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Unsymmetrically disubstituted urea derivatives: A potent class of antiglycating agents

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ABSTRACT

A series of unsymmetrically disubstituted urea derivatives **1**–**28** has been synthesized and screened for their antiglycation activity in vitro. Compounds **26** (IC_{50} = 4.26 ± 0.25 μM), **1** (IC_{50} = 5.8 ± 0.08 μM), **22** (IC_{50} = 4.26 ± 0.25 μM), **6** (IC_{50} = 6.4 ± 0.02 μM), **5** (IC_{50} = 6.6 ± 0.26 μM), **2** (IC_{50} = 7.02 ± 0.31 μM), **3** (IC_{50} = 7.14 ± 0.84 μM), **27** (IC_{50} = 7.27 ± 0.36 μM), **4** (IC_{50} = 8.16 ± 1.04 μM), **21** (IC_{50} = 8.4 ± 0.15 μM), **23** (IC_{50} = 9.0 ± 0.35 μM) and **13** (IC_{50} = 15.22 ± 6.7 μM) showed an excellent antiglycation activity far better than the standard (rutin, IC_{50} = 41.9 ± 2.3 μM). This study thus provides a series of potential molecules for further studies of antiglycation agents.

1. Introduction

Urea is a functional moiety that is commonly found in natural products and often displays a wide range of biological activities.[1] In particular; substituted ureas have attracted attention due to their range of applications as agricultural pesticides,[2] dyeing material for hair and cellulose fibres, antioxidants in gasoline and additives in detergents to prevent carbon deposits and corrosion inhibitors.[3] They can also serve as uron herbicides,[1,2] plant growth regulators, agroprotectives, tranquillizers and as anticonvulsants.[3] An unsymmetrically substituted urea is a common structural feature of many biologically active compounds such as enzyme inhibitors and pseudopeptides.[4] Unsymmetrically substituted ureas at amino groups have also been shown to have a potent HIV-1 protease inhibitory activity,[3,4] equipotent towards both wild and mutant types.[4] Sulfonylureas have found applications as oral antidiabetic drugs and as herbicides.[5] Some urea derivatives are useful as active ingredients in antimicrobial, antifungal and algaecides agents.[6]

Among them diaryl urea derivatives have been patented for treatment of protein kinase dependent diseases of the animal or human body.[7]

Some novel urea derivatives found to be useful for the treatment of inflammatory diseases.[8,9] A series of ureas and thioureas was synthesized, and their inhibitory activities against $NO^{\cdot}$ production in lipopolysaccharide-activated macrophages were evaluated.[10]

In the present study in vitro antiglycation effect of a series of unsymmetrically disubstituted urea derivatives **1**–**28** was evaluated. Discovery of antiglycation agents is now considered to be an important area of pharmaceutical research for the treatment of late diabetic complications.

The incident of type-2 diabetes is increasing at an alarming rate and about 1–2% of the world's population is affected with over 100 million diabetic patients are worldwide. A large number of studies focused the factors involving in the pathogenesis of diabetic complications but the exact molecular basis of these complications is not fully understood yet. Hyperglycemia is one of the causative factors of diabetes complications, that is, atheroma, hypertension and microangiopathy. Its detrimental effects are mostly attributed to the formation of sugar-derived substances called advanced glycation end products (AGEPs).[11] AGEPs are a group of molecules formed in a non-enzymatic way from combination of reducing sugars with free amino groups of proteins, lipids and nucleic acids. An enormous amount of evidence suggests that AGEPs are significant pathogenic mediators of almost all increasing diabetic complications.[12]

Initial product of protein–glucose interaction is Schiff base which forms without any enzyme, whereas the rearrangement of Schiff base intermediate to Amadori product takes number of days. Extent of glycation of vital biochemicals is dependent on the degree and duration of hyperglycemia in vivo. Considerable effort

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has been focussed on the discovery of new inhibitors of glycation because of their therapeutic potential.[13] Antiglycating compounds may react with carbonyl group of reducing sugars, Amadori products and 3-deoxyglucosones to inhibit the formation of AGEPs. Certain molecules have been developed that can cleave AGEP cross-links and perhaps open the possibility of reversing the steady process of diabetic complications.[14] Antioxidants may provide protection against glycation-derived free radicals, while chelators are helpful in removing the transition metals to prevent autoxidation of glucose and Amadori products.[15]

Compounds with both antiglycation and antioxidant properties may offer therapeutic potential. It has been found that aged garlic extract (AGE) inhibit the formation of AGEPs in vitro and it also prevents the formation of glycation-derived free radicals. *S*-Allylcysteine is a key component of aged garlic extract that acts as a potent antioxidant and may inhibit the AGEPs formation.[16]

Aminoguanidine, an inhibitor of AGEP formation was found to prevent retinopathy in diabetic animals and protect them from developments of diabetic vascular complications. However, aminoguanidine has encountered some toxicity problems in phase III of clinical trials so this drug can serve as a prototype for many new molecules that are being synthesized and tried in vitro at present.[17] Efforts have now been intensely dedicated to the search of new and safe synthetic antiglycation agents.[18] It has been demonstrated that polyamines, spermine and spermidine have potent antiglycation effects. Results of protection against glycation were comparable to those of aminoguanidine and carnosine.[19]

2. Results and discussion

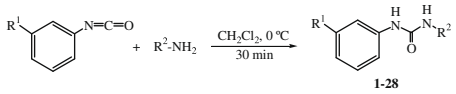
2.1. Chemistry

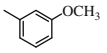
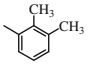
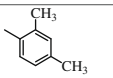
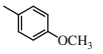
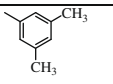
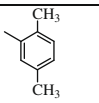
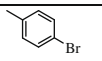
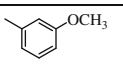
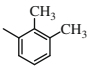
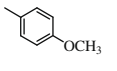
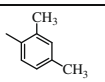
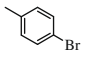
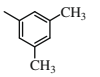
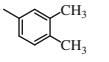
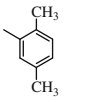
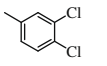
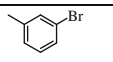
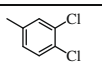
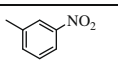
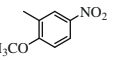
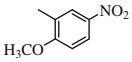
We have previously reported the tertiary amine promoted synthesis of symmetrical 1,3-disubstituted ureas in high yields.[20] In the present study we prepared a series of twenty-eight (28) unsymmetrical N,N′-disubstituted aryl urea **1–28** by reacting substituted phenyl isocyanates with respective amines in dichloromethane at 0 °C for 30 min, as shown in Scheme 1. In all experiments, solid materials were formed which were filtered, washed with minimum amount of ethanol, dried under vacuum and recrystallized with ethanol. The structures of compounds were confirmed by using ^{1}H NMR and mass spectroscopy.

2.2. Biology

All the synthesized compounds **1–28** were randomly evaluated for their antiglycation activity according to literature protocol.[21] Unsymmetrical N,N′-disubstituted ureas **1–28** exhibited a varying degree of antiglycation activity, when compared to standard rutin (IC_{50} = 41.9 ± 2.3 μM) (Table 1). Compounds **26** (IC_{50} = 4.26 ± 0.25 μM), **1** (IC_{50} = 5.8 ± 0.08 μM), **22** (IC_{50} = 4.26 ± 0.25 μM), **6** (IC_{50} = 6.4 ± 0.02 μM), **5** (IC_{50} = 6.6 ± 0.26 μM), **2** (IC_{50} = 7.02 ± 0.31 μM), **3** (IC_{50} = 7.14 ± 0.84 μM), **27** (IC_{50} = 7.27 ± 0.36 μM), **4** (IC_{50} = 8.16 ± 1.04 μM), **21** (IC_{50} = 8.4 ± 0.15 μM), **23** (IC_{50} = 9.0 ± 0.35 μM) and 1**3** (IC_{50} = 15.22 ± 6.7 μM) showed excellent antiglycation activities, far better than standard (rutin, IC_{50} = 41.9 ± 2.3 μM). The compounds with IC_{50} values more than 100 μM were considered to be inactive. Additionally compounds **7**, **8**, **10–12** and **15–19** initially showed less than 50% activity and thus were not further evaluated for their IC_{50}. Compound **26** having a bromine moiety *meta* to urea bond found to be the most active among the series with an IC_{50} value 4.26 ± 0.25 μM, however, its *para* analogue **22** exhibited an IC_{50} value 6.1 ± 0.13 μM, surprisingly the *ortho* analogue **25** showed an IC_{50} value 120.4 ± 14.6 μM. This difference in activity of different analogues reveals that the substitution at one of the aromatic rings has pronounced effect in glucose or protein binding activity. Compound **27** having a nitro group at its *meta* position showed an IC_{50} value 7.27 ± 0.36 μM, whereas compounds **21** having *para* methoxy and **23** containing *meta* and *para* dimethyl groups showed IC_{50} values 8.4 ± 0.15 and 9.0 ± 0.35 μM, respectively. These results also showed that a suitable substituent at aromatic ring is responsible for enhancing the glucose or protein binding ability of the molecule.

In chloro-containing unsymmetrical urea derivatives, compound **1** having a methoxy group at *meta* position of the second aromatic ring was found to be active with an IC_{50} value of 5.8 ± 0.08 μM, however, its *ortho* **2** and *para* **3** analogues showed



S. No.	R^1	R^2	Yield (%)	S. No.	R^1	R^2	Yield (%)
1	Cl		55	**15**	H		81
2	Cl		91	**16**	H		88
3	Cl		52	**17**	H		57
4	Cl		60	**18**	H		83
5	Cl		77	**19**	H		50
6	Cl		84	**20**	H		85
7	Cl		86	**21**	H		32
8	Cl		94	**22**	H		63
9	Cl		26	**23**	H		41
10	Cl		96	**24**	H		32
11	Cl		87	**25**	H		66
12	Cl		84	**26**	H		33
13	Cl		47	**27**	H		22
14	Cl		61	**28**	H		53

Scheme 1. Synthesis of unsymmetrically disubstituted urea derivatives.

Table 1
Anti-glycation activities of the compounds **1**–**28**

Compounds	IC_{50} ± SEM (μM)
1	5.8 ± 0.08
2	7.02 ± 0.31
3	7.14 ± 0.84
4	8.16 ± 1.04
5	6.6 ± 0.26
6	6.4 ± 0.02
7	Inactive
8	Inactive
9	905.9 ± 3.8
10	Inactive
11	Inactive
12	Inactive
13	15.22 ± 6.7
14	407.6 ± 1.7
15	Inactive
16	Inactive
17	Inactive
18	Inactive
19	Inactive
20	229 ± 11.8
21	8.4 ± 0.15
22	6.1 ± 0.13
23	9 ± 0.35
24	838.3 ± 3.14
25	120.4 ± 14.6
26	4.26 ± 0.25
27	7.27 ± 0.36
28	162.5 ± 16.8
Rutin (std.)	41.9 ± 2.3

slightly higher IC_{50} values 7.02 ± 0.31, and 7.14 ± 0.84 μM, respectively. *Para* bromo-containing compound **6** demonstrated an IC_{50} value 6.4 ± 0.02 μM, whilst its *meta* analog **4** showed an IC_{50} = 8.16 ± 1.04 μM also indicating that the presence of suitable substituent(s) on both or either of the aromatic ring of unsymmetrically disubstituted ureas are assisting factors in glucose or protein binding affinity. When a compound **13** having *meta* and *para* dichloro groups was screened, it showed an IC_{50} value 15.22 ± 6.7 μM, which clearly demonstrated that disubstitution may result in lowering of antiglycation activity.

In conclusion, compounds **26** (IC_{50} = 4.26 ± 0.25 μM), **1** (IC_{50} = 5.8 ± 0.08 μM), **26** (IC_{50} = 4.26 ± 0.25 μM), **22** (**I**C_{50} = 6.1 ± 0.13 μM), **6** (IC_{50} = 6.4 ± 0.02 μM), **5** (IC_{50} = 6.6 ± 0.26 μM), **2** (IC_{50} = 7.02 ± 0.31 μM), **3** (IC_{50} = 7.14 ± 0.84 μM), **27** (IC_{50} = 7.27 ± 0.36 μM), **4** (IC_{50} = 8.16 ± 1.04 μM), **21** (IC_{50} = 8.4 ± 0.15 μM), **23** (IC_{50} = 9.0 ± 0.35 μM) and 1**3** (IC_{50} = 15.22 ± 6.7 μM) showed excellent antiglycation activities, far better than standard (rutin, IC_{50} = 41.9 ± 2.3 μM). These compounds therefore, may serve as powerful lead compounds for further studies.

3. Experimental

Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel plates (Kieselgel 60 F_{254}, E. Merck, Germany) and chromatograms were visualized under UV light at 254 and 365 nm or iodine vapors. Solvents for extraction and chromatography were of reagent grade. Solvents used for chemical reactions were distilled before use. ^{1}H NMR spectroscopy was performed on a Bruker AVANCE 300, 400 MHz and 500 MHz, δ in ppm related to $SiMe_4$ (0 ppm) as internal standard. Mass spectra were recorded either on MAT-312 or on JEOL JMS-HX 110 instruments. All the biological screenings were done in laboratories of the H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan.

3.1. Materials and methods

Bovine serum albumin (BSA) was purchased from Research Organics, Cleveland, while other chemicals, glucose anhydrous, trichloroacetic acid (TCA), sodium azide (NaN_3), dimethyl sulfoxide (DMSO), sodium dihydrogen phosphate (NaH_2PO_4), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCL), potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide (NaOH) were purchased from Sigma Aldrich.

Sodium phosphate buffer (pH 7.4) was prepared by mixing Na_2HPO_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.

3.2. Anti-glycation assay (in vitro)[21]

In 96-well plate assays, each well contain 60 μL of reaction mixtures {20 μL bovine serum albumin (BSA) (10 mg/mL + 20 μL of glucose anhydrous (50 mg/mL) + 20 μL, test sample}. Glycated control contains 20 μL BSA + 20 μL glucose + 20 μL sodium phosphate buffer, while blank control contains 20 μL BSA and 40 μL sodium phosphate buffer. Reaction mixture was incubated at 37 °C for 7-days. After incubation, 6 μL (100%) of trichloroacetic acid (TCA) was added in each well 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 advance glycated end product (AGE)-BSA were dissolved in 60 μL phosphate buffer solution (PBS). Assessment 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 through the following formula:

$$\%\ \text{Inhibition} = [1 - (\text{Fluorescence of sample}/\text{Fluorescence of glycated sample}) \times 100]$$

3.3. General procedure for preparation of N,N′-(disubstituted)-urea 1–28

Aniline (0.184 mL, 1.66 mmol) was dissolved in 5 mL dichloromethane. Temperature was maintained at 0 °C, then substituted phenyl isocyanate (0.20 mL, 1.65 mmol) was added drop wise with constant stirring. After 30 min, formation of white solid was occurred and the resultant solid product was filtered, washed with little ethanol and dried under vacuum. Recrystallization with ethanol afforded the desired solid urea.

3.3.1. *N*-(3-Chlorophenyl)-*N*′-(3-methoxyphenyl)-urea (1)

Yield: 0.25 g, 55%; R_f = 0.67 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 185 °C; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.82 (s, 1H, N–H), 8.71 (s, 1H, N–H), 7.69 (s, 1H, Ar-H), 7.27 (m, 2H, Ar-H), 7.17 (m, 2H, Ar-H), 7.0 (d, 1H, J = 8.0 Hz, Ar-H), 6.92 (d, 1H, J = 8.0 Hz, Ar-H), 6.55 (d, 1H, J = 6.3 Hz, Ar-H), 3.72 (s, 3H, OCH_3); EI MS: *m/z* (rel. abund. %), 278 (M^{2+}, 26.9), 276 (M^+, 83), 155 (19.4), 153 (56.8), 149 (46.8), 129 (31.7), 127 (100), 123 (65.1), 119 (10).

3.3.2. *N*-(3-Chlorophenyl)-*N*′-(2-methoxyphenyl)-urea (2)

Yield: 0.52 g, 91%; R_f = 0.87 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 158 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 9.49 (s, 1H, N–H), 8.25 (s, 1H, N–H), 8.09 (d, 1H, J = 6.2 Hz, Ar-H), 7.72 (s, 1H, Ar-H), 7.10

(m, 6H, Ar-H), 3.87 (s, 3H, OCH_3); EI MS: *m/z* (rel. abund. %), 278 (M^{2+}, 32.7), 276 (M^+, 100), 155 (4.7), 153 (11.7), 149 (18.1), 129 (28), 127 (100), 123 (98), 108 (74.2).

3.3.3. *N*-(3-Chlorophenyl)-*N′*-(4-methoxyphenyl)-urea (3)

Yield: 0.30 g, 52%; R_f = 0.64 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 176 °C; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.76 (s, 1H, N–H), 8.50 (s, 1H, N–H), 7.68 (s, 1H, Ar-H), 7.34 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.25 (m, 2H, Ar-H), 6.83 (d, 1H, *J* = 7.6 Hz, Ar-H), 6.86 (d, 2H, *J* = 8.6 Hz, Ar-H), 3.71 (s, 3H, OCH_3); EI MS: *m/z* (rel. abund. %), 278 (M^{2+}, 32.7), 276 (M^+, 95.7), 155 (4.6), 153 (13.5), 149 (49.8), 129 (18.5), 127 (55.6), 123 (100), 108 (91.4).

3.3.4. *N*-(3-Bromophenyl)-*N′*-(3-chlorophenyl)-urea (4)

Yield: 0.41 g, 60%; R_f = 0.80 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 235 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.95 (s, 1H, N–H), 8.94 (s, 1H, N–H), 7.83 (s, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 7.19 (m, 6H, Ar-H); EI MS: *m/z* (rel. abund. %), 326 (M^+, 12.24), 328 (M^{2+}, 3.08), 171 (54.1), 173 (51.7), 127 (100), 129 (36.5), 92.1 (45.7), 65.1 (55.7).

3.3.5. *N*-(2-Bromophenyl)-*N′*-(3-chlorophenyl)-urea (5)

Yield: 0.52 g, 77%; R_f = 0.93 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 189 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 9.64 (s, 1H, N–H), 8.18 (s, 1H, N–H), 8.03 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.73 (s, 1H, Ar-H), 7.62 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.30 (m, 3H, Ar-H), 7.00 (m, 2H, Ar-H); EI MS: *m/z* (rel. abund. %), 326 (M^+, 9), 328 (M^{2+}, 2.2), 245 (38), 171 (77.3), 173 (74), 127 (100), 129 (33.3), 92.1 (54), 65.1 (60.6).

3.3.6. *N*-(4-Bromophenyl)-*N′*-(3-chlorophenyl)-urea (6)

Yield: 0.57 g, 84%; R_f = 0.77 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 209 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.91 (s, 1H, N–H), 8.89 (s, 1H, N–H), 7.68 (s, 1H, Ar-H), 7.43 (m, 4H, Ar-H), 7.28 (m, 2H, Ar-H), 7.01 (m, 1H, Ar-H); EI MS: *m/z* (rel. abund. %), 326 (M^+, 20), 328 (M^{2+}, 5.2), 197 (7.3), 199 (7.4), 171 (100), 173 (7.85), 127 (97), 129 (36.2), 92.1 (63.1), 65.1 (71.4).

3.3.7. *N-(3-Chlorophenyl)-N′-(2,3-dimethylphenyl)-urea (7)*

Yield: 0.49 g, 86%; R_f = 0.87 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 196 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 9.10 (s, 1H, N–H), 8.01 (s, 1H, N–H), 7.72 (s, 1H, Ar-H), 7.47 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.26 (m, 2H, Ar-H), 6.97 (m, 3H, Ar-H), 2.44 (s, 3H, CH_3), 2.12 (s, 3H, CH_3); EI MS: *m/z* (rel. abund. %), 274 (M^+, 93.2), 276 (M^{2+}, 32.2), 153 (12.4), 147 (20.3), 129 (33.8), 127 (100), 121 (52.7), 106 (21.3).

3.3.8. *N*-(3-Chlorophenyl)-*N′*-(2,4-dimethylphenyl)-urea (8)

Yield: 0.43 g, 94%; R_f = 0.87 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 215 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 9.11 (s, 1H, N–H), 7.90 (s, 1H, N–H), 7.72 (s, 1H, Ar-H), 7.59 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.25 (m, 2H, Ar-H), 7.69 (m, 3H, Ar-H), 2.22 (s, 3H, CH_3), 2.18 (s, 3H, C-H_3); EI MS: *m/z* (rel. abund. %), 274 (M^+, 100), 276 (M^{2+}, 31.8), 147 (13.9), 129 (33.8), 127 (69), 129 (24.6), 121 (72), 106 (21.4).

3.3.9. *N*-(3-Chlorophenyl)-*N′*-(3,5-dimethylphenyl)-urea (9)

Yield: 0.15 g, 26%; R_f = 0.78 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 204 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.84 (s, 1H, N–H), 8.58 (s, 1H, N–H), 7.71 (s, 1H, Ar-H), 7.25 (m, 2H, Ar-H), 7.00 (m, 3H, Ar-H), 6.62 (s, 1H, Ar-H), 2.22 (s, 6H, CH_3); EI MS: *m/z* (rel. abund. %), 274 (M^+, 65.8), 276 (M^{2+}, 21.9), 153 (36.8), 155 (12.5), 147 (34.5), 129 (34.3), 127 (100), 121 (78.9), 106 (21.6).

3.3.10. *N*-(3-Chlorophenyl)-*N′*-(2,5-dimethylphenyl)-urea (10)

Yield: 0.55 g, 96%; R_f = 0.75 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 216 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 9.22 (s, 1H, N–H), 7.94 (s, 1H, N–H), 7.74 (s, 1H, Ar-H), 7.62 (s, 1H, Ar-H), 7.26 (m, 2H, Ar-H), 7.03 (m, 2H, Ar-H), 6.79 (d, 1H, *J* = 7.7 Hz, Ar-H), 2.24 (s, 3H, CH_3), 2.17 (s, 3H, CH_3); EI MS: *m/z* (rel. abund. %), 274 (M^+, 46), 276 (M^{2+}, 15.8), 153 (33.4), 155 (11.3), 147 (52.9), 129 (43.7), 127 (100), 121 (82.9), 106 (27.8).

3.3.11. *N*-(3-Chlorophenyl)-*N′*-(2,6-dimethylphenyl)-urea (11)

Yield: 0.50 g, 87%; R_f = 0.71 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 218 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.95 (s, 1H, N–H), 7.79 (s, 1H, N–H), 7.26 (s, 1H, Ar-H), 7.01 (m, 6H, Ar-H), 2.19 (s, 6H, CH_3); EI MS: *m/z* (rel. abund. %), 274 (M^+, 20.5), 276 (M^{2+}, 9.9), 153 (5.5), 147 (19), 127 (100), 121 (50.7), 106 (20.8).

3.3.12. *N*-(3-Chlorophenyl)-*N′*-(3,4-dimethylphenyl)-urea (12)

Yield: 0.48 g, 84%; R_f = 0.76 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 200 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.81 (s, 1H, N–H), 8.54 (s, 1H, N–H), 7.70 (s, 1H, Ar-H), 7.22 (m, 6H, Ar-H), 2.18 (s, 3H, CH_3), 2.14 (s, 3H, CH_3); EI MS: *m/z* (rel. abund. %), 274 (M^+, 57.8), 276 (M^{2+}, 19.7), 155 (14.3), 153 (42.5), 147 (76.1), 132 (57.1), 127 (100), 121 (93.4), 106 (34.1).

3.3.13. *N*-(3-Chlorophenyl)-*N′*-(3,4-chlorophenyl)-urea (13)

Yield: 0.31 g, 47%; R_f = 0.74 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 196 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 9.09 (s, 1H, N–H), 9.04 (s, 1H, N–H), 7.86 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.68 (s, 1H, Ar-H), 7.52 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.32 (m, 3H, Ar-H), 7.03 (m, 1H, Ar-H); EI MS: *m/z* (rel. abund. %), 274 (M^+, 57.8), 276 (M^{2+}, 19.7), 155 (14.3), 153 (42.5), 147 (76.1), 132 (57.1), 127 (100), 121 (93.4), 106 (34.1).

3.3.14. *N*-(3-Chlorophenyl)-*N′*-(2-methoxy-5-nitrophenyl)-urea (14)

Yield: 0.45 g, 61%; R_f = 0.76 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 203 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 9.63 (s, 1H, N–H), 9.08 (s, 1H, N–H), 8.63 (s, 1H, Ar-H), 7.93 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.73 (s, 1H, Ar-H), 7.25 (m, 3H, Ar-H), 7.05 (d, 1H, *J* = 7.8 Hz, Ar-H), 4.03 (s, 3H, OCH_3); EI MS: *m/z* (rel. abund. %), 321 (M^+, 19.5), 323 (M^{2+}, 5.9), 194 (66.2), 168 (95), 153 (89.4), 155 (24.7), 127 (100), 129 (30.8).

3.3.15. *N*-(2,3-Dimethylphenyl)-*N′*-phenyl-urea (15)

Yield: 0.36 g, 81%; R_f = 0.96 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 190 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.89 (s, 1H, N–H), 7.91 (s, 1H, N–H), 7.52 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.43 (d, 2H, *J* = 7.9 Hz Ar-H), 7.25 (t, 2H, *J* = 7.8 Hz Ar-H), 6.95 (t, 1H, *J* = 7.7 Hz, Ar-H), 6.94 (d, 1H, *J* = 7.3 Hz, Ar-H), 6.89 (t, 1H, *J* = 7.4 Hz, Ar-H), 2.24 (s, 3H, CH_3), 2.12 (s, 3H, CH_3); EI MS: *m/z* (rel. abund. %), 240 (M^+, 74.3), 147 (12.9), 106 (25.9), 93 (100).

3.3.16. *N*-(2,4-Dimethylphenyl)-*N′*-phenyl-urea (16)

Yield: 0.39 g, 88%; R_f = 0.94 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 207 °C ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.90 (s, 1H, N–H), 7.81 (s, 1H, N–H), 7.63 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.43 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.25 (t, 2H, *J* = 7.8 Hz, Ar-H), 6.97 (t, 1H, *J* = 7.7 Hz, Ar-H), 6.93 (d, 1H, *J* = 6.5 Hz, Ar-H), 6.72 (s, 1H, Ar-H), 2.21 (s, 3H, CH_3), 2.18 (s, 3H, CH_3); EI MS: *m/z* (rel. abund. %), 240 (M^+, 77), 147 (18), 121 (100), 106 (27.6), 93 (88.3).

3.3.17. *N*-(2,6-Dimethylphenyl)-*N′*-phenyl-urea (17)

Yield: 0.25 g, 57%; R_f = 0.8 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 220 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.69 (s, 1H, N–H), 7.68 (s, 1H, N–H), 7.42 (d, 2H, *J* = 7.7 Hz, Ar-H), 7.23 (t, 2H, *J* = 7.8 Hz, Ar-H), 7.05 (d, 2H, *J* = 7.6 Hz, Ar-H), 6.90 (t, 1H, Ar-H *J* = 7.3 Hz), 6.87 (t, 1H, *J* = 7.0 Hz, Ar-H), 2.19 (s, 3H, CH_3); EI MS: *m/z* (rel. abund. %), 240 (M^+, 93.80), 147 (42), 121 (90.2), 106 (65.3), 93 (100).

3.3.18. *N*-(3,5-Dimethylphenyl)-*N′*-phenyl-urea (18)

Yield: 0.36 g, 83%; R_f = 0.76 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 165 °C; ^{1}H NMR: (400 MHz, CD_3OD): δ 8.57 (s, 1H, N–H), 8.45 (s, 1H, N–H), 7.42 (d, 2H, J = 5.8 Hz, Ar-H), 7.25 (t, 2H, J = 6.0 Hz, Ar-H), 7.05 (s, 6H, CH_3), 6.94 (t, 1H, J = 5.5 Hz, Ar-H), 6.59 (s, 3H,CH_3); EI MS: *m/z* (rel. abund. %), 240 (M^+, 100), 147 (20), 121 (83.7), 106 (25.3), 93 (85).

3.3.19. *N*-(2,3-Dimethylphenyl)-*N′*-phenyl-urea (19)

Yield: 0.22 g, 50%; R_f = 0.70 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 217 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.96 (s, 1H, N–H), 7.81 (s, 1H, N–H), 7.66 (s, 1H, Ar-H), 7.44 (d, 2H, J = 7.6 Hz, Ar-H), 7.25 (t, 2H, J = 7.8 Hz, Ar-H), 7.02 (d, 1H, J = 7.6 Hz, Ar-H), 6.93 (d, 1H, J = 7.3 Hz, Ar-H), 6.74 (d, 1H, J = 7.5 Hz, Ar-H), 2.23 (s, 3H, CH_3), 2.17 (s, 3H, CH_3); EI MS: *m/z* (rel. abund. %), 240 (M^+, 24.6), 147 (4.32), 121 (73.68), 106 (55.06), 93 (100).

3.3.20. *N*-(3-Methoxyphenyl)-*N′*-phenyl-urea (20)

Yield: 0.38 g, 85%; R_f = 0.62 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 152 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.62 (s, 1H, N–H), 8.59 (s, 1H, N–H), 7.42 (d, 2H, Ar-H), 7.26 (t, 2H, J = 7.8 Hz, Ar-H), 7.16 (t, 1H, J = 2.9 Hz, Ar-H), 7.13 (s, 1H, Ar-H), 6.95 (t, 1H, J = 7.3 Hz, Ar-H), 6.90 (d, 1H, J = 2.7 Hz, Ar-H), 6.53 (d, 1H, J = 4.5 Hz, Ar-H), 3.72 (s, 3H, OCH_3); EIMS: *m/z* (rel. abund. %), 242 (M^+, 100), 149 (30), 123 (76.7), 119 (22.7), 93 (82.9).

3.3.21. *N*-(4-Methoxyphenyl)-*N′*-phenyl-urea (21)

Yield: 0.14 g, 32%; R_f = 0.78 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 195 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.54 (s, 1H, N–H), 8.43 (s, 1H, N–H), 7.41 (d, 2H, J = 10.2 Hz, Ar-H), 7.33 (d, 2H, J = 11.3 Hz, Ar-H), 7.25 (t, 2H, J = 10.4 Hz, Ar-H), 6.94 (t, 1H, J = 9.7 Hz, Ar-H), 6.84 (d, 1H, J = 12.0 Hz, Ar-H), 3.72 (s, 3H, OCH_3); EI MS: *m/z* (rel. abund. %), 242 (M^+, 59.10), 149 (24.23), 123 (88.6), 119 (11.55), 93 (87.08).

3.3.22. *N*-(4-Bromophenyl)-*N′*-phenyl-urea (22)

Yield: 0.34 g, 63%; R_f = 0.72 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 226 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.78 (s, 1H, N–H), 8.67 (s, 1H, N–H), 7.41 (d, 2H, J = 9.0 Hz, Ar-H), 7.26 (t, 2H, J = 10.0 Hz, Ar-H), 7.24 (d, 2H, J = 7.3 Hz, Ar-H), 7.01 (d, 2H, J = 7.1 Hz, Ar-H), 6.96 (t, 1H, J = 7.3 Hz, Ar-H); EI MS: *m/z* (rel. abund. %), 291 (M^+, 13.29), 290 (12.35), 172 (6.52), 171 (76.40), 119 (5.63), 93 (100).

3.3.23. *N*-(3,4-Dimethylphenyl)-*N′*-phenyl-urea (23)

Yield: 0.18 g, 41%; R_f = 0.82 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 187 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.54 (s, 1H, N–H), 8.43 (s, 1H, N–H), 7.41 (d, 2H, J = 7.7 Hz, Ar-H), 7.26 (t, 2H, J = 7.5 Hz, Ar-H), 7.21 (s, 1H, Ar-H), 7.15 (d, 1H, J = 8.0 Hz, Ar-H), 7.0 (d, 1H, J = 8.1 Hz, Ar-H), 6.93 (t, 1H, J = 7.3 Hz, Ar-H), 2.17 (s, 3H, CH_3), 2.14 (s, 3H, CH_3); EI MS: *m/z* (rel. abund. %), 240 (M^+, 38.93), 147 (5.63), 121 (88.68), 106 (35.43), 93 (100).

3.3.24. *N*-(3,4-Dichlorophenyl)-*N′*-phenyl-urea (24)

Yield: 0.16 g, 32%; R_f = 0.74 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 200 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.95 (s, 1H, N–H), 8.76 (s, 1H, N–H), 7.86 (d, 2H, J = 7.54 Hz, Ar-H), 7.50 (d, 1H, J = 8.8 Hz, Ar-H), 7.43 (d, 1H, J = 7.6 Hz, Ar-H), 7.30 (m, 1H, Ar-H), 6.98 (t, 1H, J = 7.3 Hz, Ar-H), 7.27 (s, 1H, Ar-H); EI MS: *m/z* (rel. abund. %), 284 (M^{2+}, 4.5), 282 (M^+, 26.2), 280 (39.8), 244 (1.4), 187 (65.4), 163 (100), 161 (100), 93 (91.5).

3.3.25. *N*-(2-Bromophenyl)-*N′*-phenyl-urea (25)

Yield: 0.35 g, 66%; R_f = 0.86 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 180 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 9.42 (s, 1H, N–H), 8.10 (s, 1H, N–H), 8.05 (d, 2H, J = 8.3 Hz, Ar-H), 7.53 (d, 1H, J = 7.1 Hz, Ar-H), 7.27 (t, 2H, J = 8.8 Hz, Ar-H), 7.21 (t, 1H, J = 10.1 Hz, Ar-H), 7.15 (d, 1H, J = 7.3 Hz, Ar-H), 7.05 (t, 1H, J = 7.3 Hz, Ar-H), 7.01 (t, 1H, J = 7.06 Hz, Ar-H); EI MS: *m/z* (rel. abund. %), 292 (M^{2+}, 10.7), 290 (M^+, 10), 199 (58.8), 197 (58.6), 173 (86), 171 (100), 93 (87.4).

3.3.26. *N*-(3-Bromophenyl)-*N′*-phenyl-urea (26)

Yield: 0.18 g, 33%; R_f = 0.65 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 173 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.82 (s, 1H, N–H), 8.70 (s, 1H, N–H), 7.83 (d, 2H, J = 7.6 Hz, Ar-H), 7.43 (d, 1H, J = 7.9 Hz, Ar-H), 7.27 (t, 2H, J = 7.5 Hz, Ar-H), 7.04 (t, 1H, J = 7.1 Hz, Ar-H), 7.02 (s, 1H, Ar-H), 7.00 (t, 1H, J = 7.1 Hz, Ar-H); EI MS: *m/z* (rel. abund. %), 292 (M^{2+}, 29.7), 290 (M^+, 30.5), 199 (49.7), 197 (51.5), 119 (66.2), 173 (94.7), 171 (100), 93 (87.4).

3.3.27. *N*-(3-Nitrophenyl)-*N′*-phenyl-urea (27)

Yield: 0.10 g, 22%; R_f = 0.85 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 190 °C; ^{1}H NMR: (400 MHz, DMSO-d_6): δ 9.17 (s, 1H, N–H), 9.79 (s, 1H, N–H), 8.53 (s, 1H, Ar-H), 7.80 (d, 1H, J = 6.34 Hz, Ar-H), 7.69 (d, 1H, J = 7.0 Hz, Ar-H), 7.46 (d, 1H, J = 7.8 Hz, Ar-H), 7.55 (t, 2H, J = 8.14 Hz, Ar-H), 7.29 (t, 2H, J = 7.66 Hz, Ar-H), 6.99 (t, 1H, J = 7.3 Hz, Ar-H); EI MS: *m/z* (rel. abund. %), 257 (M^+, 25.4), 164 (59.9), 138 (89.7), 119 (100), 93 (81.5).

3.3.28. *N*-(2-Methoxy-5-nitrophenyl)-*N′*-phenyl-urea (28)

Yield: 0.28 g, 53%; R_f = 0.58 (CH_2Cl_2/MeOH, 9.5:0.5); mp: 218 °C; ^{1}H NMR: (400 MHz, DMSO-d_6): δ 9.41 (s, 1H, N–H), 9.10 (s, 1H, N–H), 8.58 (s, 1H, Ar-H), 7.90 (d, 2H, J = 7.8 Hz, Ar-H), 7.46 (d, 1H, J = 7.8 Hz, Ar-H), 7.29 (t, 2H, J = 7.6 Hz, Ar-H), 7.23 (d, 1H, J = 9.06 Hz, Ar-H), 6.99 (t, 1H, J = 7.3 Hz, Ar-H), 4.03 (s, 3H, OCH_3); EI MS: *m/z* (rel. abund. %), 287 (M^+, 21), 194 (24.6), 168 (100), 153 (21.1), 119 (68), 93 (42.4).

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