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Synthesis of imidazole-schiff base analogues: SAR studies of potent antiglycation and urease activities

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ABSTRACT

A series of imidazole-Schiff base derivatives (**4-23**) were synthesized, spectroscopically characterized and evaluated for their *in vitro* antiglycation and urease inhibitory activities. The results shown that compounds **9, 10, 11, 15, 16, 22** and **23** showed excellent antiglycation and urease activities with IC_{50} values are lower than the standards rutin and thiourea respectively. Preliminary structure-activity relationship revealed that the compounds **9, 10, 11, 15, 16, 22** and **23** with electron donating moiety (OH, OCH_3) were found to be excellent antiglycation and urease activity and compounds **5, 6, 7, 8, 12, 13, 14 and 21** with electron withdrawing groups (Cl, F, NO_2 and Br) were found to be least antiglycation and urease activity.

Keywords: Imidazole; Schiff's bases; Antiglycation; Urease activity; Electronic effect.

1. INTRODUCTION

Antiglycation agents play an impartment role in the treatment of diabetic complications. The number of effective antiglycation agents is still very limited. [1] The formation of advanced glycation end products (AGEs), is believed to play important roles in pathogenesis of diabetic and aging, [2] are considered these advanced glycarion end products as vital mediators of approximately all diabetic complications. [3] Non-enzymatic reaction between reducing sugar and free amino group of proteins, leads to glycated protein termed Amadori product. Schiff bases of glucose are produced primarily by the reaction between glucose and protein without any enzyme, this intermediate rearranges to Arnadori products. [4] Few molecules have been found that can cleave AGEPs cross-links and reverse the balanced process of diabetic complications. [5] *S*-Allylcysteine is an important component of aged garlic extract that inhibits the AGEPs formation. [6] Many efforts have been made to develop new safe and sound synthetic antiglycation agents. [7]

The metalloenzyme urease (urea amidohydrolase; EC 3.5.1.5) catalyzes the hydrolysis urea into ammonia and carbon dioxide. It is present in a variety of plants, algae, fungi, and bacteria. [8] Urease is involved in the pathogenesis of hepatic encephalopathy, hepatic coma urolithiasis, pyelo nephritis, ammonia, and urinary catheter encrustation. [8.9] It is also a major cause of pathologies induced by Helicobacter pylori as this allows bacteria to survive at the low pH of the stomach and hence plays an important role in producing peptic and gastric ulcers. [8] As a result, ureases have been identified as important targets in research both for human and animal health, as well as in agriculture.

Heterocyclic nucleus imparts an important function in medicinal chemistry and serves as a key template for the development of various therapeutic agents. [10] Imidazoles have occupied a unique position in heterocyclic chemistry, and its derivatives have attracted considerable interests in recent years for their versatile properties in chemistry and pharmacology. It improves pharmacokinetic characteristics of lead molecules and thus is used as a remedy to optimize solubility and bioavailability parameters of proposed poorly soluble lead molecules. The imidazole derivatives possess extensive spectrum of biological activities such as anti-inflammatory, [11] antioxidant, [12] antibacterial, [13] anticancer, [14] antitubercular [15]

and anti-HIV [16] activities. It is also present in the structure of many natural or synthetic drug molecules, that is, cimetidine, azomycin, and metronidazole. Imidazole-containing drugs have a broader scope in remedying various dispositions in clinical medicine.

Based on the above facts and in continuation of our drug development program, the present work involves the synthesis of a series of small and simple imidazole derived Schiff's base analogues as potential urease and antiglycation agents.

2. EXPERIMENTAL

2.1 Chemistry

General

All chemicals and reagents obtained from Sigma Aldrich (India), Merck (India) and Avra Synthesis (India) were used without further purification. Melting points were determined on a Superfit melting point apparatus (India) and are uncorrected. FT-IR was performed using a Jasco spectrometer (Japan) using nujol media. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Agilent Technologies (USA) using DMSO (d_6) as solvent. High resolution mass spectroscopic analysis was performed on a Bruker MicroTOF QII mass spectrometer in positive mode. Progress of the reaction was monitored by TLC using silica gel coated on glass plates with the solvent system comprising chloroform/ methanol/acetic acid in the ratio 98:02:03 and the compounds on the TLC plates were detected by under UV light.

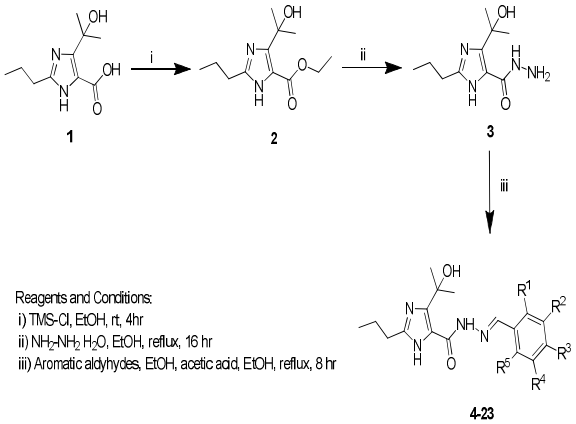
2.1.1. Ethyl 4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carboxylate (2)

To a solution of imidazole (0.05 mol, 10.6 g) in ethanol (100 mL), trimethylsilylchloride (0.05 mol, 5.43 g) was added slowly. The reaction mixture was stirred for 4 hrs to complete the reaction (monitored by TLC). The solvent was removed under reduced pressure and the resultant precipitate was washed with ice cold water and filtered to yield the desired products **2.** Yield 10.8 g, 90.1%

Yield 10.8 g, 90.1 %, R_f^a = 0.66, R_f^b = 0.71, m.p. 184-185 °C, IR KBr (cm^{-1}): 1660, 3214, 3510; ^{1}H NMR (DMSO-d_6) δ ppm: 0.91-0.95 (t, 3H, CH_3), 1.30 (t, 3H, CH_3), 1.59 (s, 6H, $2CH_3$), 1.68-1.73 (m, 2H, CH_2), 2.62-2.66 (t, 2H, CH_2), 4.30-4.35 (t, 2H, CH_2), 5.84 (s, 1H, OH), 9.9 (s, 1H, ring NH); HRMS m/z, (M+1): 241.1750

2.1.2. 4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (3)

To a solution of **2** (0.04 mol, 9.6 g) in ethanol (100 mL), hydrazine hydrate (0.048 mol, 2.4 g) was added. The reaction mixture was refluxed for 16 hrs for completion of the reaction (monitored by TLC). The solvent was removed under reduced pressure and cooled by adding ice cold water. The resulting precipitate was filtered, washed with cold water and recrystallized from ethanol to get the desired compounds **3**. Yield 7.7 g, 85.5 %



Entry	R^1	R^2	R^3	R^4	R^5	Entry	R^1	R^2	R^3	R^4	R^5
4	H	H	H	H	H	14	NO_2	H	NO_2	H	H
5	H	H	Cl	H	H	15	H	OH	OH	H	H
6	H	H	NO_2	H	H	16	H	OCH_3	OCH_3	H	H
7	H	H	F	H	H	17	H	Br	OH	Br	H
8	H	H	Br	H	H	18	H	Br	OH	H	H
9	H	H	OCH_3	H	H	19	H	Br	OCH_3	H	H
10	H	H	OH	H	H	20	H	OCH_3	OH	Br	H
11	H	OCH_3	OH	H	H	21	Cl	H	H	H	F
12	Cl	H	Cl	H	H	22	H	OCH_3	OCH_3	OCH_3	H
13	F	H	F	H	H	23	H	OH	OH	OH	H

Scheme - 1: Synthesis of target compounds 4-23.

Yield 7.7 g, 85.5 %, R_f^a = 0.36, R_f^b = 0.40, m.p. 201-202 °C, IR KBr (cm^{-1}): 1674, 2983, 3117, 3525; ^{1}H NMR (DMSO-d_6) δ ppm: 0.81-0.99 (t, 3H, CH_3), 1.22-1.58 (s, 6H, $2CH_3$), 1.67-1.95 (m, 2H, CH_2), 2.58-2.62 (t, 2H, CH_2), 4.09-4.11 (d, 2H, NH_2), 7.09 (s, 1H, OH), 8.37 (t, 1H, NH), 8.84 (s, 1H, ring NH); HRMS m/z, (M+1): 227.1758

2.1.3. General procedure for the synthesis of Schiff's bases (4-23)

An equimolar amount of **3** (1 mmol) was dissolved in ethanol (10 mL/g of compound) and treated with appropriate aldehydes (1 mmol) in the presence of catalytic amount of glacial acetic acid. The reaction mixtures were refluxed for 7–8

hr and the completion of reaction was monitored by TLC. After completion of the reaction, the solvent was removed under reduced pressure and cooled by adding ice cold water. The resulting precipitate was filtered, washed with water and recrystallized from ethanol to obtain the desired Schiff's bases **(4-23)**.

2.1.3.1. *N′*-Benzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (4)

Yield 88.40%, R_f^a = 0.64, R_f^b = 0.71, m.p. 189-190 °C, IR KBr (cm^{-1}): 1614, 1750, 3214, 3315, 3510; ^{1}H NMR (DMSO-d_6) δ ppm: 0.98 (s, 3H, CH_3), 1.27 (s, 6H, $(CH_3)_2$), 1.71 (m, 2H, CH_2), 2.92 (t, 2H, CH_2), 7.41-7.85 (m, 5H, Ar-H), 7.91 (s, 1H, -N=CH), 9.12 (s, 1H, OH), 10.21 (s, 1H, NH), 11.22 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.9, 24.1, 29.8, 31.3, 76.2, 128.6, 129.4, 131.1, 133.7, 136.4, 142.8, 143.6, 157.1, 160.1; HRMS m/z: 315.1548 [M+1]

2.1.3.2. *N′*-(4-Chlorobenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (5)

Yield 91.10%, R_f^a = 0.52, R_f^b = 0.56, m.p. 159-160 °C, IR KBr (cm^{-1}): 1619, 1768, 3215, 3320, 3560; ^{1}H NMR (DMSO-d_6) δ ppm: 0.90 (s, 3H, CH_3), 1.29 (s, 6H, $(CH_3)_2$), 1.75 (m, 2H, CH_2), 2.87 (t, 2H, CH_2), 7.32-7.80 (m, 4H, Ar-H), 7.88 (s, 1H, -N=CH), 9.01 (s, 1H, OH), 10.21 (s, 1H, NH), 11.52 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.5, 24.4, 29.5, 31.8, 75.9, 128.9, 129.8, 132.1, 136.0, 136.9, 143.1, 144.2, 157.2, 160.1; HRMS m/z: 349.1236 [M+1], 351.6245 [M+3].

2.1.3.3. *N′*-(4-Nitrobenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (6)

Yield 86.23%, R_f^a = 0.42, R_f^b = 0.50, m.p. 172-174 °C, IR KBr (cm^{-1}): 1606, 1730, 3212, 3355, 3588; ^{1}H NMR (DMSO-d_6) δ ppm: 0.85 (s, 3H, CH_3), 1.30 (s, 6H, $(CH_3)_2$), 1.74 (m, 2H, CH_2), 2.72 (t, 2H, CH_2), 7.60-8.12 (m, 4H, Ar-H), 7.88(s, 1H, -N=CH), 8.90 (s, 1H, OH), 10.09 (s, 1H, NH), 11.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.6, 23.9, 28.6, 31.4, 76.2, 124.6, 125.7, 130.1, 136.1, 143.0, 143.9, 151.3, 157.8, 159.9; HRMS m/z: 360.6215 [M+1].

2.1.3.4. *N′*-(4-Fluorobenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (7)

Yield 88.10%, R_f^a = 0.49, R_f^b = 0.51, m.p. 168-169 °C, IR KBr (cm^{-1}): 1610, 1770, 3250, 3370, 3568; ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (s, 3H, CH_3), 1.27 (s, 6H, $(CH_3)_2$), 1.77 (m, 2H, CH_2), 2.80 (t, 2H, CH_2), 7.12-7.42 (m, 4H, Ar-H), 7.95 (s, 1H, -N=CH), 8.51 (s, 1H, OH), 10.17 (s, 1H, NH), 11.30 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 12.9, 23.8, 29.1, 31.6, 75.8, 115.6, 128.7, 129.1, 136.7, 142.1, 144.5, 157.0, 157.9, 164.1; HRMS m/z: 333.4512 [M+1].

2.1.3.5. *N′*-(4-Bromobenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (8)

Yield 86.20%, R_f^a = 0.39, R_f^b = 0.42, m.p. 185-187 °C, IR KBr (cm^{-1}): 1628, 1745, 3269, 3377, 3590; ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (s, 3H, CH_3), 1.32 (s, 6H, $(CH_3)_2$), 1.83 (m, 2H, CH_2), 2.69 (t, 2H, CH_2), 7.55-7.80 (m, 4H, Ar-H), 7.99(s, 1H, -N=CH), 8.79 (s, 1H, OH), 10.56 (s, 1H, NH), 11.31 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.0, 24.1, 28.5, 30.5, 76.0, 124.9, 125.6, 131.1, 132.6, 136.5, 142.0, 143.5, 156.9, 160.1; HRMS m/z: 394.1254 [M+1], 396.5642 [M+3].

2.1.3.6. 4-(2-Hydroxypropan-2-yl)-*N′*-(4-methoxybenzylidene--2-propyl-1*H*-imidazole-5-carbohydrazide (9)

Yield 85.24%, R_f^a = 0.45, R_f^b = 0.51, m.p. 180-182 °C, IR KBr (cm^{-1}): 1606, 1733, 3310, 3395, 3555; ^{1}H NMR (DMSO-d_6) δ ppm: 0.92 (s, 3H, CH_3), 1.35 (s, 6H, $(CH_3)_2$), 1.90 (m, 2H, CH_2), 2.88 (t, 2H, CH_2), 3.78 (s, 3H, OMe), 7.10-7.82 (m, 4H, Ar-H), 7.91 (s, 1H, -N=CH), 8.56 (s, 1H, OH), 10.68 (s, 1H, NH), 11.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.5, 24.3, 29.4, 31.6, 55.6, 75.9, 114.9, 125.5, 130.6, 136.5, 142.8, 144.1, 157.0, 160.3, 163.5; HRMS m/z: 345.1254 [M+1].

2.1.3.7. *N′*-(4-Hydroxybenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (10)

Yield 90.56%, R_f^a = 0.35, R_f^b = 0.39, m.p. 166-168 °C, IR KBr (cm^{-1}): 1610, 1740, 3260, 3380, 3565, 3590; ^{1}H NMR (DMSO-d_6) δ ppm: 0.90 (s, 3H, CH_3), 1.35 (s, 6H, $(CH_3)_2$), 1.90 (m, 2H, CH_2), 2.87 (t, 2H, CH_2), 6.80-7.45 (m, 4H, Ar-H), 7.88 (s, 1H, -N=CH), 8.90 (s, 1H, OH), 9.20 (s, 1H, OH), 10.61 (s, 1H, NH), 11.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.7, 24.6, 29.5, 31.3, 76.8, 116.9, 126.5, 130.2, 136.1, 142.5, 144.6, 157.5, 160.0, 160.9; HRMS m/z: 331.2364 [M+1].

2.3.1.8.*N′*-(4-Hydroxy-3-methoxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (11)

Yield 82.15%, R_f^a = 0.41, R_f^b = 0.47, m.p. 158-160 °C, IR KBr (cm^{-1}): 1612, 1780, 3336, 3341, 3562, 3585; ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (s, 3H, CH_3), 1.31 (s, 6H, $(CH_3)_2$), 1.86 (m, 2H, CH_2), 2.81 (t, 2H, CH_2), 3.78 (s, 3H, OMe), 6.91-7.62 (m, 3H, Ar-H), 8.02 (s, 1H, -N=CH), 9.12 (s, 1H, OH), 9.45 (s, 1H, OH), 10.37 (s, 1H, NH), 11.16 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 14.1, 25.2, 30.3, 31.0, 56.2, 76.1, 112.1, 117.0, 122.6, 130.6, 136.1, 143.5, 144.3, 149.8, 151.6, 157.6, 160.1; HRMS m/z: 361.4562 [M+1].

2.3.1.9. *N′*-(2,4-Dichlorobenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (12)

Yield 87.28%, R_f^a = 0.43, R_f^b = 0.50, m.p. 175-176 °C, IR KBr (cm^{-1}): 1622, 1758, 3312, 3379, 3545; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.35 (s, 6H, $(CH_3)_2$), 1.80 (m, 2H, CH_2), 2.84 (t, 2H, CH_2), 7.20-7.82 (m, 3H, Ar-H), 8.10 (s, 1H, -N=CH), 9.52 (s, 1H, OH), 10.52 (s, 1H, NH), 11.13 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.3, 24.5, 30.6, 31.4, 76.3, 126.9, 128.2, 129.4, 129.9, 131.4, 132.0, 136.1, 140.5, 142.3, 157.6, 160.3; HRMS m/z: 384.1546 [M+1], 386.4569 [M+3].

2.3.1.10. *N′*-(2,4-Difluorobenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (13)

Yield 84.20%, R_f^a = 0.46, R_f^b = 0.52, m.p. 181-182 °C, IR KBr (cm^{-1}): 1630, 1755, 3324, 3318, 3569; ^{1}H NMR (DMSO-d_6) δ ppm: 0.85 (s, 3H, CH_3), 1.32 (s, 6H, $(CH_3)_2$), 1.83 (m, 2H, CH_2), 2.79 (t, 2H, CH_2), 6.92 (s, 1H, Ar-H), 7.13-7.70 (m, 2H, Ar-H), 7.84 (s, 1H, -N=CH), 8.99 (s, 1H, OH), 10.60 (s, 1H, NH), 11.17 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 14.3, 24.9, 29.5, 31.1, 76.1, 111.3, 112.9, 113.4, 132.1, 136.4, 142.8, 143.6, 157.2, 160.3, 161.4, 163.2; HRMS m/z: 351.4521 [M+1].

2.3.1.11. *N′*-(2,4-Dinitrobenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (14)

Yield 81.98%, R_f^a = 0.51, R_f^b = 0.57, m.p. 191-192 °C, IR KBr (cm^{-1}): 1622, 1768, 3375, 3398, 3514; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.29 (s, 6H, $(CH_3)_2$), 1.77 (m, 2H, CH_2), 2.86 (t, 2H, CH_2), 7.92 (s, 1H, N=CH), 8.20-840 (m, 2H, Ar-H), 8.84 (s, 1H, Ar-H), 9.99 (s, 1H, OH), 10.29 (s, 1H, NH), 11.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.9, 23.9, 29.3, 31.5, 76.4, 120.5, 130.4, 131.2, 133.1, 136.1, 142.4, 143.9, 148.2, 151.6, 157.2, 160.3; HRMS m/z: 405.3542 [M+1].

2.3.1.12. *N′*-(3,4-Dihydroxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (15)

Yield 86.77%, R_f^a = 0.32, R_f^b = 0.37, m.p. 166-167 °C, IR KBr (cm^{-1}): 1610, 1710, 3318, 3374, 3520, 3599; ^{1}H NMR (DMSO-d_6) δ ppm: 0.84 (s, 3H, CH_3), 1.22 (s, 6H, $(CH_3)_2$), 1.82 (m, 2H, CH_2), 2.78 (t, 2H, CH_2), 6.80-7.45 (m, 3H, Ar-H), 7.88 (s, 1H, -N=CH), 8.84 (s, 1H, 0H), 9.57 (s, 2H, OH), 10.56 (s, 1H, NH), 11.09 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.8, 23.7, 29.3, 31.0, 76.1, 116.2, 118.3, 123.5, 131.1, 136.3, 142.7, 143.1, 148.1, 151.0, 157.3, 160.7; HRMS m/z: 347.6524 [M+1].

2.3.1.13. *N′*-(3,4-Dimethoxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (16)

Yield 83.15%, R_f^a = 0.44, R_f^b = 0.51, m.p. 157-158 °C, IR KBr (cm^{-1}): 1612, 1770, 3317, 3384, 3566; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.20 (s, 6H, $(CH_3)_2$), 1.90 (m, 2H, CH_2), 2.90 (t, 2H, CH_2), 3.82 (s, 6H, 2OMe), 6.92-7.51 (m, 3H, Ar-H), 7.93 (s, 1H, -N=CH), 8.99 (s, 1H, OH), 10.12 (s, 1H, NH), 11.16 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.9, 23.8 29.4, 31.4, 56.4, 76.7, 109.3, 111.3, 122.6, 130.8, 136.8, 142.8, 144.2, 150.1, 152.4, 157.8, 160.4; HRMS m/z: 375.2654 [M+1].

2.3.1.14. *N′*-(3,5-Dibromo-4-hydroxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (17):

Yield 85.10%, R_f^a = 0.40, R_f^b = 0.44, m.p. 168-169 °C, IR KBr (cm^{-1}): 1608, 1778, 3330, 3398, 3547; ^{1}H NMR (DMSO-d_6) δ ppm: 0.93 (s, 3H, CH_3), 1.28 (s, 6H, $(CH_3)_2$), 1.94 (m, 2H, CH_2), 2.84 (t, 2H, CH_2), 7.60-7.72 (m, 2H, Ar-H), 7.88 (s, 1H, -N=CH), 8.78 (s, 1H, OH), 9.45 (s, 1H, OH), 10.19 (s, 1H, NH), 11.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.4, 23.6 29.5, 31.3, 76.3, 110.3, 129.3, 130.6, 136.8, 142.8, 145.8, 157.2, 158.1, 160.6; HRMS m/z: 489.2314 [M+1], 491.2654 [M+3].

2.3.1.15. *N′*-(3-Bromo-4-hydroxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (18)

Yield 87.10%, R_f^a = 0.46, R_f^b = 0.51, m.p. 174-175 °C, IR KBr (cm^{-1}): 1616, 1788, 3320, 3399, 3565; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.33 (s, 6H, $(CH_3)_2$), 1.87 (m, 2H, CH_2), 2.74 (t, 2H, CH_2), 6.90-7.77 (m, 3H, Ar-H), 7.97 (s, 1H, -N=CH), 8.88 (s, 1H, OH), 9.45 (s, 1H, OH), 10.82 (s, 1H, NH), 11.13 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 14.4, 24.1 29.8, 31.6, 76.4, 113.3, 118.6, 128.4, 129.4, 130.4, 136.7, 142.7, 145.4, 157.4, 158.4, 160.9; HRMS m/z: 410.2654 [M+1], 412.2654 [M+3].

2.3.1.16. *N′*-(3-Bromo-4-methoxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (19)

Yield 88.17%, R_f^a = 0.51, R_f^b = 0.57, m.p. 179-181 °C, IR KBr (cm^{-1}): 1607, 1729, 3314, 3347, 3558; ^{1}H NMR (DMSO-d_6) δ ppm: 0.91 (s, 3H, CH_3), 1.37 (s, 6H, $(CH_3)_2$), 1.88 (m, 2H, CH_2), 2.82 (t, 2H, CH_2), 3.81 (s, 3H, OMe), 6.92-7.71 (m, 3H, Ar-H), 7.89 (s, 1H, -N=CH), 9.10 (s, 1H, OH), 10.22 (s, 1H, NH), 11.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.5, 24.3 29.7, 31.3, 56.4, 76.5, 111.3, 112.6, 128.1, 129.0, 129.4, 136.0, 142.3,

144.1, 157.1, 158.3, 160.6; HRMS m/z: 424.1264 [M+1], 426.4597 [M+3].

2.3.1.17. *N′*-(3-Bromo-4-hydroxy-5-methoxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (20)

Yield 89.27%, R_f^a = 0.42, R_f^b = 0.47, m.p. 165-168 °C, IR KBr (cm^{-1}): 1611, 1735, 3322, 3354, 3560; ^{1}H NMR (DMSO-d_6) δ ppm: 0.87 (s, 3H, CH_3), 1.34 (s, 6H, $(CH_3)_2$), 1.84 (m, 2H, CH_2), 2.77 (t, 2H, CH_2), 3.77 (s, 3H, OMe), 7.32-7.42 (m, 2H, Ar-H), 7.87 (s, 1H, -N=CH), 9.10 (s, 1H, OH), 9.88 (s, 1H, OH), 10.21 (s, 1H, NH), 11.22 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.2, 24.1 29.6, 31.8, 56.2, 76.1, 111.0, 114.4, 122.1, 129.9, 136.7, 142.1, 143.4, 145.6, 153.6, 157.4, 159.9; HRMS m/z: 440.1654 [M+1], 442.1564 [M+3].

2.3.1.18. *N′*-(2-Chloro-6-fluorobenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (21)

Yield 86.38%, R_f^a = 0.52, R_f^b = 0.59, m.p. 170-171 °C, IR KBr (cm^{-1}): 1602, 1758, 3310, 3359, 3566; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.39 (s, 6H, $(CH_3)_2$), 1.89 (m, 2H, CH_2), 2.90 (t, 2H, CH_2), 7.22-7.49 (m, 3H, Ar-H), 7.90 (s, 1H, -N=CH), 9.28 (s, 1H, OH), 10.12 (s, 1H, NH), 11.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.8, 24.3 29.1, 31.0, 76.0, 113.8 118.4, 125.7, 134.5, 135.4, 136.9, 142.0, 143.7, 156.5, 160.5, 161.5; HRMS m/z: 367.4521 [M+1], 369.2451 [M+3].

2.3.1.19. 4-(2-Hydroxypropan-2-yl)-2-propyl-*N′*-(3,4,5-trimethoxybenzylidene)-1*H*-imidazole-5-carbohydrazide (22)

Yield 85.41%, R_f^a = 0.42, R_f^b = 0.48, m.p. 168-169 °C, IR KBr (cm^{-1}): 1610, 1766, 3352, 3369, 3588; ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (s, 3H, CH_3), 1.36 (s, 6H, $(CH_3)_2$), 1.84 (m, 2H, CH_2), 2.77 (t, 2H, CH_2), 3.82 (s, 9H, 3OMe), 7.12-7.18 (m, 2H, Ar-H), 7.99 (s, 1H, -N=CH), 9.38 (s, 1H, OH), 10.11 (s, 1H, NH), 11.56 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.2, 24.8 29.7, 31.6, 56.2, 60.6, 76.7, 104.2, 128.4, 136.4, 141.5, 142.4, 144.9, 153.5, 156.0, 160.4; HRMS m/z: 405.1265 [M+1].

2.3.1.20. 4-(2-Hydroxypropan-2-yl)-2-propyl-*N′*-(3,4,5-trihydroxybenzylidene)-1*H*-imidazole-5-carbohydrazide (23)

Yield 84.09%, R_f^a = 0.30, R_f^b = 0.34, m.p. 174-175 °C, IR KBr (cm^{-1}): 1615, 1719, 3349, 3359, 3562; ^{1}H NMR (DMSO-d_6) δ ppm: 0.84 (s, 3H, CH_3), 1.30 (s, 6H, $(CH_3)_2$), 1.80 (m, 2H, CH_2), 2.81 (t, 2H, CH_2), 5.01 (s, 1H, OH), 6.88-7.10 (m, 2H, Ar-H), 7.78 (s, 1H, -N=CH), 8.12 (s, 2H, 2OH), 9.38 (s, 1H, OH), 10.11 (s, 1H, NH), 11.56 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.1, 24.5 29.6, 31.0, 76.4, 108.2, 129.4, 136.1, 138.5, 141.7, 143.3, 146.1, 157.5, 160.1; HRMS m/z: 363.1265 [M+1]

2.4. Biology

2.4.1. *In vitro* Antiglycation assay [17]

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.

Bovine serum albumin (10 mg/mL) was incubated with glucose anhydrous (50 mg/mL) 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, while blank control contains 20 µL BSA and 40 µL sodium phosphate buffer. The mixture was incubated at 37°C for 7 days. After incubation, 6 µL (100%) of TCA was added into 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 advanced glycated end product-BSA were dissolved in 60 µL phosphate buffer solution (PBS). Evaluation of fluorescence spectrum (excitation 370 nm), and change in fluorescence intensity (excitation 370 nm to emission 440 nm), based on AGEs were monitored by using spectrofluorimeter (RF-1500, Shimadzu, Japan). % Inhibition was calculated using the formula:

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

Rutin was used as the standard antiglygation agent.

2.4.2. *In vitro* urease inhibition assay [18]

Reaction mixtures comprising 25 µl of jack bean urease enzyme (10mg/ml of 0.2M SPB) solution and 55 µL of buffers containing 100 µM urea were incubated with 5 µl of the test compounds (0.5-500 µM concentration) at 30°C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [20]. Briefly, 45 µl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µl of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min using a micro

plate reader (RF-1500, Shimadzu, Japan). All the reactions were performed in triplicate in a final volume of 200 µl. The entire assays were performed at pH 6.8. Percentage inhibition was calculated from the formula

$$\% \text{ Inhibition} = 1 - \left(\frac{\text{OD test well}}{\text{OD control}} \right) \text{ x } 100$$

Thiourea was used as the standard inhibitor of urease assay.

3. RESULTS AND DISCUSSION

3.1. Chemistry

Syntheses of the desired compounds were achieved according to the steps illustrated in

3.2. Scheme

4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carboxylic acid (**1**) were ethylated using trimethylsilylchloride (TMS-Cl) and ethanol at room temperature, which upon reaction with excess of hydrazine hydrate afforded the corresponding imidazole hydrazide (**3**). The Schiff's bases (**4-23**) were obtained by reacting **3** with different aromatic aldehydes in presence of catalytic amount of glacial acetic acid. All the derivatives were obtained in high yield and the methods employed are very simple. The structures of all the newly synthesized compounds including intermediates were confirmed by IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis. The formations of ethyl ester (**2**) were confirmed by the appearance of a triplet at 1.30-1.33δ for ethyl CH_3 and multiplet at 4.30-4.35δ for ethyl CH_2 and absence of COOH proton peak at 11.80δ in ^{1}HNMR spectrum. In IR spectra, bands at 2983 and 3117cm^{-1} for NH_2-NH groups indicates the conversion of ethyl esters into hydrazides. The formation of Schiff's bases were confirmed by the presence of absorption at 1604-1635 for imines i.e., -N=CH- in IR spectra. The presence of all requisite peaks and absence of extraneous peaks in ^{1}HNMR and ^{13}CNMR confirms the structures.

3.3. Biological activity

3.3.1. Antiglycation and Urease activity

We have synthesized imidazole-Schiff base (**4-23**) analogues and their subjected to antiglycation and urease inhibitory activities. The results obtained are presented in table 1 and the data represents average values from triplicate runs. Activity of the test compounds was compared with rutin and thiourea, which served as reference standards for antiglycation and urease inhibitory activities respectively. Most of the synthesized compounds showed potent antiglycation and urease inhibition activity. Compounds **9, 10, 11, 15, 16, 22** and **23** showed excellent antiglycation and urease activities with IC_{50} values are lower than the standards rutin and thiourea respectively. The reaming compounds **4-8**, **12-14**, **17**, **18**, **19-21** and **24** showed least antiglycation and urease inhibition activities with IC_{50} values are more than standards. Results revealed that compounds containing electron donating (OH and OCH_3) groups (**9, 10, 11, 15, 16, 22** and **23**) are more active than electron withdrawing (Cl, NO_2, F and Br) groups (**5-8**, **12-14**, **17**, **18**, **19-21** and **24).**

Table - 1: Antiglycation and urease inhibition of synthesized compounds

Entry	Antiglycation activity[a] (IC_{50} µM)	Urease inhibitory activity[a] (IC_{50} µM)
1	176±1.2	44±0.3
2	Inactive	Inactive
3	Inactive	Inactive
4	158±1.2	40±0.4
5	90±0.6	34±1.2
6	111±1.0	30±0.4
7	55±0.1	24±0.6
8	120±1.8	29±1.0
9	32±0.9	18±1.6
10	26±1.5	15±0.9
11	19±0.5	12±0.3
12	126±0.7	31±0.4
13	74±1.6	36±1.0
14	141±0.3	39±1.6
15	17±1.3	11±0.6
16	21±0.8	15±0.1
17	51±0.2	21±1.3
18	45±1.3	25±0.8
19	55±0.9	28±0.3
20	39±0.4	20±0.6
21	60±0.4	30±1.3
22	15±0.1	8±0.5
23	10±0.2	6±0.4
Rutin	41±0.45	-
Thiourea	-	21±0.53

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

The antiglycation and urease activities of Schiff bases of imidazoles C_6H_5CHO without any substituent on the phenyl ring were almost same

as that of imidazole moiety. The introduction of OH or OCH_3 on the phenyl ring drastically increases the antiglycation and urease activity and even the magnitude of increase is in accordance with the number of OH and OCH_3 groups. The Schiff bases with three OH groups or three OCH_3 groups or two OH or two OCH_3 groups on phenyl rings exhibited striking antiglycation and urease activities. The present investigation reveals that OH and OCH_3 were capable of increasing the activity even in the presence of electron withdrawing groups such as F, Cl, Br and NO_2. On the basis of present observation, it was very clear that the presence of OH or OCH_3 or both makes the molecules as potent antiglycation and urease activities. The compounds with electron withdrawing groups F, Cl, Br and NO_2 showed least antiglycation and urease activities. To study the structure activity relationship (SAR), compounds having OH (phenolic) and OCH_3 (anisole) groups in the phenyl ring (**9, 10, 11, 15, 16, 22** and **23)** were found to be the most potent antiglycation and urease activities. In phenyl ring the number of hydroxy and methoxy group increases the activities also increases. The activity order is OCH_3 > OH > 2 OCH_3 > 2 OH > 3 OCH_3 > 3 OH. On the other hand, it seems to be interesting to point out that among the halogen substituted derivatives (Cl, F, NO_2 and Br), compounds containing fluoro proved to be an activity enhancer which is in well agreement with earlier reports. [19,20] This may be due to the more electron withdrawing nature of the fluoro compared to other halogens.

4. CONCLUSION

In conclusion, we have designed and synthesized a series of small and simple imidazole derived Schiff base analogues with different groups in benzene ring. From the biological activities studies, compounds **9, 10, 11, 15, 16, 22** and **23** with OH and OCH_3 groups in benzene ring (electron donating) exhibited excellent antiglycation and urease inhibition activitiy. Compounds **5, 6, 7, 8, 12, 13, 14 and 21** with Cl, F, NO_2 and Br in benzene ring (electron withdrawing) showed least antiglycation and urease inhibition activitiy.

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