Journal of Saudi Chemical Society (2017) **21**, S248–S257

King Saud University

Journal of Saudi Chemical Society

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ORIGINAL ARTICLE

Synthesis and evaluation of novel ureido/thioureido derivatives of amino acid conjugated 2,3-dichlorophenyl piperazine as highly potent antiglycating agents

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Received 14 September 2013; revised 10 February 2014; accepted 18 February 2014
Available online 12 March 2014

KEYWORDS

Amino acids;
Piperazine;
Conjugation;
Urea/thiourea;
Antiglycation

Abstract We report the synthesis and *in vitro* antiglycation activity of more than 80 amino acid–heterocycle conjugate derived ureas and thioureas. They were characterized by physical and spectroscopical methods. Many of the analogues synthesized showed activity at the sub-micro molar level. Introduction of different amino acids as linker and systematic variation of the substituents on the aromatic ring revealed promising leads. In particular, compounds containing Glu and Tyr as the linkers exhibited high antiglycating potency with IC_{50} 1–4 μM as against the reference, rutin with IC_{50} 41.9 μM.

Conclusions: Compounds bearing halogen atoms emerged as the most active analogues and serve as lead for future studies.

1. Introduction

Diabetes mellitus, especially type 2 diabetes is increasing at an alarming rate and is considered as one of the main threats to human health in the 21st century, in both developed and developing nations [38]. Over the last 30 years, an enormous number of studies have been dedicated to unravel the pathophysiology of type 2 diabetes mellitus [28]. The AGEs are a class of compounds which are generated during protein glycation [26]. This process starts with the formation of an initial adduct between the carbonyl group of reducing sugar and the $-NH_2$ group of a protein, a Maillard reaction. Accumulation of AGEs is associated with aging, diabetes, Alzheimer's disease, renal failure and many other chronic diseases [11].

Abbreviations: AGE, advanced glycation end products; Boc, *t*-butoxycarbonyl; BSA, bovine serum albumin; EDCI, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide·HCl; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methyl morpholine; PBS, phosphate buffer saline; PZN, 1-(2,3-dichlorophenyl)piperazine; TCA, trichloroacetic acid; TFA, trifluoroacetic acid

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2014.02.006

One very interesting and promising class of heterocycle is piperazine and its derivatives. They have been extensively investigated by the organic chemists due to their close association with various types of biological activities. Moreover they have wide clinical applications in the therapy of functional diseases and exhibit insecticidal [4], antidiabetic [9], antimicrobial [3], acetyl cholinesterase inhibitors [10], antimalarial [23], dopamine transporter [19], D_2/D_4 antagonist (He [12], MC_4 receptor [2] and HIV-protease inhibitor [24]. On the other hand, the therapeutical applications of amino acids have received a considerable attention in respiratory physiology, cardiology, renal failure, neurological disorders and congenital defects. According to earlier studies, amino acids have also proven to be antidiabetic, antiglycating and anticataractogenic [27,34]. Several amino acids have found to be beneficial in diabetes mellitus (DM) through their antiglycating functions [22]. Furthermore, previous reports from our research group have revealed that conjugation of different amino acids/peptides to various biologically active scaffolds and their derivatization led to promising leads [35,29,31–33,25,37].

An unsymmetrically substituted urea is a common structural feature of many biological active compounds such as enzyme inhibitors and pseudopeptides [8]. Sulfonylureas have found applications as oral antidiabetic drugs and as herbicides [13]. However, urea can also be used as a protector of glycation, especially against fluorescent advanced glycation end products [14]. Moreover, recently unsymmetrically disubstituted urea derivatives, bis-Schiff bases of and also 3,5-di(trifluoromethyl)-1,2,4-triazolesulfonyl ureas and thioureas have been reported as potent class of antiglycation and antidiabetic agents, respectively [15,16,6].

Encouraged by the above facts, the present investigation involves the synthesis and antiglycation studies of four series of amino acid-2,3-dichlorophenyl piperazine conjugated urea/thiourea derivatives.

2. Materials and methods

2.1. General

Amino acids used were of *L*-configuration unless otherwise mentioned. TFA was purchased from Advanced Chem. Tech. (Louisville, KY, USA). NMM and phenyl isocyanates/isothiocyanates were purchased from Sigma Chemical Co. (St. Louis, MO). Melting points were 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). Bovine serum albumin was purchased from Research Organics, Cleveland, while other chemicals were purchased from Sigma Aldrich.

2.2. Chemistry

2.2.1. General procedure for the conjugation of Boc–Xaa–OH [where Xaa = Phe, Glu(OBzl), Tyr(2,6-Cl_2-OBzl) and Lys(Z)] to 1-(2,3-dichlorophenyl)piperazine (**1**–**4**)

1-(2,3-Dichlorophenyl)piperazine·HCl was synthesized by previously reported method [21]. 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 (3×20 mL), water (3×20 mL), 0.1 N cold HCl (3×20 mL) followed by brine. The organic layer was dried over anhydrous Na_2SO_4, the solvent was removed under reduced pressure, triturated with ether, filtered and dried to get the conjugates **1**–**4**.

2.2.2. General procedure for the synthesis of ureido and thioureido derivatives (**8**–**55**)

Each time, Boc–Xaa–PZN (0.150 g) [where Xaa = side chain of Phe, Glu, Tyr and Lys] 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 or isothiocyanates (0.0012 mol) were added drop-wise while maintaining the temperature at 0 °C. The reaction mixture was stirred for 8 h 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 (2×10 mL), water (2×10 mL), 0.1 N citric acid (2×10 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 under vacuum.

2.2.3. General procedure for side chain deprotection of OBzl of Glu, 2,6-Cl_2-Bzl of Tyr and Z of Lys (**56**–**91**)

To a solution of the derivatives (0.001 mol) in methanol (10 mL/g of compound), 10% Pd–C (100 mg) and ammonium formate (1 g) were added and the mixture was stirred at room temperature for 2 h. After completion of the reaction monitored by TLC, catalyst was filtered

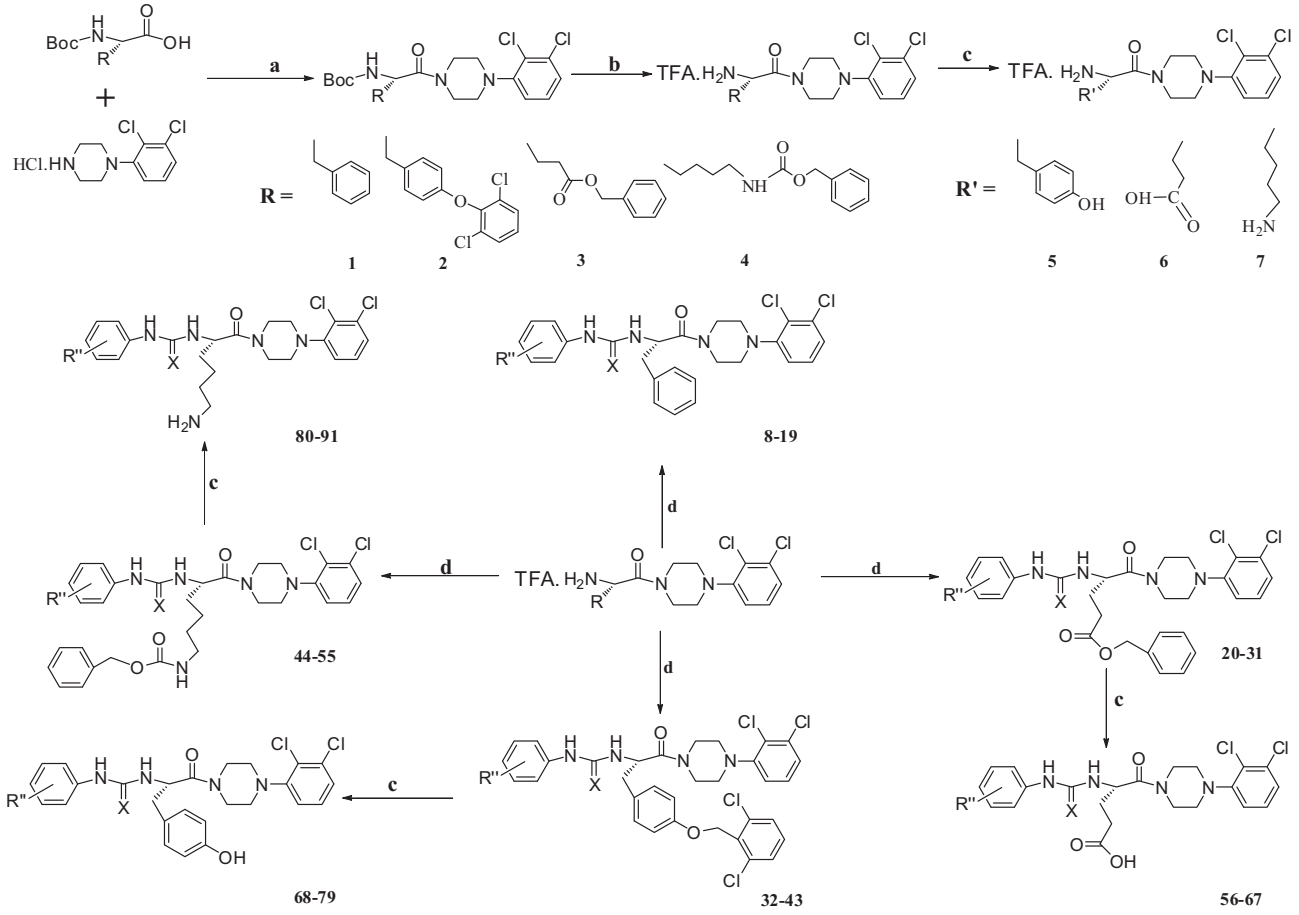
and washed with methanol. The solvent was evaporated under reduced pressure and the product was taken into $CHCl_3$, washed with saturated NaCl and the solvent was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and triturated with ether, filtered and dried to get side chain deprotected derivatives (**56–91**).

2.3. Pharmacology

Antiglycation assay (in vitro) [20]. 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 to 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 was 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:

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



Scheme 1 Schematic representation of the synthesis of ureido/thioureido derivatives of amino acid-conjugated 2,3-dichlorophenyl piperazine. Reagents and conditions: (a) EDCI/HOBt, NMM, 0 °C; (b) TFA, 40 min, rt; (c) $HCOONH_4$/10% Pd–C; (d) Phenyl isocyanates/isothiocyanates, NMM, THF, 8 h, 0 °C to rt; R = Side chain of Phe, Tyr, Glu and Lys; R″ = H, Br, F, Cl; X = O or S.

Table 1 Physical and mass data of the synthesized compounds.

Entry	R	X	R_f^a	R_f^b	Yield (%)	MP (°C)	Mol. For.	Mass (M + 1)
8	H	O	0.58	0.69	91.5	Gum	$C_{26}H_{26}Cl_2N_4O_2$	483.1
9	H	S	0.54	0.65	92.6	Gum	$C_{26}H_{26}Cl_2N_4OS$	499.1
10	2Br	S	0.53	0.62	94.2	62–65	$C_{26}H_{25}Cl_2BrN_4OS$	578.0
11	3Br	O	0.56	0.69	92.5	84–86	$C_{26}H_{25}Cl_2BrN_4O_2$	562.0
12	3Br	S	0.53	0.66	90.3	63–65	$C_{26}H_{25}Cl_2BrN_4OS$	578.0
13	4Br	O	0.52	0.67	94.2	75–77	$C_{26}H_{25}Cl_2BrN_4O_2$	562.4
14	3F	O	0.54	0.65	90.5	89–91	$C_{26}H_{25}Cl_2FN_4O_2$	501.1
15	4F	O	0.55	0.63	92.5	87	$C_{26}H_{25}Cl_2FN_4O_2$	501.1
16	4F	S	0.51	0.62	94.5	Gum	$C_{26}H_{25}Cl_2FN_4OS$	517.1
17	3Cl	O	0.53	0.63	90.4	90	$C_{26}H_{25}Cl_3N_4O_2$	515.0
18	3Cl	S	0.56	0.68	96.5	Gum	$C_{26}H_{25}Cl_3N_4OS$	533.0
19	4Cl	S	0.52	0.67	87.5	72	$C_{26}H_{25}Cl_3N_4OS$	532.0
20	H	O	0.45	0.56	90.4	95	$C_{29}H_{30}Cl_2N_4O_4$	569.1
21	H	S	0.42	0.51	86.3	82	$C_{29}H_{30}Cl_2N_4O_3S$	585.1
22	2Br	S	0.48	0.52	90.7	58	$C_{29}H_{29}Cl_2BrN_4O_3S$	664.0 664.5
23	3Br	O	0.44	0.53	94.7	70–72	$C_{29}H_{29}Cl_2BrN_4O_4$	648.0 648.1
24	3Br	S	0.41	0.54	90.8	62	$C_{29}H_{29}Cl_2BrN_4O_3S$	664.5 664.6
25	4Br	O	0.43	0.57	94.5	65	$C_{29}H_{29}Cl_2BrN_4O_4$	648.0 648.2
26	3F	O	0.40	0.55	92.5	75	$C_{29}H_{29}Cl_2FN_4O_4$	587.1
27	4F	O	0.46	0.59	94.3	78–80	$C_{29}H_{29}Cl_2FN_4O_4$	587.1
28	4F	S	0.43	0.56	91.4	67–69	$C_{29}H_{29}Cl_2FN_4O_3S$	604.1
29	3Cl	O	0.41	0.54	93.2	78	$C_{29}H_{29}Cl_3N_4O_4$	604.1
30	3Cl	S	0.44	0.57	88.5	70–72	$C_{29}H_{29}Cl_3N_4O_3S$	619.1
31	4Cl	S	0.42	0.58	95.3	74	$C_{29}H_{29}Cl_2FN_4O_3S$	619.1
32	H	O	0.62	0.71	90.2	75	$C_{33}H_{30}Cl_4N_4O_3$	671.1
33	H	S	0.59	0.69	94.5	68	$C_{33}H_{30}Cl_4N_4O_2S$	687.3
34	2Br	S	0.58	0.68	88.6	58–60	$C_{33}H_{29}Cl_4BrN_4O_2S$	766.0 766.3
35	3Br	O	0.63	0.71	86.2	68–70	$C_{33}H_{29}Cl_4BrN_4O_3$	750.0 750.5
36	3Br	S	0.64	0.72	92.3	60	$C_{33}H_{29}Cl_4BrN_4O_2S$	765.9 765.5
37	4Br	O	0.61	0.70	86.3	72	$C_{33}H_{29}Cl_4BrN_4O_3$	750.0 750.5
38	3F	O	0.60	0.69	94.0	115–118	$C_{33}H_{29}Cl_4FN_4O_3$	689.1
39	4F	O	0.58	0.68	92.1	120–123	$C_{33}H_{29}Cl_4FN_4O_3$	689.1
40	4F	S	0.63	0.71	96.3	75	$C_{33}H_{29}Cl_4FN_4O_2S$	705.1
41	3Cl	O	0.65	0.73	89.3	110–112	$C_{33}H_{29}Cl_5N_4O_3$	705.1
42	3Cl	S	0.64	0.72	94.2	95	$C_{33}H_{29}Cl_5N_4O_3$	721.0
43	4Cl	S	0.62	0.70	87.5	98–100	$C_{33}H_{29}Cl_5N_4O_2S$	721.1
44	H	O	0.54	0.60	94.3	Gum	$C_{31}H_{35}Cl_2N_5O_4$	612.2
45	H	S	0.52	0.59	96.0	Gum	$C_{31}H_{35}Cl_2N_5O_3S$	628.1
46	2Br	S	0.53	0.61	88.3	67–70	$C_{31}H_{34}Cl_2BrN_5O_3S$	707.1 707.4
47	3Br	O	0.54	0.63	89.2	78	$C_{31}H_{34}Cl_2BrN_5O_4$	691.6 691.3
48	3Br	S	0.56	0.62	90.3	71	$C_{31}H_{34}Cl_2BrN_5O_3S$	707.1 707.5
49	4Br	O	0.52	0.59	92.5	73	$C_{31}H_{34}Cl_2BrN_5O_4$	691.8 691.5
50	3F	O	0.55	0.60	95.6	Gum	$C_{31}H_{34}Cl_2FN_5O_4$	630.2
51	4F	O	0.54	0.62	87.4	Gum	$C_{31}H_{34}Cl_2FN_5O_4$	630.2
52	4F	S	0.57	0.64	95.2	Gum	$C_{31}H_{34}Cl_2FN_5O_3S$	646.1
53	3Cl	O	0.50	0.59	91.3	92	$C_{31}H_{34}Cl_3N_5O_4$	646.1
54	3Cl	S	0.54	0.62	89.5	84–86	$C_{31}H_{34}Cl_3N_5O_3S$	662.1

(*continued on next page*)

Table 1 *(continued)*

Entry	R	X	R_f[a]	R_f[b]	Yield (%)	MP (°C)	Mol. For.	Mass (M + 1)
55	4Cl	S	0.51	0.60	93.2	82	$C_{31}H_{34}Cl_3N_5O_3S$	662.1
56	H	O	0.31	0.45	86.5	90	$C_{22}H_{24}Cl_2N_4O_4$	479.1
57	H	S	0.33	0.42	92.5	84–86	$C_{22}H_{24}Cl_2N_4O_3S$	495.0
58	2Br	S	0.35	0.48	93.2	82	$C_{22}H_{23}Cl_2BrN_4O_3S$	574.0 574.2
59	3Br	O	0.32	0.44	90.2	85	$C_{22}H_{23}Cl_2BrN_4O_4$	558.0 558.3
60	3Br	S	0.30	0.41	87.5	78–80	$C_{22}H_{23}Cl_2BrN_4O_3S$	574.1 574.5
61	4Br	O	0.32	0.43	93.5	87	$C_{22}H_{23}Cl_2BrN_4O_4$	558.0 558.2
62	3F	O	0.34	0.40	89.3	71	$C_{22}H_{23}Cl_2FN_4O_4$	497.1
63	4F	O	0.33	0.46	86.5	68–70	$C_{22}H_{23}Cl_2FN_4O_4$	497.1
64	4F	S	0.30	0.43	95.6	78	$C_{22}H_{23}Cl_2FN_4O_3S$	513.1
65	3Cl	O	0.31	0.41	89.5	88	$C_{22}H_{23}Cl_3N_4O_4$	513.0
66	3Cl	S	0.34	0.44	95.6	74	$C_{22}H_{23}Cl_3N_4O_3S$	529.1
67	4Cl	S	0.32	0.42	90.5	76–78	$C_{22}H_{23}Cl_3N_4O_3S$	529.1
68	H	O	0.54	0.60	92.5	105	$C_{26}H_{26}Cl_2N_4O_3$	512.1
69	H	S	0.51	0.59	93.4	88–90	$C_{26}H_{26}Cl_2N_4O_2S$	529.1
70	2Br	S	0.49	0.62	89.5	118	$C_{26}H_{25}Cl_2BrN_4O_2S$	608.0 608.2
71	3Br	O	0.52	0.58	89.5	143	$C_{26}H_{25}Cl_2BrN_4O_3$	592.0 592.4
72	3Br	S	0.53	0.60	90.6	120	$C_{26}H_{25}Cl_2BrN_4O_2S$	608.1 608.5
73	4Br	O	0.50	0.59	89.6	132	$C_{26}H_{25}Cl_2BrN_4O_3$	592.0 592.4
74	3F	O	0.54	0.62	90.2	152	$C_{26}H_{25}Cl_2FN_4O_3$	531.4
75	4F	O	0.52	0.58	88.9	154	$C_{26}H_{25}Cl_2FN_4O_3$	531.3
76	4F	S	0.51	0.59	92.5	145–147	$C_{26}H_{25}Cl_2FN_4O_2S$	547.4
77	3Cl	O	0.53	0.61	94.6	135–137	$C_{26}H_{25}Cl_3N_4O_3$	548.8
78	3Cl	S	0.51	0.59	95.7	114	$C_{26}H_{25}Cl_3N_4O_2S$	563.9
79	4Cl	S	0.52	0.60	93.5	114–116	$C_{26}H_{25}Cl_3N_4O_2S$	563.8
80	H	O	0.43	0.54	89.6	Gum	$C_{23}H_{29}Cl_2N_5O_2$	478.4
81	H	S	0.40	0.55	87.2	Gum	$C_{23}H_{29}Cl_2N_5OS$	494.4
82	2Br	S	0.42	0.51	94.5	60	$C_{23}H_{28}Cl_2BrN_5OS$	574.3 574.4
83	3Br	O	0.41	0.53	92.5	65	$C_{23}H_{28}Cl_2BrN_5O_2$	557.1 557.5
84	3Br	S	0.43	0.56	95.7	58	$C_{23}H_{28}Cl_2BrN_5OS$	574.3 574.6
85	4Br	O	0.42	0.50	93.6	70	$C_{23}H_{28}Cl_2BrN_5O_2$	557.1 557.4
86	3F	O	0.41	0.52	88.3	Gum	$C_{23}H_{28}Cl_2FN_5O_2$	496.4
87	4F	O	0.45	0.55	89.4	Gum	$C_{23}H_{28}Cl_2FN_5O_2$	496.3
88	4F	S	0.40	0.52	91.2	Gum	$C_{23}H_{28}Cl_2FN_5OS$	512.4
89	3Cl	O	0.43	0.50	92.8	72	$C_{23}H_{28}Cl_3N_5O_2$	512.8
90	3Cl	S	0.45	0.53	94.1	Gum	$C_{23}H_{28}Cl_3N_5OS$	528.9
91	4Cl	S	0.43	0.55	92.4	Gum	$C_{23}H_{28}Cl_3N_5OS$	528.8

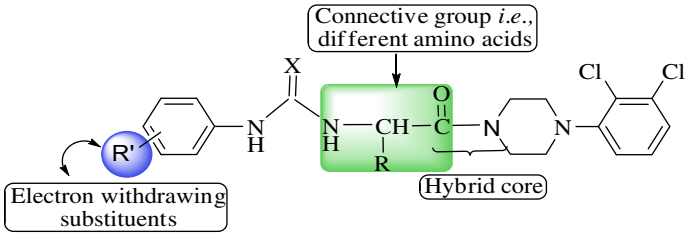


Figure 1 General features of the compounds proposed in this work.

3. Results and discussion

In the present study we have synthesized four series of disubstituted urea and thiourea derivatives. The synthesis was started by conjugating piperazine moiety to different amino acids using EDCI/HOBt as coupling agent and NMM as base. TLC indicated the completion of the reaction. Further the conjugation was confirmed by the absence of the –COOH group and appearance of the amide group (CONH) in the PMR spectrum. The Boc group of the conjugates was removed using TFA (which was confirmed by TLC) and was further reacted with respective substituted phenyl isocyanates/isothiocyanates to get the title compounds. Further, OBzl of Glu, 2,6-Cl_2-Bzl of Tyr and Z of Lys were removed by $HCOONH_4$/10% Pd–C to obtain corresponding side chain free derivatives (Scheme 1). Yields of the compounds were found to be >85%. All the synthesized compounds 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 $\delta \sim 8.75$ and multiplet for another –NH proton at $\delta \sim 8.31$ for urea derivatives. On the other hand, δ singlet at ~9.70 (−NH) and multiplet at $\delta \sim 8.49$ (NH) were 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 physical and mass data of the derivatives are presented in Table 1. Structural IR and ^{1}H NMR data have been provided as Supplementary Data.

From our previous investigations we knew that the amide bond (obtained by conjugating amino acids/peptides with heterocycle) [29–31] is beneficial for enhancing the biological properties. Therefore we first started with the conjugation of amino acids with 2,3-dichlorophenyl piperazine to obtain hybrid molecules **1**–**7**. To get the structural feature shown in Fig. 1 it was necessary to introduce NH–CO–NH/NH–CS–NH groups by using amino acids and isocyanates/isothiocyanates. The resultant 84 title compounds were subjected to antiglycation activity and the data are presented in Table 2. Rutin served as positive control in the assay with IC_{50} 41.9 µM.

In the earlier work [37], it was noticed that compounds containing simple amino acids like glycine and proline revealed promising antiglycation activity. Further, it was observed that the presence of electron withdrawing substituents enhances the activity compared to electron donating groups. This formed the basis for the present work wherein, we have chosen representative amino acid from different classes and also felt necessary to consider only electron withdrawing groups.

In this direction, we have synthesized a small library of 84 compounds in order to identify potent antiglycating agents. Among the amino acids, compounds containing Glu (**20**–**31**/**56**–**67**) and Tyr (**32**–**43**/**68**–**79**) showed a significant improvement in the activity compared to Phe (**8**–**19**) and Lys (**44**–**55**/**80**–**91**) analogues. From this it can be inferred that the presence of acidic instead of basic amino acid and phenolic instead of the phenyl group containing amino acid is favorable for better antiglycation activity.

Table 2 Antiglycation activity of the synthesized compounds.

R	Z	With protection							Without protection							
		IC_{50} ± SEM[a] (µM)							IC_{50} ± SEM[a] (µM)							
		Entry	Phe	Entry	Glu(OBzl)	Entry	Tyr(2,6-Cl_2-Bzl)		Entry	Lys(Z)	Entry	Glu	Entry	Tyr	Entry	Lys
H	O	**8**	176.2 ± 1.65	**20**	100.0 ± 3.05	**32**	131.19 ± 3.23		**44**	265.15 ± 4.25	**56**	92.34 ± 1.32	**68**	125.0 ± 2.08	**80**	215.18 ± 5.05
H	S	**9**	145.0 ± 1.21	**21**	60.25 ± 2.95	**33**	180.24 ± 4.08		**45**	250.10 ± 5.13	**57**	54.21 ± 2.65	**69**	175.98 ± 1.92	**81**	224.56 ± 4.52
2Br	S	**10**	2.00 ± 0.9	**22**	4.09 ± 2.09	**34**	1.96 ± 0.79		**46**	2.00 ± 1.19	**58**	3.16 ± 1.15	**70**	1.79 ± 0.95	**82**	1.62 ± 0.91
3Br	O	**11**	5.28 ± 0.83	**23**	1.35 ± 0.95	**35**	1.80 ± 0.96		**47**	2.20 ± 1.09	**59**	1.18 ± 0.62	**71**	1.60 ± 1.00	**83**	2.08 ± 1.05
3Br	S	**12**	4.58 ± 1.22	**24**	1.85 ± 0.86	**36**	4.40 ± 1.12		**48**	3.12 ± 1.95	**60**	1.45 ± 0.74	**72**	3.42 ± 1.02	**84**	3.02 ± 1.12
4Br	O	**13**	4.12 ± 1.25	**25**	3.85 ± 2.12	**37**	4.72 ± 2.25		**49**	4.18 ± 2.25	**61**	2.80 ± 0.99	**73**	4.22 ± 2.12	**85**	3.42 ± 1.39
3F	O	**14**	1.89 ± 0.90	**26**	3.85 ± 1.18	**38**	2.00 ± 1.09		**50**	4.10 ± 2.38	**62**	2.72 ± 1.18	**74**	1.59 ± 0.52	**86**	3.59 ± 2.13
4F	O	**15**	1.75 ± 0.71	**27**	1.90 ± 0.63	**39**	2.60 ± 1.03		**51**	3.09 ± 1.08	**63**	1.75 ± 0.90	**75**	2.48 ± 1.05	**87**	2.75 ± 1.05
4F	S	**16**	5.00 ± 1.65	**28**	1.96 ± 0.90	**40**	3.10 ± 1.24		**52**	3.89 ± 2.25	**64**	1.71 ± 0.79	**76**	2.72 ± 1.49	**88**	3.31 ± 1.23
3Cl	O	**17**	1.15 ± 0.85	**29**	2.29 ± 1.32	**41**	1.82 ± 0.95		**53**	2.75 ± 0.82	**65**	1.67 ± 0.68	**77**	1.40 ± 0.52	**89**	1.89 ± 0.80
3Cl	S	**18**	4.00 ± 2.15	**30**	4.10 ± 1.11	**42**	1.92 ± 0.83		**54**	3.79 ± 1.25	**66**	3.94 ± 1.09	**78**	1.47 ± 0.98	**90**	3.02 ± 1.09
4Cl	S	**19**	4.45 ± 2.20	**31**	3.90 ± 1.27	**43**	4.59 ± 2.17		**55**	4.00 ± 2.04	**67**	3.57 ± 1.18	**79**	3.59 ± 2.07	**91**	3.19 ± 1.10
		I	Boc–Phe–PZN		> 500	**II**	Boc-Glu(OBzl)-PZN			325.21 ± 3.23	**III**	Boc-Tyr(2,6-Cl_2-Bzl)-PZN				238.41 ± 2.36
		IV	Boc–Lys(Z)–PZN		213.50 ± 6.9	**V**	Boc-Glu-PZN			317.09 ± 2.5	**VI**	Boc-Tyr-PZN				220.0 ± 2.8
		VII	Boc–Lys–PZN		205.49		Standard, rutin			41.9 ± 2.3						

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.

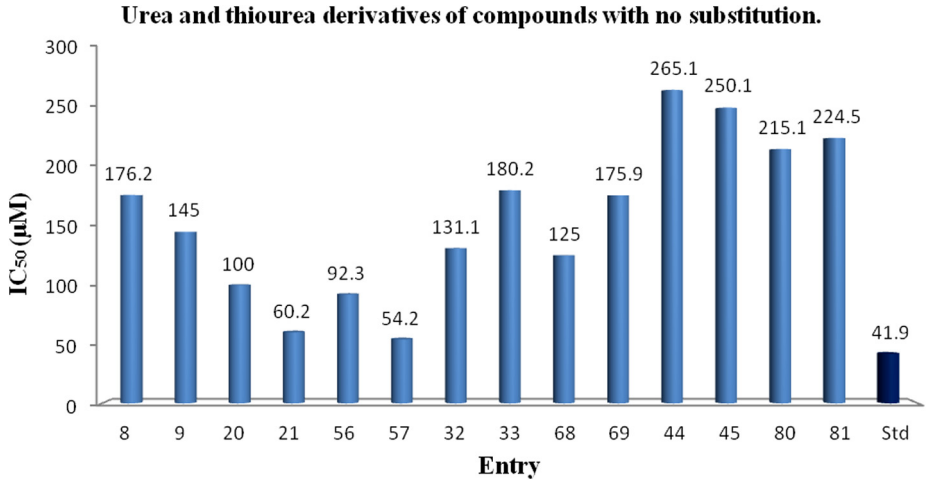


Figure 2 Diagrammatic representation of antiglycation activity of urea and thiourea derivatives of compounds with no substitution.

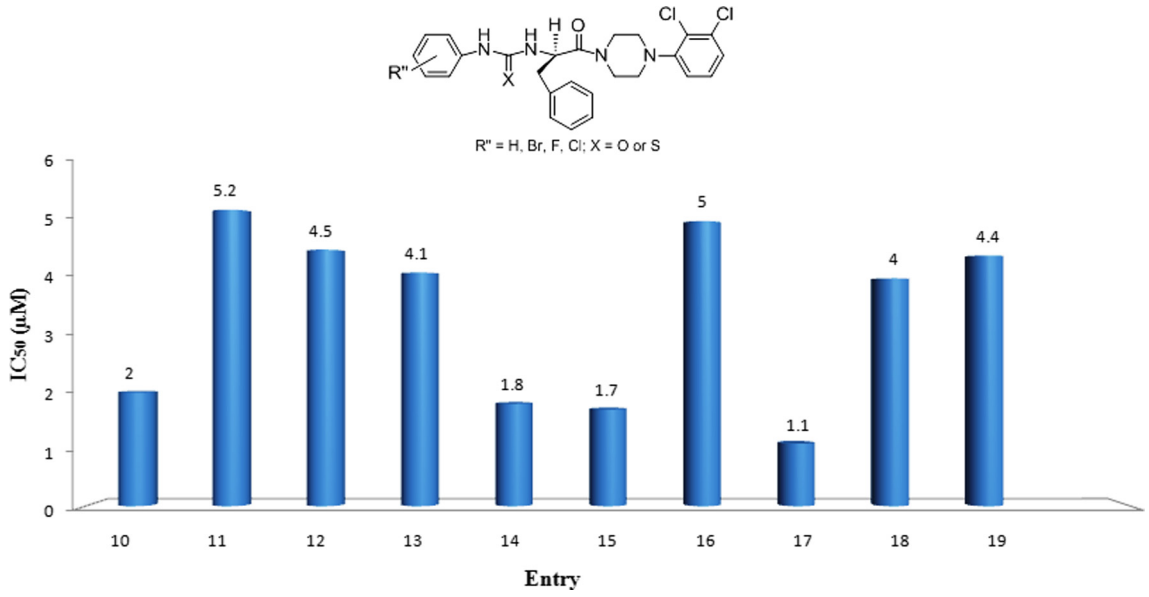


Figure 3 Diagrammatic representation of antiglycation activity of urea and thiourea derivatives of phenylalanine containing compounds.

In medicinal chemistry thiourea moiety is considered as isostere for urea bond possessing more rigidity and stability [5,7]. Further several articles [17,1] report that compounds containing the thiourea group exhibit more activity than urea. So, we synthesized two sets of compounds containing these functionalities. It was noticed that there was no difference in activity if O of urea is replaced by S. Hence, it may be inferred that for this set of compounds both urea and thiourea groups hold promise significantly in protein glycation.

As a next step, we varied substituents of different electronegativities on the aromatic ring of the phenyl isocyanates/isothiocyanates. It was noticed that the activity is electronegativity dependent i.e., compounds containing halogen moieties (**10**–**19**, **22**–**31**/**58**–**67**, **34**–**43**/**70**–**79**, **46**–**55**/**82**–**91**) exerted highly potent activity than the compounds without halogen on the aromatic ring of the urea/thiourea group (**8**, **9**, **20**, **21**, **32**, **33**, **44**, **45**, **56**, **57**, **68**, **69**, **80**, **81**). This revealed that the presence of halogen is very much essential for good activity [18]. Further the compounds have demonstrated that polarity plays a role in the inhibition studies. This is supported by the fact that compounds having free amino acid side chain (**56**–**67**, **68**–**79** and **80**–**91**) have shown enhanced activity compared to those compounds bearing

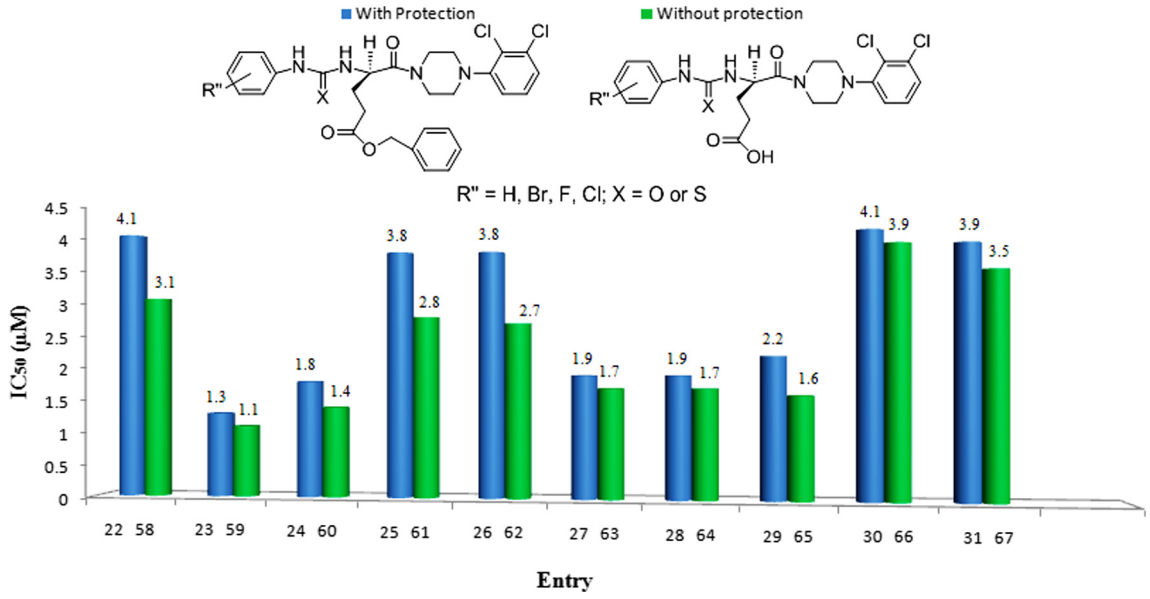


Figure 4 Diagrammatic representation of antiglycation activity of urea and thiourea derivatives of glutamic acid containing compounds.

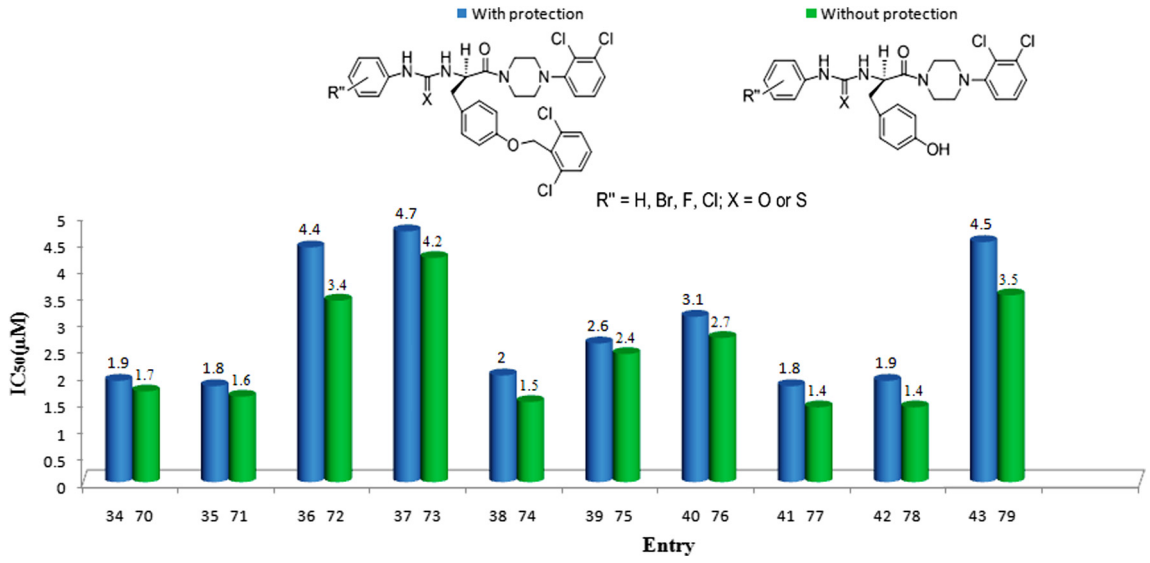


Figure 5 Diagrammatic representation of antiglycation activity of urea and thiourea derivatives of tyrosine containing compounds.

side chain protected amino acids (**20**–**31**, **32**–**43** and **44**–**51**) which is in accordance with the earlier article [36] (Figs. 2–6). In Fig. 2, it may be observed that compounds having no substitution displayed high IC_{50} values compared to standard rutin. Fig. 3 explains the activity profile obtained for phenylalanine derivatives. It can be seen that those derivatives possessing Cl and F substitutions exhibited high activity. Figs. 4–6 explain more or less the same trend of activity fetched for Glu, Tyr and Lys derivatives respectively. It is clearly seen that compounds after deprotection of the side chain functions possessed high activity compared to their counterparts.

4. Conclusion

The current study demonstrates the structure–activity guided synthesis of antiglycating agents. We further developed the general scaffold of the title compounds with respect to glycation inhibition studies. The resulting analogues showed an up to 20-fold higher activity than the reference compound. The activity was dependent on the amino acids. Moreover, the data demonstrated that halogen substituent on the aromatic ring was favorable for activity. On the other hand, the presence of side chain free amino acids in the

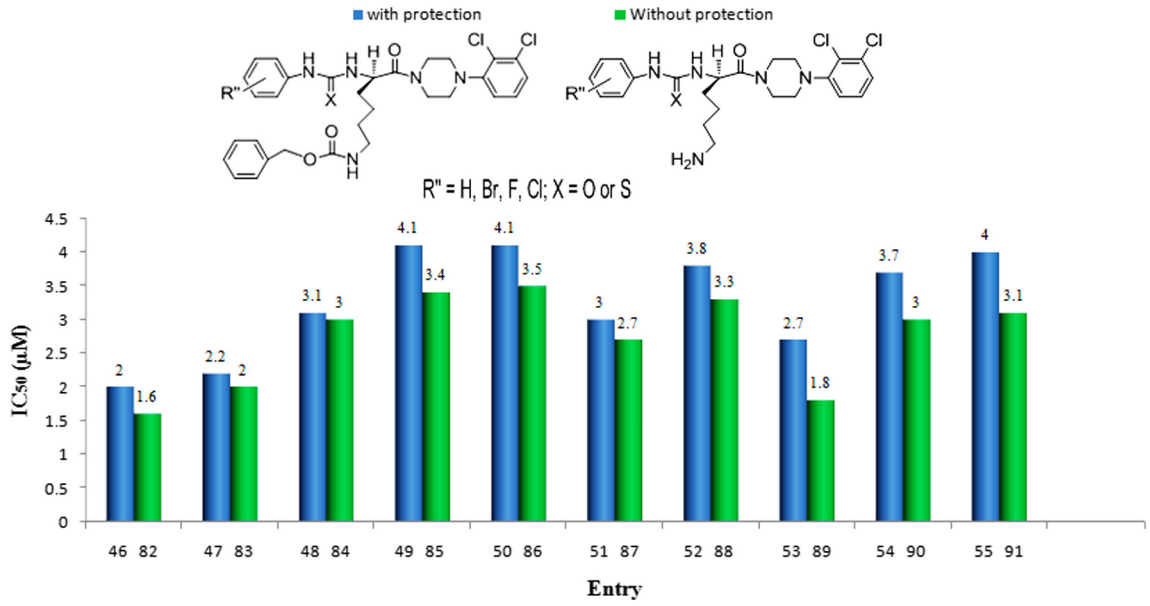


Figure 6 Diagrammatic representation of antiglycation activity of urea and thiourea derivatives of lysine containing compounds.

compounds possessed enhanced activity. However, the results showed that the compounds containing both urea and thiourea groups significantly inhibit protein glycation. The results obtained in this work further encourage us to continue in this line.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jscs.2014.02.006.

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