1,3-Disubstituted Ureas as Antiglycating Agents

[1]Shahnaz Perveen*, [2]Sana Mustafa,
[3]Khalid Mohammed Khan and [3]Muhammad Iqbal Choudhary
[1]*PCSIR Laboratories Complex, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.*
[2]*Department of Chemistry, University of Karachi, Karachi-75270, Pakistan.*
[3]*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.*
dr_shahnaz_perveen@yahoo.com*

(Received on 15th March 2013, accepted in revised form 9th May 2013)

Summary: Twenty one (21) 1,3-disubstituted urea derivatives were screened for their antiglycating potential and some of them displayed promising activity. Compound *N*-butyl-*N'*-(4-nitrophenyl)urea (**6**) and *N*-isopropyl-*N'*-(4-nitrophenyl)urea (**18**) exhibited excellent activity and could be investigated in search of medicines treating diabetes and associated complications.

Keywords: Symmetric urea, unsymmetric urea, antiglycation, structure-activity relationship, diabetes.

Introduction

Nitrogenous compounds not only play a significant role in pharmaceutical industry but also vital in many other industrial fields related to special and fine chemistry. Among them urea derivatives are extensively used and this wonderful class of compounds having multifocal applications in several fields [1]. A diverse number of urea derivatives are reported to reveal wide range of biological and pharmacological activities [2, 3]. Synthetic approaches are putting due to their numerous applications including hair dyeing and cellulose fibers, antioxidants in gasoline for automobiles, additive in detergents to avoid carbon deposition, corrosion inhibitors, and growth regulators in plants. They are also used as anticonvulsant, tranquillizing, anti-HIV-1 protease, anti-interleukin-8 (IL8) inhibitors, anthelmintics, anti-malarial, diuretic, analgesic, and antibacterial. In agrochemicals they are used as intermediates for the production of carbamates [4-7]. *N,N'*-Disubstituted ureas, amides and carbamates are reported as new powerful and stable inhibitors of soluble epoxide hydrolase both *in vivo* and *in vitro* [8] . The urea derivatives have urease and α-chymotrypsin enzyme inhibitory properties; it was found that the symmetrical *N*-(4-nitrophenyl)-*N*'-(4-nitrophenyl)urea has an excellent urease inhibitory property having an IC_{50} value 1.25 μM, in addition it is also found to be an effective α-chymotrypsin inhibitor having an IC_{50} value 3.15 μM [9]. Numerous substituted urea derivatives used for controlling undesired plant growth [10]. Among them *N,N'*-diphenylurea have reproducible cytokinin activity [11]. *N*-Benzoyl-*N'*-(3-nitrophenyl)urea found to be useful in antitumorous composition [12]. Some 1,3-disubstituted urea or thiourea are heat stabilizers for oil-extended [13]. Urea derivatives with piperazine rings and additionally substituted aromatic groups are fruitful in complications correspond to undesired calcium ion channel activity such as ameliorating conditions [14].

Type-2 diabetes is very common nowadays and millions of people are affected with a number of complications by this disease but unfortunately the exact molecular basis of complications is not completely understood. High blood sugar is one of the factor, that originates microangiopathy, hypertension and atheroma [15]. These are fundamentally associate with the advanced glycation end products in hyperglycemic state that contributes to the complications of diabetes, nephropathy, vascular diseases, aging, and Alzheimer's disease *etc* [16].

The chemistry and biology of the Maillard (glycation) reaction [17] and potential role of aminoguanidine and other inhibitors towards its advanced glycation end products (AGEs) in aging as well as the role of oxidative stress have been reviewed extensively [18-20]. AGEs are actually molecules which produced due to a non-enzymatic reaction of reducing sugar with free amino groups of proteins, nucleic acids and lipids. Basically they are sugar-derived substances and their elevated level is an indication of diabetes [16]. There are lots of evidences which proposed that AGEPs are responsible for almost all ever-increasing diabetic complications [21]. The initial product, Schiff base is formed non-enzymatically by the interaction of protein molecule with glucose, the Schiff base

*To whom all correspondence should be addressed.

intermediate by rearrangement is change to Amadori product and it is well known that anaerobically and oxidatively formed advanced stuff put harmful effects on biological system [22]. Dietary control reduced the level of Amadori products; pentosidine and (carboxymethyl) lysine (CML) [23-25]. Glycation-derived free radicals may be protected by antioxidants and chelators prevent auto-oxidation of glucose and Amadori products [26, 27]. Therefore, the compounds which have both antiglycating and antioxidant properties have good therapeutic applications. In this fashion, aged garlic extract which have a potent antioxidant *S*-allylcysteine, prevents the formation of AGEs and also inhibit the development of glycation-derived free radicals [27, 28]. Insulin C-peptide prevent albumin permeation and it was examined in diabetic rats to reduce motor nerve conduction velocity. Specific receptors of AGEs may function as metal chelator and tested for the treatment of neural and vascular dysfunction [29]. An inhibitor of AGEPs formation, aminoguanidine, has ability to prevent retinopathy in diabetic animals and inhibit diabetic vascular complications [30]. Polyamine, spermidine and spermine have effective antiglycation activity, as compare to aminoguanidine, and carnosine [31]. A number of research groups have been engaged in the search of new and safe antiglycation molecules around the globe [32-35]. In present study a series of twenty one (21) urea derivatives were synthesized and *in vitro* antiglycation activity were evaluated, in continuation of our efforts to find out more effective antiglycation agents [36-40].

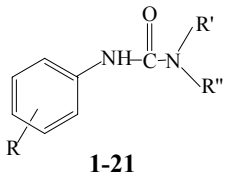
Since centuries investigations for discovery of chemical agents that could be used for the treatment of almost all human ailments consequently countless remedies are in use as medicines in everyday life. The available data also suggests that the search is still required for the isolation of bioactive substances from natural source in combating human diseases. As well as pharmaceutical companies are putting equal contribution and synthesizing novel compounds of therapeutic importance, and could lead to the discovery of novel compounds. In this way we have synthesized a good number of urea derivatives and subject them antiglycation assays.

Results and Discussion

Chemistry

Compounds (**1-21**) were prepared by treating *ortho*, *meta* and *para* nitrophenyl isocyanates with primary, secondary and tertiary amines in the presence of non-polar solvent like 1,4-dioxane, hexane, THF, ether, etc, at room temperature and progress of reaction was checked by TLC. Completion of reaction showed by remarkable difference in R_f values with respect to starting materials, the reaction mixture was poured into ice cold water with continuous stirring. The solid obtain was filtered and on purification afforded desire product (Table-1).

The structures of compounds were confirmed by EI MS, ^{1}H NMR, ^{13}C NMR, IR and UV spectroscopy. The synthetic compounds were finally submitted for antiglycation activities.



1-21

Bioactivities of Synthetic Urea Derivatives

The specific aims of this studies is first to synthesize and then to carry out a broad random high throughput screening of a large number of 1,3-disubstituted urea derivatives to identify new and specific antiglycating agents. On the basis of the experimental data and the structure of the inhibitors, the structure-activity relationships of the new inhibitors are studied.

The IC_{50} values is the concentration of inhibitor necessary to produce a 50% reduction in enzyme compared to the uninhibited system and is determined by using EZ-Fit 5 Program. A good number of them found to exhibit excellent and promising activities. These compounds could be further studies for pharmaceutical applications in near future (Table-2).

Structure-Activity Relationship

In the light of our previous report [36], all the synthetic compounds were evaluated for their antiglycation potential. 1,3-Disubstituted ureas **1-21** having nitro group showed a diversified antiglycation activity when compared to standard drug rutin (IC_{50} = 294.50 ± 1.50 μM). Compound **18** (IC_{50} = 147.55 ± 2.07 μM) and **6** (IC_{50} = 281.36 ± 6.07 μM) exhibited excellent activity better than the standard, both of these compounds are *para* nitro derivatives and have an isopropyl and butyl moiety on urea *N*-bridge, respectively. Compound **18** was found to be most potent among the series, however, its *ortho* (**16**) and *meta* (**14**) analogue with IC_{50} = 624.84 ± 11.43 μM

and 985.56 ± 4.89 μM, respectively showed comparatively low activity. Compound **6** also showed better activity to its *ortho* (**12**) analogue with an IC_{50} = 723.15 ± 5.31 μM and *meta* (**3**) analogue was found to be inactive. Nevertheless, compounds **7** (IC_{50} = 408.44 ± 27.05 μM), **16** (IC_{50} = 624.84 ± 11.43 μM), **12** (IC_{50} = 723.15 ± 5.31 μM), **10** (IC_{50} = 903.49 ± 6.73 μM), **5** (IC_{50} = 909.71 ± 2.63 μM) and **17** (IC_{50} = 910.07 ± 14.31 μM) showed moderate activity. Since, compound **9** and **19** showed less than 50% inhibition therefore were not further studied for their IC_{50} values. This difference in activities demonstrated that substitution of aromatic ring at *para* position by a nitro group and presence of an alkyl group at nitrogen of urea-bridge enhanced the effect of urea derivatives towards its antiglycation capabilities.

The result also showed that compound **17** with a nitro group at *para* position demonstrate some activity while it's *ortho* (**2**) and *meta* (**4**) analogue found inactive. Compound **10** with a nitro group at *meta* position exhibited better activity to its inactive *ortho* (**1**) and *para* (**13**) counterparts. Compound **7** with 1-phenylethyl moiety and nitro group at the *ortho* position exhibited slight better activity as compared its *para* nitro analogue **5**.

Table-1: 1,3-Disubstituted urea derivatives **1-21**.

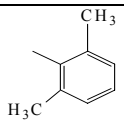
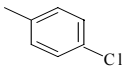
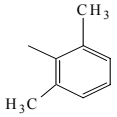
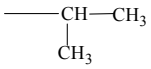
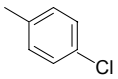
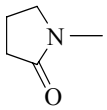
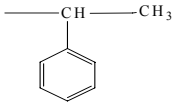
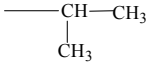
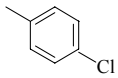
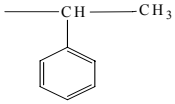
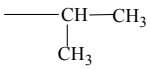
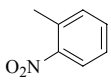
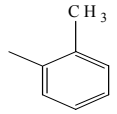
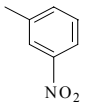
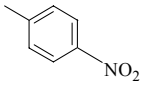
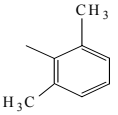
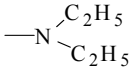
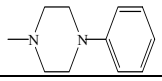
Compound	R	R′	R″	Compound	R	R′	R″
1	***o*-NO$_2$**		**H**	**12**	***o*-NO$_2$**	—$(CH_2)_3CH_3$	**H**
2	***o*-NO$_2$**		**H**	**13**	***p*-NO$_2$**		**H**
3	***m*-NO$_2$**	—$(CH_2)_3CH_3$	**H**	**14**	***m*-NO$_2$**		**H**
4	***m*-NO$_2$**		**H**	**15**	***m*-NO$_2$**		-
5	***p*-NO$_2$**		**H**	**16**	***o*-NO$_2$**		**H**
6	***p*-NO$_2$**	—$(CH_2)_3CH_3$	**H**	**17**	***p*-NO$_2$**		**H**
7	***o*-NO$_2$**		**H**	**18**	***p*-NO$_2$**		**H**
8	***o*-NO$_2$**		**H**	**19**	***p*-NO$_2$**		**H**
9	***m*-NO$_2$**		**H**	**20**	***p*-NO$_2$**		**H**
10	***m*-NO$_2$**		**H**	**21**	***p*-NO$_2$**		-
11	***o*-NO$_2$**		-	-	-	-	-

Table-2: Antiglycation Activity of Urea Derivatives **1-21**.

Compound	% Inhibition	IC_{50} ± SEM (μM)	Compound	% Inhibition	IC_{50} ± SEM (μM)
1	**< 50**	**-**	**12**	**63.33**	**723.15 ± 5.31**
2	**< 50**	**-**	**13**	**< 50**	**-**
3	**< 50**	**-**	**14**	**50.69**	**985.56 ± 4.89**
4	**< 50**	**-**	**15**	**< 50**	**-**
5	**57.88**	**909.71 ± 2.63**	**16**	**63.24**	**624.84 ±11.43**
6	**82.83**	**281.36 ± 6.07**	**17**	**63.44**	**910.07 ± 14.31**
7	**56.86**	**408.44 ± 27.05**	**18**	**89.89**	**147.55 ± 2.07**
8	**< 50**	**-**	**19**	**43.49**	**-**
9	**45.59**	**-**	**20**	**< 50**	**-**
10	**63.44**	**903.49 ± 6.73**	**21**	**< 50**	**-**
11	**< 50**	**-**	**Rutin***	**-**	**294.50 ± 1.50**

* = Standard

On the basis of above finding it could be concluded that not only the specific moieties but also the position of nitro group in urea derivatives is responsible for enhancing or suppressing the glucose or protein binding ability of the molecule and nitro group at *para* position is most promising among the series.

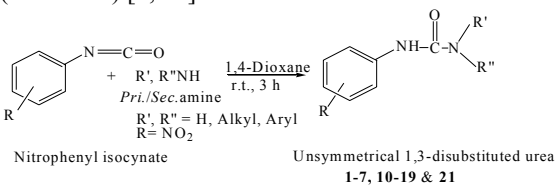
Experimental

Melting points were taken on Gallenkamp melting point apparatus and were uncorrected. Thin layer chromatography was performed on pre-coated silica gel plates (Kieselgel 60 F_{254}, E. Merk, Germany) and spots were visualized under UV (Dual range, UK, Germany) at 254 and 365nm. IR spectra were taken on a Thermo Nicolet Avatar 370 DTGS FT-IR Spectrometer. EI-MS were performed at MAT-312 and on JEOLJMS-HX 110 instrument ^{1}H-NMR spectra were recorded at Bruker AVANCE 400, 500 MHz, δ in ppm related $SiMe_4$ (0 ppm) as internal standard. Elemental analyses were carried out on a Perkin Elmer 2400 CHN Elemental analyzer. Reagent grade solvents were used. The compounds were synthesized in Pharmaceutical Research Centre, PCSIR Laboratories Complex, Karachi, Pakistan. While bioscreening were done in PCMD, H. E. J Research Institute of Chemistry, International Center for Chemical and Biological Science, University of Karachi, Pakistan.

General Procedure for the Preparation of N,N'-1,3-Disubstituted Urea

Method 1

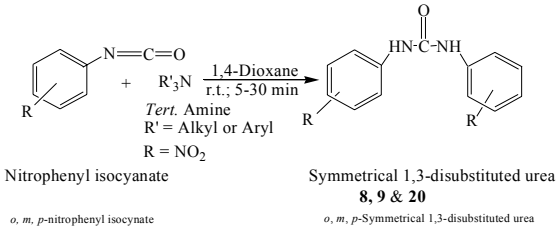
For the synthesis of unsymmetrical 1,3-disubstituted ureas, 2.44 of primary/secondary amine was taken in a round bottom flask containing 5-10 mL of 1,4-dioxane and than 1.22 mmol of nitrophenyl isocyanate was added drop wise at 30-40^{o}C with continuous stirring. The reaction was completed in 3 hours than the whole reaction mixture was transferred into ice cold water with continuous stirring; solid obtained was filtered and washed several times with distilled water and on purification by crystallization desire product was obtained (Scheme-1) [9, 41].



Scheme -1: Synthesis of *o*, *m*, *p*-nitro unsymmetrical 1,3-disubstituted urea.

Method 2

Symmetrical 1,3-disubstituted ureas are prepared by taking 1.22 mmol of nitrophenyl isocyanates in 5 to 10 mL of 1,4-dioxane, 2.44 mmol of triethylamine was added at room temperature and progress of reaction was monitored *via* TLC. After completion of reaction, the reaction mixture was poured into ice cold water with continuous stirring, solid was filtered and crystallized by appropriate solvent to afford desired product (Scheme-2) [42,43].



Scheme-2: Synthesis of *o*, *m*, *p*-nitro symmetrical 1,3-disubstituted urea.

N-(2,6-Dimethylphenyl)-N'-(2-nitrophenyl)urea **(1)**

Yield: 98%; R_f = 0.60 (CH_2Cl_2/C_6H_{14}, 6:4); m.p: 209 $^\circ$C; IR (Solid): ν_{max} 3322, 3256, 1646, 1585, 1548, 1499, 1336, 1283, 1225, 1152, 1090, 858, 764 cm^{-1}; ^{1}H-NMR: (500 MHz, $CDCl_3$): δ 9.63 (1H, br. s, NH), 8.75 (1H, d, $J_{3',4'}$ = 8.2 Hz, H-3'), 8.10 (1H, d, $J_{6',5'}$ = 8.2 Hz, H-6'), 7.57 (1H, dt, $J_{5'(4',\ 6')}$ = 7.3 Hz, $J_{5',3'}$ = 0.8 Hz, H-5'), 7.26 (1H, t, $J_{4'(3',5')}$ = 7.3 Hz, H-4'), 7.19 (2H, br. d, $J_{3,4}$ = $J_{5,4}$ = 7.3 Hz, H-3/H-5),

7.04 (1H, t, $J_{4(3,5)}$ = 7.3 Hz, H-4), 6.03 (1H, br. s, NH), 2.33 (3H, s, CH_3), 1.50 (3H, s, CH_3); EI MS: m/z (rel. abund. %), 285 (M^+, 45), 255 (2), 239 (5), 222 (2), 164 (5), 147 (88), 138 (100), 121 (70), 106 (26), 92 (37). Anal. Calcd for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.12; H, 5.32; N, 14.71.

N-(4-Chlorophenyl)-N'-(2-nitrophenyl)urea **(2)**

Yield: 77%; R_f = 0.68 (CH_2Cl_2/CH_3OH, 9:1); m.p: 241 °C; IR (Solid): ν_{max} 3334, 16667, 1618, 1585, 1540, 1491, 1334, 1344, 1274, 1205, 1095, 1017 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 9.97 (1H, br. s, NH), 8.62 (1H, d, $J_{3',4'}$ = 8.5 Hz, H-3'), 8.18 (1H, d, $J_{6',5'}$ = 7.5 Hz, H-6'), 7.61 (1H, t, $J_{4'(3',5')}$ = 7.5 Hz, H-4'), 7.37 (2H, d, $J_{3,4} = J_{5,6}$ = 8.7 Hz, H-3/H-5), 7.32 (2H, d, $J_{2,3} = J_{6,5}$ = 8.7 Hz, H-2/H-6), 7.09 (1H, t, $J_{5'(4',6')}$ = 7.5 Hz, H-5'), 6.76 (1H, br. s, NH); EI MS: m/z (rel. abund. %), 293 (M^{2+}, 4), 291 (M^+, 11), 155 (6), 153 (21), 148 (3), 138 (100), 127 (89), 125 (15), 111 (9), 108 (14), 92 (39), 90 (52). Anal. Calcd for $C_{13}H_{10}N_3O_3Cl$: C, 53.63; H, 3.46; N, 14.43. Found: C, 53.60; H, 3.44; N, 14.40.

N-Butyl-N'-(3'-nitrophenyl)urea **(3)**

Yield: 91%; R_f = 0.72 (CH_2Cl_2/C_6H_{14}, 6:4); m.p: 138 °C; IR (Solid): ν_{max} 3342, 2942, 1712, 1638, 1524, 1422, 809, 735cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.14 (1H, t, $J_{2'(4',6')}$ = 2.0 Hz, H-2'), 7.85 (1H, dd, $J_{6',5'}$ = 8.1 Hz, $J_{6',4'}$ = 1.4 Hz, H-6'), 7.78 (1H, dd, $J_{4',5'}$ = 8.1 Hz, $J_{4',6'}$ = 1.4 Hz, H-4'), 7.41 (1H, t, $J_{5'(4',6')}$ = 8.1 Hz, H-5'), 6.50 (1H, br. s, NH), 4.66 (1H, br. s, NH), 3.27 (2H, q, $J_{1(2,NH)}$ = 6.9 Hz, CH_2), 1.53 (2H, qin, $J_{2(1,3)}$ = 6.9 Hz, CH_2), 1.37 (2H, sex, $J_{3(2,4)}$ = 7.2 Hz, CH_2), 0.93 (3H, t, $J_{4,3}$ = 7.2 Hz, CH_3); EI MS: m/z (rel. abund. %), 237 (M^+, 3), 270 (3), 165 (1), 138 (100), 108 (7), 92 (59), 65 (24). Anal. Calcd for $C_{11}H_{15}N_3O_3$: C, 55.69; H, 6.37; N, 17.71. Found: C, 55.71; H, 6.34; N, 17.68.

N-(4-Chlorophenyl)-N'-(3'-nitrophenyl)urea **(4)**

Yield: 79%; R_f = 0.88 (CH_2Cl_2/C_6H_{14}, 6:4); m.p: 241°C; IR (Solid): ν_{max} 3363, 1687, 1593, 1540, 1487, 1344, 1230, 1095, 8784 cm^{-1}; ^{1}H-NMR: (500 MHz, $CDCl_3$): δ 8.21 (1H, t, $J_{2'(4',6')}$ = 1.9 Hz, H-2'), 7.91 (2H, dd, $J_{2,3} = J_{6,5}$ = 8.4 Hz, $J_{2,6} = J_{6,2}$ = 1.4 Hz, H-2/H-6), 7.82 (2H, dd, $J_{3,2} = J_{5,6}$ = 8.4 Hz, $J_{3,5} = J_{5,3}$ = 1.3 Hz, H-3/H-5), 7.4 (1H, d, $J_{4',5'}$ = 8.1 Hz, H-4'), 7.44 (1H, dd, $J_{6',5'}$ = 8.5 Hz, $J_{6',4'}$ = 1.7 Hz, H-6'), 7.32 (1H, m, H-5'), 6.66 (1H, br. s, NH), 6.45 (1H, br. s, NH); EI MS: m/z (rel. abund. %), 293 (M^{+2}, 4), 291 (M^+,16), 165 (5), 155 (3), 153 (12), 138 (31), 127 (100), 111 (13), 99 (21), 92 (40). Anal. Calcd for $C_{13}H_{10}N_3O_3Cl$: C, 53.63; H, 3.46; N, 14.43. Found: C, 53.60; H, 3.48; N, 14.41.

N-(4-Nitrophenyl)-N'-(1''-phenylethyl)urea **(5)**

Yield: 64%; R_f = 0.74 (CH_2Cl_2/CH_3OH, 9:1); m.p: 186 °C; IR (Solid): ν_{max} 3350, 3277, 2978, 1659, 1565, 1503, 1450, 1344, 1283, 1156 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.11 (2H, d, $J_{2,3} = J_{6,5}$ = 9.1 Hz, H-2/H-6), 7.41 (2H, d, $J_{3,2} = J_{5,6}$ = 9.1 Hz, H-3/H-5), 7.35 (2H, m, H-2''/H-3''/H-5''/H-6''), 7.30 (1H, m, H-4''), 6.57 (1H, br. s, NH), 4.93 (1H, m, CH), 3.68 (1H, s, NH), 1.52 (3H, d, $J_{2'',1''}$ = 6.6 Hz, CH_3); EI MS: m/z (rel. abund. %), 285 (M^+, 2), 270 (4), 164 (4), 148 (53), 138 (97), 132 (16), 120 (11), 105 (100), 92 (16). Anal. Calcd for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.21; H, 5.33; N, 14.69.

N-Butyl-N'-(4'-nitrophenyl)urea **(6)**

Yield: Quantitative; R_f = 0.67 (CH_2Cl_2/C_6H_{14}, 7:3); m.p: 137 °C; IR (Solid): ν_{max} 3379, 3305, 2845, 1650, 1556, 1503, 1319, 1225, 1111, 849, 747, 678 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 9.35 (1H, br. s, NH), 8.15 (2H, d, $J_{3',2'} = J_{5',6'}$ = 8.7 Hz, H-3'/H-5'), 7.50 (2H, d, $J_{2',3'} = J_{6',5'}$ = 8.7 Hz, H-2/H-6), 6.68 (1H, br. s, NH), 3.27 (2H, t, $J_{1,2}$ = 7.2 Hz, CH_2), 1.52 (2H, qin, $J_{2(1,3)}$ = 7.2 Hz, CH_2), 1.36 (2H, sex, $J_{3(2,4)}$ = 7.2 Hz, CH_2), 0.92 (3H, t, $J_{4,3}$ = 7.2 Hz, CH_3); EI MS: m/z (rel. abund. %), 237 (M^+, 17), 219 (2), 219 (2), 207 (2), 164 (6), 138 (100), 122 (6), 108 (30), 92 (17), 78 (21). Anal. Calcd for $C_{11}H_{15}N_3O_3$: C, 55.69; H, 6.37; N, 17.71. Found: C, 55.71; H, 6.39; N, 17.68.

N-(2-Nitrophenyl)-N'-(1''-phenylethyl)urea **(7)**

Yield: 68%; R_f = 0.81 (CH_2Cl_2/C_6H_{14}, 9:1); m.p: 209 °C; IR (Solid): ν_{max} 3374, 3305, 1658, 1552, 1503, 1315, 1225, 1111, 849, 747 cm^{-1}; ^{1}H–NMR: (400 MHz, $CDCl_3$): δ 9.72 (2H, br. s, NH), 8.62 (1H, d, $J_{3,4}$ = 7.6 Hz, H-3), 8.14 (1H, d, $J_{6,5}$ = 7.3 Hz, H-6), 7.53 (1H, t, $J_{5(4,6)}$ = 7.3 Hz, H-5), 7.36 (4H, m, H-2''/H-3''/H-5''/H-6''), 7.29 (1H, m, H-4''), 7.01 (1H, t, $J_{4,3}$ = 7.3 Hz, H-4), 5.00 (1H, m, CH), 1.52 (3H, m, CH_3); EI MS: m/z (rel. abund. %), 285 (M^+, 6), 268 (2), 164 (57), 147 (32), 138 (89), 132 (85), 120 (10), 106 (100), 92 (33), 90 (37). Anal. Calcd for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.19; H, 5.28; N, 14.75.

N-(2-Nitrophenyl)-N'-(2'-nitrophenyl)urea **(8)**

Yield: 56%; R_f = 0.70 (CH_2Cl_2/C_6H_{14}, 6:4); m.p: 227 °C; IR (Solid): ν_{max} 3366, 1659, 1589, 1556, 1499, 1344, 1344, 1288, 1209, 1160, 849, 739, 727, 682 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 10.13 (1H, br. s, NH), 8.55 (1H, d, $J_{3,4}$ = 7.5 Hz, H-3), 8.23 (1H,

d, $J_{3',4'}$ = 7.6 Hz, H-3'), 8.10 (1H, d, $J_{6,5}$ = 8.5 Hz, H-6), 7.65 (1H, t, $J_{4(3,5)}$ = 7.5 Hz, H-4), 7.34 (1H, t, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.16 (1H, t, $J_{4'(3',5')}$ = 7.6 Hz, H-4'), 6.78 (1H, d, $J_{6',5'}$ = 8.3 Hz, H-6'), 6.69 (1H, t, $J_{5'(4',6')}$ = 7.6 Hz, H-5'), 6.01 (1H, br. s, NH) ; EI MS: m/z (rel. abund. %), 302 (M^+, 2), 256 (2), 164 (4), 138 (79), 121 (7), 92 (63), 90 (59), 80 (33), 78 (12), 77 (9), 76 (6), 66 (18), 65 (100), 52 (13). Anal. Calcd for $C_{13}H_{10}N_4O_5$: C, 51.66; H, 3.33; N, 18.54. Found: C, 51.68; H, 3.31; N, 18.52.

N-(3-Nitrophenyl)-N'-(3'-nitrophenyl)urea **(9)**

Yield: Quantitative; R_f = 0.77 (CH_2Cl_2/C_6H_{14}, 7:3); m.p: 242 °C; IR (Solid): ν_{max} 3346, 1720, 1691, 1524, 1417, 1352, 809, 756, 682 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.02 (2H, t, $J_{2(4,6)} = J_{2'(4',6')}$ = 1.9 Hz, H-2/H-2'), 7.78 (2H, dd, $J_{4,5} = J_{4',5'}$ = 8.2 Hz, $J_{4,6} = J_{4',6'}$ = 1.6 Hz, H-4/H-4'), 7.80 (2H, dd, $J_{6,5} = J_{6,'5'}$ = 8.2 Hz, $J_{6,4} = J_{6',4'}$ = 1.6 Hz, H-6/H-6'), 7.38 (2H, t, $J_{5(4,6)} = J_{5'(4',6')}$ = 8.2 Hz, H-5/H-5'), 7.23 (1H, s, NH), 3.08 (1H, s, NH); EI MS: m/z (rel. abund. %), 302 (M^+, 3), 164 (23), 138 (86), 118 (15), 108 (5), 106 (12), 92 (73), 90 (97), 65 (100). Anal. Calcd for $C_{13}H_{10}N_4O_5$: C, 51.66; H, 3.33; N, 18.54. Found: C, 51.64; H, 3.30; N, 18.51.

N-(2,6-Dimethylphenyl)-N'-(3'-nitrophenyl)urea **(10)**

Yield: 92%; R_f = 0.74 (CH_2Cl_2/C_6H_{14}, 6:4); m.p: 243 °C; IR (Solid): ν_{max} 3326, 1638, 1524, 1340, 1217, 1074, 764, 730 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.06 (1H, br. s, H-2'), 7.84 (2H, dt, $J_{5',4'} = J_{6',5'}$ = 7.9 Hz, $J_{5'(4',6')}$ = 1.6 Hz, H-5'/H-6'), 7.41 (1H, t, $J_{4(3,5)}$ = 8.2 Hz, H-4), 7.20 (1H, d, $J_{4',5'}$ = 7.9 Hz, H-4'), 7.19 (2H, m, H-3, 5), 6.27 (1H, br. s, NH), 5.87 (1H, br. s, NH), 2.34 (6H, s, $2CH_3$); EI MS: m/z (rel. abund. %), 285 (M^+, 92), 270 (2), 255 (2), 238 (3), 164 (4), 147 (86), 138 (97), 121 (100), 106 (49), 92 (40). Anal. Calcd for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.17; H, 5.35; N, 14.70.

N-(2-Nitrophenyl)-N'-(N-phenylpiperazinyl)urea **(11)**

Yield: 71%; R_f = 0.76 (CH_2Cl_2/C_6H_{14}, 7:3); m.p: 269 °C; IR (Solid): ν_{max} 3366, 2835, 1666, 1599, 1589, 1499, 1450, 1344, 1270, 1229, 1139 cm^{-1}; ^{1}H-NMR: (400 MHz, DMSO): δ 9.3 (1H, br. s, NH), 7.92 (2H, d, $J_{3,4} = J_{6,5}$ = 8.0 Hz, H-3/H-6), 7.66 (2H, dd, $J_{3',2'} = J_{5',6'}$ = 8.1 Hz, $J_{3',4'} = J_{5',4'}$ = 7.0 Hz, H-3'/H-5'), 7.23 (2H, t, $J_{4(3,5)} = J_{5,(4,6)}$ = 8.0 Hz, H-4/H-5), 6.97 (2H, d, $J_{2',3'} = J_{6',5'}$ = 8.1 Hz, H-2'/H-6'), 6.81 (1H, t, $J_{4'(3',5')}$ = 7.0 Hz, H-4'), 3.60 (4H, t, $J_{1'',2''}$ = 4.9 Hz, $2CH_2$), 3.17 (4H, t, $J_{2'',1''}$ = 4.9 Hz, $2CH_2$) ; EI MS: m/z (rel. abund. %), 326 (M^+, 1), 162 (27), 146.9 (7), 132 (35), 119 (28), 104.9 (76), 104 (86), 90.9 (100). Anal. Calcd for $C_{17}H_{18}N_4O_3$: C, 62.57; H, 5.56; N, 17.17. Found: C, 62.54; H, 5.58; N, 17.19. 8.14.

N-Butyl-N'-(2'-nitrophenyl)urea **(12)**

Yield: Quantitative; R_f = 0.71 (CH_2Cl_2/CH_3OH, 9:1); m.p: 127 °C; IR (Solid): ν_{max} 3342, 2970, 1665, 1507, 1465, 1340, 1262, 1148, 743 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 9.76 (1H, br.s, NH), 8.64 (1H, d, $J_{3',4'}$ = 8.5 Hz, H-3'), 8.16 (1H, dd, $J_{6',5'}$ = 8.5 Hz, $J_{6',4'}$ = 1.1 Hz, H-6'), 7.57 (1H, dt, $J_{4'(3',5')}$ = 8.5 Hz, $J_{4',6'}$ = 1.1 Hz, H-4'), 7.02 (1H, dt, $J_{5'(6',4')}$ = 8.5 Hz, $J_{5',3'}$ = 0.6 Hz, H-5'), 4.76 (1H, s, NH), 3.29 (2H, q, $J_{1(2,NH)}$ = 7.3 Hz, CH_2), 1.56 (2H, qin, $J_{2(1,3)}$ = 7.3 Hz, CH_2), 1.39 (2H, sex, $J_{3(2,4)}$ = 7.3 Hz, CH_2), 0.95 (3H, t, $J_{4,3}$ = 7.3 Hz, CH_3); EI MS: m/z (rel. abund. %), 237 (M^+, 3), 148 (4), 139 (7), 138 (100), 122 (3), 108 (19), 92 (29). Anal. Calcd for $C_{11}H_{15}N_3O_3$: C, 55.69; H, 6.37; N, 17.71. Found: C, 55.66; H, 6.40; N, 17.73.

N-(2,6-Dimethylphenyl)-N'-(4'-nitrophenyl)urea **(13)**

Yield: 93%; R_f = 0.65 (CH_2Cl_2/C_6H_{14}, 9:1); m.p: 186 °C; IR (Solid): ν_{max} 3326, 1663, 1540, 1499, 1332, 1230, 1103, 837, 751 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.13 (2H, d, $J_{2',3'} = J_{6',5'}$ = 9.0 Hz, H-2'/H-6'), 7.49 (2H, d, $J_{3',2'} = J_{5',6'}$ = 9.0 Hz, H-3'/H-5'), 7.22 (2H, d, $J_{3,4} = J_{5,4}$ = 8.9 Hz, H-3/H-5), 7.19 (1H, t, $J_{4(3,5)}$ = 8.9 Hz, H-4), 6.41 (1H, br. s, NH), 5.94 (1H, br. s, NH), 2.32 (6H, s, $2CH_3$); EI MS: m/z (rel. abund. %), 285 (M^+, 89), 270 (3), 255 (3), 164 (5), 147 (100), 138 (88), 132 (19), 121 (72), 108 (56), 92 (25). Anal. Calcd for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.18; H, 5.28; N, 14.75.

N-Isopropyl-N'-(3'-nitrophenyl)urea **(14)**

Yield: 82%; R_f = 0.72 (CH_2Cl_2/C_6H_{14}, 7:3); m.p: 197 °C; IR (Solid): ν_{max} 3326, 1638, 1565, 1527, 1345, 728 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.16 (1H, t, $J_{2'(4',6')}$ = 1.7 Hz, H-2'), 7.84 (1H, dd, $J_{4',5'}$ = 8.1 Hz, $J_{4',6'}$ = 1.2 Hz, H-4'), 7.76 (1H, dd, $J_{6',5'}$ = 8.1 Hz, $J_{6',4'}$ = 1.2 Hz, H-6'), 7.41 (1H, t, $J_{5'(4',6')}$ = 8.1 Hz, H-5'), 6.30 (1H, br. s, NH), 4.39 (1H, br. s, NH), 3.98 (1H, sept, $J_{1(2a,2b)}$ = 6.6 Hz, CH), 1.21 (6H, d, $J_{2a,1} = J_{2b,1}$ = 6.6 Hz, $2CH_3$); EI MS: m/z (rel. abund. %), 223 (M^+, 51), 164 (8), 139 (84), 138 (100), 92 (100), 80 (35), 58 (23). Anal. Calcd for $C_{10}H_{13}N_3O_3$: C, 53.78; H, 5.87; N, 18.83. Found: C, 53.81; H, 5.91; N, 18.86.

N-(3-Nitrophenyl)-2-oxo-1-pyrrolidinecarboxamide **(15)**

Yield: 59%; Rf = 0.56 (CH_2Cl_2/C_6H_6, 6:4); m.p: 262 °C; IR (Solid): ν_{max} 3300, 1527, 1343, 1229, 1172, 878, 735, 698 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$), δ 8.25 (1H, br. s, H-2), 7.95 (1H, d, $J_{4,5}$ = 7.9 Hz, H-4), 7.84 (1H, d, $J_{6,5}$ = 7.9 Hz, H-6), 7.50 (1H, t, $J_{5(4,6)}$ = 7.9 Hz, H-5), 6.77 (1H, br. s, NH), 3.47 (2H, t, $J_{5',4'}$ = 8.2 Hz, CH_2), 1.24 (2H, t, $J_{3',4'}$ = 8.0 Hz, CH_2), 0.85 (2H, m, CH_2); EI MS: m/z (rel. abund. %), 249 (M^+, 30), 165 (10), 164 (83), 138 (100), 136 (40), 112 (82), 106 (18), 92 (82). Anal. Calcd for $C_{11}H_{11}N_3O_4$: C, 53.01; H, 4.45; N, 42.02. Found: C, 52.99; H, 4.42; N, 42.01.

N-Isopropyl-N'-(2'-nitrophenyl)urea **(16)**

Yield: 89%; R_f = 0.65 (CH_2Cl_2/C_6H_{14}, 7:3); m.p: 198 °C; IR (Solid): ν_{max} 3396, 2960, 1641, 1557, 1503, 1348, 1285, 734 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.71(1H, br.s, NH), 8.63 (1H, d, $J_{3',4'}$ = 8.4 Hz, H-3'), 8.16 (1H, d, $J_{6',5'}$ = 8.4 Hz, H-6'), 7.56 (1H, t, $J_{4'(3',5')}$ = 8.4 Hz, H-4'), 7.01 (1H, t, $J_{5'(4',6')}$ = 8.4 Hz, H-5'), 4.70 (1H, br. s, NH), 3.98 (1H, sept, $J_{1(2a,2b)}$ = 6.5 Hz, CH), 1.22 (6H, d, $J_{2a,1} = J_{2b,1}$ = 6.5 Hz, CH_3); EI MS: m/z (rel. abund. %), 223 (M^+, 23), 167 (4), 164 (6), 149 (19), 138 (100), 92 (68), 85 (15), 83 (16), 69 (22), 57 (37). Anal. Calcd for $C_{10}H_{13}N_3O_3$: C, 53.78; H, 5.87; N, 18.83. Found: C, 53.71; H, 5.82; N, 18.88.

N-(4-Chlorophenyl)-N'-(4'-nitrophenyl)urea **(17)**

Yield: 72%; R_f = 0.70 (CH_2Cl_2/C_6H_{14}, 6:4); m.p: 250 °C; IR (Solid): ν_{max} 3366, 1723, 1595, 1539, 1492, 1345, 1270, 1173, 1113, 835cm^{-1}; ^{1}H-NMR (400 MHz, DMSO); δ 9.45 (1H, br. s, NH), 9.04 (1H, br. s, NH), 8.19 (2H, d, $J_{3,2} = J_{5,6}$ = 9.3 Hz, H-3/H-5), 7.67 (2H, d, $J_{2,3} = J_{6,5}$ = 9.3 Hz, H-2//H-6), 7.49 (2H, d, $J_{2',3'} = J_{6',5'}$ = 8.8 Hz, H-2'/H-6'), 7.35 (2H, d, $J_{3',2'} = J_{5',6'}$ = 8.8 Hz, H-3'/H-5'); EI MS: m/z (rel. abund. %), 293 (M^{+2}, 4), 291 (M^+, 10), 164 (21), 155(48), 153 (100), 138 (81), 129 (17), 127 (72), 125 (68), 108 (54), 92 (28), 90 (52), 65 (33). Anal. Calcd for $C_{13}H_{10}N_3O_3Cl$: C, 53.63; H, 3.46; N, 14.43 Found: C, 53.68; H, 3.39; N, 14.46.

N-Isopropyl-N'-(4'-nitrophenyl)urea **(18)**

Yield: 76%; R_f = 0.75 (CH_2Cl_2/C_6H_{14}, 7:3); m.p: 196 °C; IR (Solid): ν_{max} 3395, 3302, 2926, 1662, 1551, 1507, 1324, 1236, 855 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.14 (1H, d, $J_{2',3'} = J_{6',5'}$ = 9.0 Hz, H-2'/H-6'), 7.49 (2H, d, $J_{3',2'} = J_{5',6'}$ = 9.0 Hz, H-3'/H-5'), 6.56 (1H, br. s, NH), 4.54 (1H, br. s, NH), 3.99 (1H, sept, $J_{1(2a,2b)}$ = 6.5 Hz, CH), 1.20 (6H, d, $J_{2a,1} = J_{2b,1}$ = 6.5 Hz, $2CH_3$); EI MS: m/z (rel. abund. %), 223 (M^+, 52), 165 (184), 138 (100), 108 (36), 92 (98). Anal. Calcd for $C_{10}H_{13}N_3O_3$: C, 53.78; H, 5.87; N, 18.83. Found: C, 53.83; H, 5.82; N, 18.89.

N-(2-Methylphenyl)-N'-(4'-nitrophenyl)urea **(19)**

Yield: 65%; Rf = 0.73 (CH_2Cl_2/C_6H_6, 7:3); m.p: 315 °C; IR (Solid): ν_{max} 3300, 1692, 1496, 1298, 1178, 1114, 846 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$ + CD_3OD), δ 8.04 (2H, d, $J_{2',3'} = J_{6',5'}$ = 9.2 Hz, H-2'/H-6'), 7.63 (1H, d, $J_{3,4}$ = 7.9 Hz, H-3), 7.51 (2H, d, $J_{3',2'} = J_{5',6'}$ = 9.2 Hz, H-3'/H-5'), 7.10 (1H, d, $J_{6,5}$ = 7.5 Hz, H-6), 7.06 (1H, t, $J_{5(4,6)}$ = 7.4 Hz, H-5), 6.93 (1H, t, $J_{4(3,5)}$ = 7.4 Hz, H-4), 3.32 (2H, br. s, NH), 2.17 (3H, s, CH_3); EI MS: m/z (rel. abund. %), 271 (M^+, 41), 164 (2), 138 (40), 107 (100), 91 (42), 65 (48). Anal. Calcd for $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83; N, 15.50. Found: C, 61.86; H, 4.80; N, 15.47.

N-(4-Nitrophenyl)-N'-(4'-nitrophenyl)urea **(20)**

Yield: 94%; Rf = 0.78 (CH_2Cl_2/C_6H_6, 7:3); mp: 310 °C; IR (Solid): ν_{max} 3286, 3190, 1649, 1600, 1556, 1312 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD): δ 9.62 (2H, br. s, 2NH), 8.22 (4H, dd, $J_{2,3} = J_{6,5} = J_{2',3'} = J_{6',5'}$ = 8.1 Hz, $J_{2,5} = J_{6,3} = J_{2',5'} = J_{6',3'}$ = 0.2 Hz, H-2/H-6/H-2'/H-6'), 8.13 (4H, dd, $J_{3,2} = J_{5,6} = J_{3',2'} = J_{5',6'}$ = 8.1 Hz, $J_{3,6} = J_{5,2} = J_{3',6'} = J_{5',2'}$ = 0.2 Hz, H-3/H-5/H-3'/H-5'); EI MS: m/z (%) 302 (M^+, 6), 257 (15), 181 (20), 162 (100), 123 (56); Anal. Calcd for $C_{13}H_{10}N_4O_5$: C, 51.66; H, 3.34; N, 18.54. Found: C, 51.59; H, 3.28; N, 18.47.

N,N-Diethyl-N'-(4'-nitrophenyl)urea **(21)**

Yield: 90%; R_f = 0.62 (CH_2Cl_2/C_6H_{14}, 6:4); m.p: 295 °C; IR (Solid): ν_{max} 3360, 1725, 1556, 1497, 1299, 1179, 1115, 846 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.15 (2H, d, $J_{2',3'} = J_{6',5'}$ = 9.0 Hz, H-2'/H-6'), 7.54 (2H, d, $J_{3',2'} = J_{5'6'}$ = 9.0 Hz, H-3'/H-5'), 6.56 (1H, br. s, NH), 3.38 (4H, q, $J_{1,2}$ = 7.1 Hz, $2CH_2$), 1.24 (6H, t, $J_{2,1}$ = 7.1 Hz, $2CH_3$); EI MS: m/z (rel. abund. %), 237 (M^+, 12), 164 (3), 140 (2), 100 (100), 78 (20), 72 (75). Anal. Calcd for $C_{11}H_{15}N_3O_3$: C, 55.69; H, 6.37; N, 17.71. Found: C, 55.82; H, 6.40; N, 17.72.

Assay for Antiglycation
Chemicals

Bovine serum albumin (BSA) was purchased from Merk Marker Pvt. Ltd. Rutin, MGO (methyl glyoxal, 40% aqueous solution), phosphate buffers *i.e.*, sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate

(Na_2HPO_4), and dimethyl sulphoxide (DMSO) were purchased from Sigma Aldrich.

Protocol

For the determination of the ability of the candidate compounds to inhibit the methyl glyoxal (MGO) this test was used, and examine the development of fluorescence of Bovine serum albumin (BSA). The activity is carried out by using the reported protocol[16, 27] with some modifications.

To perform the antiglycation assay a control was prepared by incubating 10 mg/mL of Bovine serum albumin (BSA) with 14 mM MGO (methyl glyoxal) under sterile conditions in 0.1 M phosphate buffer of pH = 7.4 having NaN_3 (30 mM) for 9 days at 37°C.

For the preparation of positive control 10 mg/mL of BSA was incubated with 14 mM MGO and 1 mM standard drug rutin (IC_{50} = 294 ± 1.5 μM) [15] and 0.1 M phosphate buffer of pH 7.4 along with NaN_3 (30 mM) under sterile conditions at 37°C for 9 days. In the same way the test compounds of different concentrations were tested by taking triplicate samples of BSA (10 mg/mL), 14 mM MGO (methyl glyoxal), 0.1 M phosphate buffer of pH 7.4 containing NaN_3 (30 mM) were incubated under sterile conditions at 37°C for 9 days. In this way each well of 96-well plate contains 50 μL BSA solution, 50 μL MGO, and 20 μL test sample. After 9 days of incubation period, the fluorescence of the blank, control and samples were measured at the excitation (330 nm) and emission maxima (440 nm) on a microtitre plate spectrophotometer (Spectra Max, Molecular Devices, CA, USA).

The % inhibition of AGEs formation was calculated in the test sample *versus* control for each inhibiting compound by using the formula:

% Inhibition = 1- [(Fluorescence of test sample/Fluorescence of glycated control)] x 100

Conclusion

Compounds **18,** and **6** exhibited excellent activity better than the standard, both of these compounds have a nitro group at *para* position and an alkyl group *i.e.*, isopropyl and butyl moiety on nitrogen of urea-bridge, respectively. The result of activities demonstrated that substitution of aromatic ring by a nitro group at *para* position and presence of an alkyl group at nitrogen of urea-bridge improved the antiglycation activity of urea derivatives.

Acknowledgments

This work was supported by the Higher Education Commission (HEC), Government of Pakistan under the program of "5000 Ph. D Indigenous Scholarship".

References

1. I. L. 1. Finar, *Organic Chemistry*, 6th Ed., Pearson Education Ltd., **1**, 460 **(**2003).
2. P. Y. S. Lam, P. K. Jadhav, C. J. Eyermann, C. N. Hodge, Y. Ru, L. T. Bacheler, J. L. Meek, M. J. Otto, M. M. Rayner, Y. N. Wong, C. H. Chang, P. C. Weber, D. A. Jackson, T. R. Sharpe, S. Erickson-Viitanen, *Science*, **263**, 380 (1994).
3. J. L. Castro, R. G. Ball, H. B. Broughton, M. G. N. Russell, D. Rathbone, A. P. Watt, R. Baker, K. L. Chapman, A. E. Fletcher, S. Patel, A. J. Smith, G. R. Marshall, W. Ryecroft and V. G. Matassa, *Journal of Medicinal Chemistry*, **39**, 842 (1996).
4. A. R. Katritzky, D. P. M. Pleynet and B. Yang, *Journal of Organic Chemistry,* **62***,* 4155 (1997).
5. C. Sahlberg, R. Norren, P. Engelhardt, M. Hogberg, J. Kangasmetsa, L. Vrang and H. Zang, *Bioorganic and Medicinal Chemistry Letters,* **8**, 1511 (1998).
6. B. Olga, B. Chiara, F. Bondavalli, S. Schenone, A. Ranise, N. Arduino, M. B. Bertolotto, F. Montecucco, L. Ottonello, F. Dallegri, M. Tognolini, V. Ballabeni, S. Bertoni and E. Barocelli, *Journal of Medicinal Chemistry,* **50**, 3618 (2007).
7. J. N. Dominguez, C. Leon, J. Rodrigues, N. G. Dominguez, J. Gut and P. J. Rosenthal, *Journal of Medicinal Chemistry,* **48**, 3654 (2005).
8. I. H. Kim, C. Morisseau, T. Watanabe and B. D. Hammock, *Journal of Medicinal Chemistry***, 47**, 2110 (2004).
9. S. Perveen, K. M. Khan, M. A. Lodhi, M. I. Choudhary, Atta-ur-Rahman and W. Voelter, *Letters in Drug Design and Di*scovery, **5**, 401 (2008).
10. H. Johnston and W. Creek, *U.S. Patent No.* 4063928 (1977).
11. M. I. Bruce and J. A. Zwar, *Biological Science,* **165**, 245 (1966).
12. T. Haga, N. Yamada, H. Sugi, T. Koyanagi and H. Okada, *U.S. Patent No.* 4677111 (1987**)**.
13. J. A. Beckman, *U.S. Patent No.* 3654214 (1972).

14. P. Hassan, P. Hossein, D. Yanbing, S. P. Terrance, *U.S. Patent No.* 7378420 (2008).
15. M. Peppa, J. Uribarri and H. Vlassara, *Clinical Diabetes,* **21**, 186 (2003).
16. S. Rahbar and J. L. Figarola, *Archives of Biochemistry and Biophysics*, **419**, 63 (2003).
17. L. C. Maillard, *La Presse Medicale,* **71**, 546 (1912).
18. M. Brownlee, *Diabetes Care,* **15**, 1835 (1992).
19. J. W. Baynes and S. R. Thorpe, *Diabetes,* **48**, 1 (1999).
20. A. J. Bailey, G. R. Paul and L. Knott, *Mechanisms of Ageing and Development,* **106**, 1 (1998).
21. M. Brownlee, *Diabetes*, **43**, 836 (1994).
22. V. M. Monnier, *Archives of Biochemistry and Biophysics*, **419**, 1 (2003).
23. W. T. Cefalu, A. D. Bell-Farrow, Z. Q. Wang, W. E. Sonntag, M. X. Fu, J. W. Baynes and S. R. Thorpe, *Journal of Gerontology Series* A*, Biological Sciences* and *Médical Sciences,* **50**, B337 (1995).
24. D. R. Sell and V. M. Monnier, *Journal of Gerontology Series* A*, Biological Sciences and Médical Sciences,* **52**, B277 (1997).
25. D. R. Sell, N. R. Kleinman and V. M. Monnier, *Federation of American Society for Experimental Biology Journal*, **14**, 145 (2000).
26. J. V. Hunt, M. A. Bottoms and M. J. Mitchinson, *Biochemical Journal*, **291**, 529 (1993).
27. N. Ahmed, *Diabetes Research and Clinical Practice*, **67**, 3 (2005).
28. M. S. Ahmad and N. Ahmed, *American Society for Nutrition,* **136**, 796S (2006).
29. Y. Ido, A. Vindigni, K. Chang, L. Stramm, R. Chance, W. F. Heath, R. D. Di Marchi, E. Di Cera, J.R. Williamson, *Science,* **277**, 563 (1997).
30. a) A. Gugliucci, *The Journal Of The American Osteopathic Association*, **100**, 621 (2000). b) R. Singh, A. Barden, T. Mori, I. Beilin, *A Review Diabetologia*, **44**, 129 (2001).
31. A. Gugliucci and T. Menini, *Life Sciences*, **72**, 2603 (2003).
32. D. L. Price, P. M. Rhett, S. R. Thorpe and J. W. Baynes, *Journal of Biological Chemistry*, **276**, 48967 (2001).
33. T. P. Degenhardt, N. L. Alderson, D. D. Arrington, R. J. Beattie, J. M. Basgen, M. W. Steffes, S. R. Thorpe and J. W. Baynes, *Kidney International*, **61**, 939 (2002).
34. J. M. Forbes, T. Soulis, V. Thallas, S. Panagiotopoulos, D. M. Long, S. Vasan, D. Wagle, G. Jerums and M. E. Cooper, *Diabetes*, **44**, 108 (2001).
35. T. D. Lehman and B. J. Ortwerth, *Biochimica et Biophysica Acta,* **1535**, 110 (2001).
36. K. M. Khan, S. Saeed, M. Ali, M. Gohar, J. Zahid, A. Khan, S. Perveen and M. I. Choudhary, *Bioorganic and Medicinal Chemistry*, **17**, 2447 (2009).
37. K. M. Khan, U. R. Mughal, N. Ambreen, A. Khan, S. Perveen and M. I. Choudhary*, Letters in Drug Design and Discovery,* **6***,* 358 (2009).
38. K. M. Khan, M. Khan, M. Ali, M. Taha, S. Rasheed, S. Perveen and M. I. Choudhary, *Bioorganic and Medicinal Chemistry***, 17,** 7795 (2009).
39. K. M. Khan, M. Khan, N. Ambreen, M. Taha, F. Raheem, S. Rasheed, S. Saied, H. Shafi, S. Perveen and M. I. Choudhary, *Medicinal Chemistry*, **9**, 681 (2013).
40. K. M. Khan, U. R. Mughal, A. Khan, F. Naz, S. Perveen and M. I. Choudhary, *Letters in Drug Design and Discovery,* **7***,* 188 (2010).
41. S. Perveen, S. Mustafa, K. Qamar, A. Dar, K. M. Khan, M. I. Choudhary, A. Khan and W. Voelter, *Medicinal Chemistry Research*, (2013) (August On Line).
42. S. M. A. Hai, S. Perveen, R. A. Khan. K. M. Khan and N. Afza, *Natural Product Research*, **17**, 351 (2003).
43. S. Perveen, S. M. A. Hai, R. A. Khan. K. M. Khan, N. Afza and T. Sarfaraz, *Synthetic Communcation*, **35**, 166 (2005).