CH_2$)

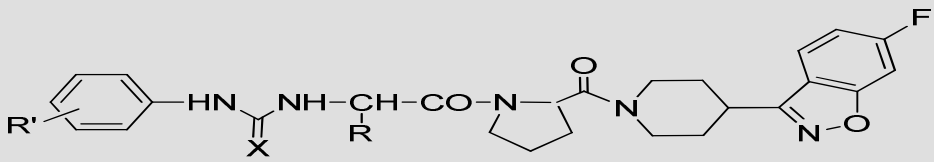
3	1622 (C=O) 3346 (NH)	Urea = 3.51 (3H, s, -OCH_3), 6.24-6.46 (1H, d, -NH), 7.09-7.61 (4H, m, Ar-H), 7.91 (1H, s, NH) Tyr = 4.86-4.92 (1H, m, $^{\alpha}CH$), 3.67-3.76 (2H, d, $^{\beta}CH_2$), 7.09-7.61 (4H, m, Ar-H), 2,6-Cl_2-Bzl = 5.09 (2H, s, CH_2), 7.09-7.61 (3H, m, Ar-H), Pro = 4.66-4.81 (1H, t, -$^{\alpha}CH$), 1.36-1.48 (4H, m, -$^{\beta,\gamma}CH_2$), 3.70-3.76 (2H, t, -$^{\delta}CH_2$)
4	1511 (C=S) 1635 (C=O) 3305 (NH)	Thiourea = 3.66 (3H, s, -OCH_3), 6.30-6.40 (1H , d, -NH), 7.04-7.40 (4H, m, Ar-H), 7.86 (1H, s, NH) Tyr = 4.90-4.95 (1H, m, $^{\alpha}CH$), 3.72-3.74 (2H, d, $^{\beta}CH_2$), 7.04-7.40 (4H, m, Ar-H), 2,6-Cl_2-Bzl = 5.04 (2H, s, CH_2), 7.04-7.40 (3H, m, Ar-H), Pro = 4.60-4.77 (1H, t, -$^{\alpha}CH$), 1.30-1.42 (4H, m, -$^{\beta,\gamma}CH_2$), 3.72-3.74 (2H, t, -$^{\delta}CH_2$)
5	1505 (C=S) 1641 (C=O) 3319 (NH)	Thiourea = 6.34-6.46 (1H, d, -NH), 6.98-7.80 (4H, m, Ar-H), 7.92 (1H, s, NH) Tyr = 4.84-4.88 (1H, m, $^{\alpha}CH$), 3.66-3.70 (2H, d, $^{\beta}CH_2$), 6.98-7.80 (4H, m, Ar-H), 2,6-Cl_2-Bzl = 5.21 (2H, s, CH_2), 6.98-7.80 (3H, m, Ar-H), Pro = 4.56-4.77 (1H, t, -$^{\alpha}CH$), 1.26-1.47 (4H, m, -$^{\beta,\gamma}CH_2$), 3.65-3.66 (2H, t, -$^{\delta}CH_2$)
6	1509 (C=O) 3307 (NH)	Urea = 6.14-6.26 (1H, d, -NH), 7.01-7.74 (4H, m, Ar-H), 8.03 (1H, s, NH) Tyr = 4.86-4.91 (1H, m, $^{\alpha}CH$), 3.60-3.71 (2H, d, $^{\beta}CH_2$), 7.01-7.74 (4H, m, Ar-H), 2,6-Cl_2-Bzl = 5.10 (2H, s, CH_2), 7.01-7.74 (3H, m, Ar-H), Pro = 4.52-4.66 (1H, t, -$^{\alpha}CH$), 1.24-1.36 (4H, m, -$^{\beta,\gamma}CH_2$), 3.59-3.67 (2H, t, -$^{\delta}CH_2$)
7	1504 (C=S) 1644 (C=O) 3360 (NH)	Thiourea = 6.24-6.42 (1H, d, -NH), 7.09-7.77 (4H, m, Ar-H), 7.96 (1H, s, NH) Tyr = 4.67-4.73 (1H, m, $^{\alpha}CH$), 3.64-3.70 (2H, d, $^{\beta}CH_2$), 7.09-7.77 (4H, m, Ar-H), 2,6-Cl_2-Bzl = 5.18 (2H, s, CH_2), 7.09-7.77 (3H, m, Ar-H), Pro = 4.56-4.71 (1H, t, -$^{\alpha}CH$), 1.26-1.44 (4H, m, -$^{\beta,\gamma}CH_2$), 3.72-3.81 (2H, t, -$^{\delta}CH_2$)
8	1620 (C=O) 3308 (NH)	Urea = 6.30-6.40 (1H, d, -NH), 7.04-7.40 (4H, m, Ar-H), 7.86 (1H, s, NH) Tyr = 4.90-4.95 (1H, m, $^{\alpha}CH$), 3.66-3.71 (2H, d, $^{\beta}CH_2$), 7.04-7.40 (4H, m, Ar-H), 2,6-Cl_2-Bzl = 5.08 (2H, s, CH_2), 7.04-7.40 (3H, m, Ar-H), Pro = 4.51-4.60 (1H, t, -$^{\alpha}CH$), 1.31-1.43 (4H, m, -$^{\beta,\gamma}CH_2$), 3.70-3.79 (2H, t, -$^{\delta}CH_2$)
9	1624 (C=O) 3352 (NH)	Urea = 3.62 (3H, s, -OCH_3), 6.26-6.39 (1H, d, -NH), 6.66-7.58 (4H, m, Ar-H), 7.79 (1H, s, NH); Phe = 4.80-4.84 (1H, m, $^{\alpha}CH$), 3.56-3.66 (2H, d, $^{\beta}CH_2$), 6.66-7.58 (5H, m, Ar-H); Pro = 4.81-4.92 (1H, t, -$^{\alpha}CH$),1.82-2.08 (4H, m, -$^{\beta,\gamma}CH_2$), 3.41-3.49 (2H, t, -$^{\delta}CH_2$)
10	1514 (C=S) 1638 (C=O) 3352 (NH)	Thiourea = 3.71 (3H, s, -OCH_3), 6.27-6.44 (1H, d, -NH), 6.74-7.63 (4H, m, Ar-H), 7.89 (1H, s, NH); Phe = 4.83-4.89 (1H, m, $^{\alpha}CH$), 3.57-3.70 (2H, d, $^{\beta}CH_2$), 6.74-7.63 (5H, m, Ar-H); Pro = 4.86-4.94 (1H, t, -$^{\alpha}CH$),1.88-2.06 (4H, m, -$^{\beta,\gamma}CH_2$), 3.39-3.46 (2H, t, -$^{\delta}CH_2$)
11	1645 (C=O) 3328 (NH)	Urea = 3.75 (3H, s, -OCH_3), 6.30-6.41 (1H, d, -NH), 6.60-7.71 (4H, m, Ar-H), 7.92 (1H, s, NH); Phe = 4.89-4.94 (1H, m, $^{\alpha}CH$), 3.63-3.76 (2H, d, $^{\beta}CH_2$), 6.60-7.71 (5H, m, Ar-H); Pro = 4.90-4.94 (1H, t, -$^{\alpha}CH$),1.91-2.13 (4H, m, -$^{\beta,\gamma}CH_2$), 3.42-3.51 (2H, t, -$^{\delta}CH_2$)
12	1511(C=S) 1636 (C=O) 3351 (NH)	Thiourea = 3.78 (3H, s, -OCH_3), 6.46-6.52 (1H, d, -NH), 6.74-7.28 (4H, m, Ar-H), 7.50 (1H, s, NH); Phe = 4.94-4.99 (1H, m, $^{\alpha}CH$), 3.64-3.73 (2H, d, $^{\beta}CH_2$), 6.74-7.28 (5H, m, Ar-H); Pro = 4.90-4.98 (1H, t, -$^{\alpha}CH$),1.86-2.10 (4H, m, -$^{\beta,\gamma}CH_2$), 3.45-3.47 (2H, t -$^{\delta}CH_2$)
13	1514 (C=S) 1628 (C=O) 3302 (NH)	Thiourea = 6.26-6.43 (1H, d, -NH), 6.60-7.66 (4H, m, Ar-H), 8.03 (1H, s, NH); Phe = 4.86-4.91 (1H, m, $^{\alpha}CH$), 3.59-3.65 (2H, d, $^{\beta}CH_2$), 6.60-7.66 (5H, m, Ar-H); Pro = 4.88-4.95 (1H, t, -$^{\alpha}CH$),1.78-2.04 (4H, m, -$^{\beta,\gamma}CH_2$), 3.41-3.46 (2H, t, -$^{\delta}CH_2$)
14	1646 (C=O) 3309 (NH)	Urea = 6.46-6.52 (1H, d, -NH), 6.62-7.81 (4H, m, Ar-H), 7.91 (1H, s, NH); Phe = 4.84-4.89 (1H, m, $^{\alpha}CH$), 3.56-3.63 (2H, d, $^{\beta}CH_2$), 6.62-7.81 (5H, m, Ar-H); Pro = 4.88-4.96 (1H, t, -$^{\alpha}CH$),1.84-2.06 (4H, m, -$^{\beta,\gamma}CH_2$), 3.38-3.51 (2H, t, -$^{\delta}CH_2$)

15	1524 (C=S) 1626 (C=O) 3337 (NH)	Thiourea = 6.30-6.41 (1H, d, -NH), 6.49-7.72 (4H, m, Ar-H), 7.92 (1H, s, NH); Phe = 4.85-4.90 (1H, m, $^{\alpha}$CH), 3.59-3.66 (2H, d, $^{\beta}CH_2$), 6.49-7.72 (5H, m, Ar-H); Pro = 4.92-4.99 (1H, t, -$^{\alpha}$CH),1.82-2.14 (4H, m, -$^{\beta,\gamma}CH_2$), 3.39-3.43 (2H, t, -$^{\delta}CH_2$)
16	1620 (C=O) 3314 (NH)	Urea = 6.22-6.34 (1H, d, -NH), 6.51-7.64 (4H, m, Ar-H), 7.81 (1H, s,NH); Phe = 4.86-4.91 (1H, m, $^{\alpha}$CH), 3.60-3.72 (2H, d, $^{\beta}CH_2$), 6.51-7.64 (5H, m, Ar-H); Pro = 4.90-4.98 (1H, t, -$^{\alpha}$CH),1.86-2.10 (4H, m, -$^{\beta,\gamma}CH_2$), 3.45-3.47 (2H, t, -$^{\delta}CH_2$);
17	1628 (C=O) 3314 (NH)	Urea = 7.11-7.61 (4H, m, Ar-H), 7.81 (1H, s, -NH), 3.74 (3H, s, -OCH_3), 6.54 (1H, d,NH); Val = 1.04-1.09 (6H, d, $(CH_3)_2$), 4.44-4.52 (1H, t, $^{\alpha}$CH), 1.86-1.89 (1H, m, $^{\beta}$CH); Pro = 4.02-4.07 (1H, t, -$^{\alpha}$CH), 1.06-1.31 (4H, m, -$^{\beta,\gamma}CH_2$), 3.40 (2H, t, -$^{\delta}CH_2$)
18	1508 (C=S) 1641 (C=O) 3319 (NH)	Thiourea = 7.04-7.72 (4H, m, Ar-H), 7.84 (1H, s, -NH), 3.70 (3H, s, -OCH_3), 6.58 (1H, d, NH); Val = 1.03-1.15 (6H, d, $(CH_3)_2$), 4.46-4.59 (1H, t, $^{\alpha}$CH), 1.86-1.92 (1H, m, $^{\beta}$CH); Pro = 4.07-4.14 (1H, t, -$^{\alpha}$CH), 1.08-1.29 (4H, m, -$^{\beta,\gamma}CH_2$), 3.52 (2H, t, -$^{\delta}CH_2$);
19	1634 (C=O) 3318 (NH)	Urea = 7.02-7.59 (4H, m, Ar-H), 7.65 (1H, s, -NH), 3.74 (3H, s, -OCH_3), 6.41 (1H, d, NH); Val = 1.07-1.18 (6H, d, $(CH_3)_2$), 4.44-4.52 (1H, t, $^{\alpha}$CH), 1.86-1.91 (1H, m, $^{\beta}$CH); Pro = 4.08-4.14 (1H, t, -$^{\alpha}$CH), 1.11-1.36 (4H, m, -$^{\beta,\gamma}CH_2$), 3.38 (2H, t, -$^{\delta}CH_2$)
20	1530 (C=S) 1650 (C=O) 3305 (NH)	Thiourea = 7.11-7.71 (4H, m, Ar-H), 7.84 (1H, s, -NH), 3.68 (3H, s, -OCH_3), 6.51 (1H, d, NH); Val = 1.02-1.06 (6H, d, $(CH_3)_2$), 4.42-4.49 (1H, t, $^{\alpha}$CH), 1.86-1.92 (1H, m, $^{\beta}$CH); Pro = 4.06-4.13 (1H, t, -$^{\alpha}$CH), 1.11-1.29 (4H, m, -$^{\beta,\gamma}CH_2$), 3.51 (2H, t, -$^{\delta}CH_2$)
21	1506 (C=S) 1644 (C=O) 3354 (NH)	Thiourea = 7.02-7.64 (4H, m, Ar-H), 7.72 (1H, s, -NH), 6.54 (1H, d, NH); Val = 1.06-1.12 (6H, d, $(CH_3)_2$), 4.39-4.56 (1H, t, $^{\alpha}$CH), 1.90-1.93 (1H, m, $^{\beta}$CH); Pro = 4.10-4.13 (1H, t, -$^{\alpha}$CH), 1.07-1.32 (4H, m, -$^{\beta,\gamma}CH_2$), 3.41 (2H, t, -$^{\delta}CH_2$);
22	1632 (C=O) 3346 (NH)	Urea = 6.98-7.62 (4H, m, Ar-H), 7.76 (1H, s, -NH), 6.54 (1H, d, NH); Val = 1.07-1.17 (6H, d, $(CH_3)_2$), 4.39-4.45 (1H, t, $^{\alpha}$CH), 1.88-1.92 (1H, m, $^{\beta}$CH); Pro = 4.01-4.06 (1H, t, -$^{\alpha}$CH), 1.07-1.29 (4H, m, -$^{\beta,\gamma}CH_2$), 3.42 (2H, t, -$^{\delta}CH_2$)
23	1528 (C=S) 1636 (C=O) 3340 (NH)	Thiourea = 7.05-7.56 (4H, m, Ar-H), 7.70 (1H, s, -NH), 6.52 (1H, d,NH); Val = 1.05-1.11 (6H, d, $(CH_3)_2$), 4.44-4.51 (1H, t, $^{\alpha}$CH), 1.88-1.93 (1H, m, $^{\beta}$CH); Pro = 4.00-4.08 (1H, t, -$^{\alpha}$CH), 1.07-1.29 (4H, m, -$^{\beta,\gamma}CH_2$), 3.40 (2H, t, -$^{\delta}CH_2$)
24	1618 (C=O) 3348 (NH)	Urea = 7.11-7.59 (4H, m, Ar-H), 7.65 (1H, s, -NH), 6.60 (1H, d, NH); Val = 0.98-1.04 (6H, d, $(CH_3)_2$), 4.49-4.56 (1H, t, $^{\alpha}$CH), 1.91-1.95 (1H, m, $^{\beta}$CH); Pro = 4.00-4.03 (1H, t, -$^{\alpha}$CH), 1.04-1.25 (4H, m, -$^{\beta,\gamma}CH_2$), 3.47 (2H, t, -$^{\delta}CH_2$)
25	1636 (C=O) 3344 (NH)	Urea = 3.66 (3H, s, -OCH_3), 7.02-7.66 (4H, m, Ar-H), 8.04 (1H, s, -NH), 6.33-6.39 (1H, d, NH); Lys = 4.47-4.51 (1H, m, $^{\alpha}$CH), 1.90-2.04 (2H, m, $^{\beta}CH_2$), 1.81-1.92 (2H, m, $^{\gamma}CH_2$), 1.65-1.73 (2H, m, $^{\delta}CH_2$), 3.28-3.41 (2H, m, $^{\epsilon}CH_2$) Z = 7.71-7.64 (1H, t, NH), 5.19 (2H, s, CH_2), 7.02-7.66 (5H, m, Ar-H) Pro = 4.34 (1H, t, -$^{\alpha}$CH), 1.82-2.26 (4H, m, -$^{\beta,\gamma}CH_2$), 3.18-3.26 (2H, t, -$^{\delta}CH_2$)
26	1506 (C=S) 1622 (C=O) 3324 (NH)	Thiourea = 3.63 (3H, s, -OCH_3), 7.01-7.68 (4H, m, Ar-H), 8.14 (1H, s, -NH), 6.18-6.26 (1H, d, NH); Lys = 4.43-4.46 (1H, m, $^{\alpha}$CH), 1.87-1.94 (2H, m, $^{\beta}CH_2$), 1.79-1.84 (2H, m, $^{\gamma}CH_2$), 1.63-1.71 (2H, m, $^{\delta}CH_2$), 3.27-3.34 (2H, m, $^{\epsilon}CH_2$) Z = 7.81-7.85 (1H, t, NH), 5.16 (2H, s, CH_2), 7.01-7.68 (5H, m, Ar-H) Pro = 4.39 (1H, t, -$^{\alpha}$CH), 1.83-2.36 (4H, m, -$^{\beta,\gamma}CH_2$), 3.24-3.32 (2H, t, -$^{\delta}CH_2$)
27	1636 (C=O) 3324 (NH)	Urea = 3.71 (3H, s, -OCH_3), 6.94-7.35 (4H, m, Ar-H), 8.08 (1H, s, -NH), 6.36-6.41 (1H, d, NH); Lys = 4.47-4.51 (1H, m, $^{\alpha}$CH), 1.95-2.04 (2H, m, $^{\beta}CH_2$), 1.80-1.85 (2H, m, $^{\gamma}CH_2$), 1.67-1.71 (2H, m, $^{\delta}CH_2$), 3.24-3.32 (2H, m, $^{\epsilon}CH_2$) Z = 7.61-7.64 (1H, t, NH), 5.22 (2H, s, CH_2), 6.94-7.35 (5H, m, Ar-H) Pro = 4.51 (1H, t, -$^{\alpha}$CH), 1.81-2.34 (4H, m, -$^{\beta,\gamma}CH_2$), 3.26-3.36 (2H, t, -$^{\delta}CH_2$)

28	1512 (C=S) 1626 (C=O) 3344 (NH)	Thiourea = 3.81 (3H, s, -OCH_3), 7.03-7.71 (4H, m, Ar-H), 8.14 (1H, s, -NH), 6.38-6.42 (1H, d, NH); Lys = 4.41-4.46 (1H, m, $^{\alpha}CH$), 1.95-2.04 (2H, m, $^{\beta}CH_2$), 1.85-1.91 (2H, m, $^{\gamma}CH_2$), 1.66-1.72 (2H, m, $^{\delta}CH_2$), 3.28-3.32 (2H, m, $^{\epsilon}CH_2$) Z = 7.82-7.86 (1H, t, NH), 5.17 (2H, s, CH_2), 7.03-7.71 (5H, m, Ar-H) Pro = 4.39 (1H, t, -$^{\alpha}CH$), 1.79-2.24 (4H, m, -$^{\beta,\gamma}CH_2$), 3.18-3.24 (2H, t, -$^{\delta}CH_2$)
29	1529 (C=S) 1648 (C=O) 3314 (NH)	Thiourea = 6.96-7.53 (4H, m, Ar-H), 8.09 (1H, s, -NH), 6.36-6.39 (1H, d, NH); Lys = 4.41-4.45 (1H, m, $^{\alpha}CH$), 1.86-1.96 (2H, m, $^{\beta}CH_2$), 1.77-1.85 (2H, m, $^{\gamma}CH_2$), 1.64-1.70 (2H, m, $^{\delta}CH_2$), 3.25-3.32 (2H, m, $^{\epsilon}CH_2$) Z = 7.55-7.66 (1H, t, NH), 5.19 (2H, s, CH_2), 6.96-7.53 (5H, m, Ar-H) Pro = 4.32 (1H, t, -$^{\alpha}CH$), 1.82-2.26 (4H, m, -$^{\beta,\gamma}CH_2$), 3.24-3.35 (2H, t, -$^{\delta}CH_2$)
30	1636 (C=O) 3326 (NH)	Urea = 6.89-7.60 (4H, m, Ar-H), 8.02 (1H, s, -NH), 6.37-6.44 (1H, d, NH); Lys = 4.45-4.50 (1H, m, $^{\alpha}CH$), 1.92-1.98 (2H, m, $^{\beta}CH_2$), 1.81-1.85 (2H, m, $^{\gamma}CH_2$), 1.63-1.69 (2H, m, $^{\delta}CH_2$), 3.34-3.40 (2H, m, $^{\epsilon}CH_2$) Z = 7.72-7.75 (1H, t, NH), 5.19 (2H, s, CH_2), 7.03-7.67 (5H, m, Ar-H) Pro = 4.39 (1H, t, -$^{\alpha}CH$), 1.75-2.25 (4H, m, -$^{\beta,\gamma}CH_2$), 3.18-3.26 (2H, t, -$^{\delta}CH_2$)
31	1514 (C=S) 1636 (C=O) 3311 (NH)	Thiourea = 7.05-7.67 (4H, m, Ar-H), 8.01 (1H, s, -NH), 6.32-6.36 (1H, d, NH); Lys = 4.42-4.48 (1H, m, $^{\alpha}CH$), 1.95-1.99 (2H, m, $^{\beta}CH_2$), 1.81-1.85 (2H, m, $^{\gamma}CH_2$), 1.64-1.67 (2H, m, $^{\delta}CH_2$), 3.24-3.29 (2H, m, $^{\epsilon}CH_2$) Z = 7.72-7.75 (1H, t, NH), 5.16 (2H, s, CH_2), 7.05-7.67 (5H, m, Ar-H) Pro = 4.36 (1H, t, -$^{\alpha}CH$), 1.76-2.24 (4H, m, -$^{\beta,\gamma}CH_2$), 3.15-3.25 (2H, t, -$^{\delta}CH_2$)
32	1620 (C=O) 3335 (NH)	Urea = 6.94-7.35 (4H, m, Ar-H), 8.10 (1H, s, -NH), 6.40-6.43 (1H, d, NH) Lys = 4.50-4.54 (1H, m, $^{\alpha}CH$), 1.93-2.00 (2H, m, $^{\beta}CH_2$), 1.83-1.89 (2H, m, $^{\gamma}CH_2$), 1.69-1.70 (2H, m, $^{\delta}CH_2$), 3.30-3.37 (2H, m, $^{\epsilon}CH_2$) Z = 7.58-7.64 (1H, t, NH), 5.27 (2H, s, CH_2), 7.03-7.67 (5H, m, Ar-H) Pro = 4.48 (1H, t, -$^{\alpha}CH$), 1.80-2.30 (4H, m, -$^{\beta,\gamma}CH_2$), 3.20-3.30 (2H, t, -$^{\delta}CH_2$)

[b] = The chemical shift values for the fragment 'Het' of the compounds V-VIII /1-32 are almost same as obtained for compound V

Table – 1: Antiglycation and Urease inhibition activities of the synthesized compounds (1-32)



		R: Side chain of Tyr(2,6-Cl_2-Bzl)			R: Side chain of Phe			R: Side chain of Val			R: Side chain of Lys(Z)		
R'	X		AG* IC_{50} (µM)	UI* IC_{50} (µM)		AG* IC_{50} (µM)	UI* IC_{50} (µM)		AG* IC_{50} (µM)	UI* IC_{50} (µM)		AG* IC_{50} (µM)	UI* IC_{50} (µM)
3-OCH_3	O	**1**	47.5 ± 0.5	21.6 ± 0.6	**9**	53.0 ± 0.3	14.5 ± 0.6	**17**	55.0 ± 0.8	24.0 ± 0.2	**25**	68.0 ± 0.8	33.0 ± 0.2
3-OCH_3	S	**2**	31.5 ± 0.4	8.6 ± 0.2	**10**	37.0 ± 0.4	11.2 ± 0.4	**18**	39.0 ± 0.2	17.0 ± 0.1	**26**	50.0 ± 0.7	27.5 ± 0.1
4-OCH_3	O	**3**	28.0 ± 0.3	16.0 ± 0.6	**11**	32.0 ± 0.4	13.0 ± 0.2	**19**	54.0 ± 0.3	18.5 ± 0.3	**27**	39.0 ± 0.3	15.0 ± 0.3
4-OCH_3	S	**4**	4.3 ± 0.5	7.2 ± 0.2	**12**	12.4 ± 0.3	4.9 ± 0.2	**20**	13.1 ± 0.5	14.0 ± 0.6	**28**	18.4 ± 0.4	11.4 ± 0.6
2-Br	S	**5**	17.1 ± 0.7	15.4 ± 0.3	**13**	19.5 ± 0.2	13.6 ± 0.7	**21**	31.0 ± 0.1	32.0 ± 0.4	**29**	37.5 ± 0.4	22.0 ± 0.4
3-Br	O	**6**	57.0 ± 0.3	62.0 ± 0.4	**14**	42.0 ± 0.8	35.0 ± 0.3	**22**	77.0 ± 0.2	41.0 ± 0.2	**30**	93.0 ± 0.3	63.0 ± 0.2

3-Br	S	**7**	40.0 ± 0.5	29.0 ± 0.4	**15**	36.0 ± 0.3	26.0 ± 0.6	**23**	55.0 ± 0.6	38.0 ± 0.1	**31**	87.0 ± 0.2	49.0 ± 0.3
4-Br	O	**8**	43.0 ± 0.4	36.0 ± 0.3	**16**	51.0 ± 0.6	41.0 ± 0.2	**24**	65.0 ± 0.2	43.0 ± 0.4	**32**	54.0 ± 0.4	80.0 ± 0.4
		V	>100	>100	**VI**	>100	>100	**VII**	>100	>100	**VIII**	>100	>100
Rutin			41.9			41.9			41.9			41.9	
Thiourea				21.0			21.0			21.0			21.0

values are mean of three determinations, the ranges of which are <5% of the mean in all case, AG = Antiglycation activity; UI* = Urease Inhibitory Activity

In our earlier work [32], we have reported the synthesis of a new series of urea/thiourea derivatives of glycine/proline conjugated benzisoxazole as promsing antiglycating agents. Further, it was found that compounds containing methoxy and bromine substituents have excerted highly potent activity. Prompted by the earlier results, the present work involves the synthesis of 32 urea/thiourea derivatives of dipeptides, Xaa-Pro [Xaa = Tyr, Phe, Val and Lys], conjugated to benzisoxazole contains only methoxy and bromine as substituents. They screened for their *in vitro* antiglycation and antiurease activities with rutin and thiourea as reference standards respectively. The results were expressed in terms of IC_{50} (Table1).

All the synthesized compounds exhibited promising activity. Further, the dipeptide conjugates with methoxy at *para* position (4, 12, 20, 28) and bromo at ortho position (5, 13, 21, 29) exhibits good antiglycation as well as antiurease activities. Best antiglycation activity was found for Tyr-Pro (4) conjugated analogue with methoxy group at the *para* position and best antiurease activity was found for Phe-Pro (12) with methoxy group at the *para* position. The results clearly indicates the importance of peptides towards the potency of protein glycation and urease inhibition. Further, the trend showed that increase in the hydrophobicity of the molecule increases the antiglycation and urease inhibition activities. In conformation to our earlier findings, the compounds with thiourea are more potent than their corresponding urea analogues. The order of the activity is found to be KP<VP<FP<YP which is in analogues to their hydrophobicities [33-36]. It is very difficult to predict the reason for increasing the activities with this preliminary and limited result. Further work in this field is in progress.

6. CONCLUSION

In summary, compounds with methoxy at *para* position and bromo at *ortho* position of the aryl moiety along with thiourea system significantly influenced the antiglycation and antiurease activities when compared to its bioisostere counterpart methoxy/bromo substituted aryl moiety along with urea system. The success in this realm has clearly encouraged researchers for further exploration and more work is needed to make the design and optimization of potent and selective antiglycation and urease inhibitors with promising levels of activity, improved stability and low toxicity.

7. REFERENCES

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