Synthesis, characterization and biological evaluation of some 5-methylpyrazine carbohydrazide based hydrazones

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Abstract: Pyrazine carbohydrazide based hydrazones were synthesized starting from 5-methylpyrazine-2-carboxylic acid. The acid was first converted to its methyl ester, which on further treatment with hydrazine hydrate transformed to carbohydrazide. The carbohydrazide was treated with differently substituted aromatic carbonyl compounds giving hydrazones. Characterization of the synthesized compounds was carried out using modern spectroscopic techniques and unambiguously confirmed through X-ray crystallographic studies of compound **3d**. The purity of the compounds was verified using elemental analysis. The target molecules were evaluated for urease inhibition, antioxidant and antimicrobial activity.

Keywords: 5-Methylpyrazine-2-carboxylic acid, hydrazones, urease inhibition, antioxidant, antimicrobial.

INTRODUCTION

Schiff bases display a wide range of biological activities including antibacterial (Abu-Hussen 2006; Karthikeyan *et al*., 2006; Singh *et al*., 2006), antifungal (Pannerselvam *et al*., 2005; Mladenova *et al*., 2002; Sridhar *et al*., 2001; Pandeya *et al*., 1999) and antitumor (Walsh *et al*., 1996; Arora and Sharma, 2003). They also play their role in coordination chemistry as ligands (Vigato and Tamburini, 2004; Katsuki, 1995; Ramesh and Sivagamasundari, 2003). Schiff base metal complexes facilitate a number of organic and inorganic transformations (Stork and Benaim, 1977). Schiff bases formed by the reaction of hydrazines with carbonyl compounds are called hydrazones and have the general structural formula $R^1R^2C=NNH_2$ (Day and Whiting, 1988; Hearn and Cynamon, 2004). Hydrazones possess very important biological activities due to the presence of the active pharmacophore (-CONH-N=C-). Isonicotinic hydrazide (INH), also known as isoniazid, is used as one of the primary standard drugs for the treatment of tuberculosis. Its Schiff base having the moiety (-CONH-N=C-) shows strong activity with low toxicity and excellent bioavailability (Jin, *et al*., 2006). Similarly, a number of other hydrazone compounds show anticancer activities (Mohareb, *et al*., 2010). Apart from their biological activities, hydrazones are very useful intermediates towards the synthesis of a variety of heterocycles (Mohareb and Samir, 2012; Sharshira and Hamada, 2012; Dalloul, *et al*., 2005; Benke, 1983; Ganjali, 2004). A 5% ruthenium (III) complex of a salen ligand has been reported as a very efficient and selective reagent for the determination of chloride in serum samples, apart from its use as an indicator (Miniyar and Makhija, 2009).

Keeping in view the importance of hydrazones, we herein report the synthesis, characterization and biological screening of some 5-methylpyrazine-2-carbohydrazide based hydrazones.

MATERIALS AND METHODS

General

Analytical grade solvents and reagents were used in the present study. Melting point apparatus (model MFB-595, Gallenkamp) was employed for the determination of melting temperatures in open capillaries. IRPrestige-21 spectrophotometer (Shimadzu) was used to record the IR spectral data of the compounds in the range 4000-400cm^{-1}. The NMR spectra (^{1}H and ^{13}C) were measured on Avance 300 MHz NMR Spectrometer (Bruker) in deuterated solvents using residual solvent signal as an indirect reference. EIMS was performed using JEOL JMS 600-H machine. Vario EL III CHNS-O Elemental Analyzer was used to perform elemental analyses for verification of purity of the synthesized compounds. The elemental analyses data is given in table 1.

Nutrient agar was used for the culturing and growth of the microorganisms (bacteria and fungi). Nutrient broth was used for inoculation and for shaking incubation and standardization of the microorganisms. Tazocine was used

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as the standard drug. The tested microorganisms included eight bacterial stains, *E. faecalis*, *E. Coli*, *P. mirabilis*, *Staph aureus*, *P. Aeruginosa*, *K. pneumoniae*, *B. cereus* and *C. freundii*, and two fungal strains, *A. niger* and *A. fulvus*. Urease inhibition activity of the target molecules was performed against jack bean urease using thiourea as the standard inhibitor. The free radical scavenging ability of the target molecules was evaluated employing DPPH method. Gallic acid was used as the standard.

Synthesis of methyl 5-methylpyrazine-2-carboxylate (1)

5-Methylpyrazine-2-carboxylic acid (8.0g, 56.0 mmol) was taken in excess CH_3OH, a few drops of conc. sulphuric acid (cat.) added and the mixture brought to reflux. After 5 hrs, the solvent was evaporated to dryness *in vacuo* (on the rotary) and the residue was transferred to 1L beaker followed by addition of water (400 mL). Solid $NaHCO_3$ was added slowly in portions till effervescence ceased and the pH was ~9. The extraction with $CHCl_3$ (3 × 400 mL) followed by evaporation of the solvent afforded the crude product. The purification through recrystallization (CH_3Cl in petroleum benzene) gave off-white crystals. Yield: 8.4g, 55.3 mmol, 95%; R_f: 0.51 (50% ethyl acetate in petroleum ether); soluble in chloroform, acetonitrile, methanol; mp: 88-89°C; FT-IR (cm^{-1}): 1716, 1367, 1294, 1257, 1151, 1031; ^{1}H NMR ($CDCl_3$): δ 2.60 (s, 3H, C<u>H</u>$_3$), 3.95 (s, 3H, OC<u>H</u>$_3$), 8.51 (s, 1H, H-6 pyrazine), 9.11 (s, 1H, H-3 pyrazine); ^{13}C NMR ($CDCl_3$): δ 21.91 (<u>C</u>H$_3$), 52.93 (O<u>C</u>H$_3$), 140.30 (C-2 pyrazine), 144.18 (C-6 pyrazine), 145.28 (C-3 pyrazine), 157.86 (C-5 pyrazine), 164.57 (<u>C</u>O); EIMS: *m/z* (%) 152 [M^+] (9), 122 (54), 94 (100), 66 (23), 53 (17).

Synthesis of 5-methylpyrazine-2-carbohydrazide (2)

5-Methylpyrazine-2-carbohydrazide **(2)** was synthesized by the reaction of methanolic solution of ester 1 (8.3g, 54.5 mmol) with 80% hydrazine hydrate (5.45g, 109 mmol) under reflux conditions (4 hrs). Methanol was distilled off on the rotary and the concentrated stuff was recrystallized from chloroform in petroleum ether giving creamy coloured crystals. Yield: 7.8g, 51.3 mmol, 94%; R_f: 0.40 (40% acetone in petroleum ether); soluble in chloroform, acetonitrile, methanol; mp: 131-132°C; FT-IR (cm^{-1}): 3302, 3205, 3036, 1647, 1616, 1510, 1471, 1286, 1116, 1033; ^{1}H NMR ($CDCl_3$): δ 2.66 (s, 3H, C<u>H</u>$_3$), 8.40 (s, 1H, H-6 pyrazine), 9.24 (s, 1H, H-3 pyrazine); ^{13}C NMR ($CDCl_3$): δ 21.94 (<u>C</u>H$_3$), 140.94 (C-2 pyrazine), 142.65 (C-6 pyrazine), 143.11 (C-3 pyrazine), 157.58 (C-5 pyrazine), 163.73 (<u>C</u>O); EIMS: *m/*z 152 [M^+] (48), 137 (7), 121 (31), 94 (100), 83 (5).

General method for the synthesis of hydrazones (3a-3h)

The hydrazide 2 (0.6g, 3.6 mmol) and the corresponding carbonyl compound (3.6 mmol) were dissolved in methanol (80 mL), refluxed for 5 hrs and allowed to stay overnight. The precipitates obtained were filtered, washed with cold methanol, recrystallized from chloroform in petroleum ether and dried under vacuum over anhydrous $CaCl_2$.

(E)-5-Methyl-N´-(1-phenylethylidene)pyrazine-2-carbohydrazide (3a)

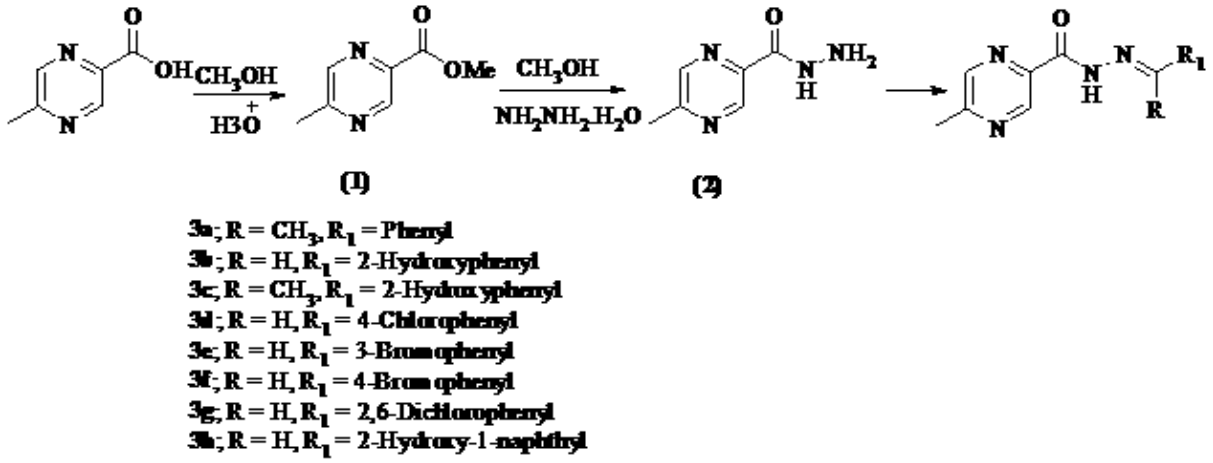
Off-white crystals; yield: 0.7g, 3.0 mmol, 80%; R_f: 0.41 (25% acetone in petroleum ether); solubility: soluble in chloroform, acetonitrile, dimethyl sulfoxide; mp: 203-204°C; FT-IR (cm^{-1}): 3336, 3043, 1693, 1604, 1581, 1373, 1026, 765; ^{1}H NMR ($CDCl_3$): δ 2.44 (s, 3H, C<u>H</u>$_3$), 2.71 (s, 3H, C<u>H</u>$_3$), 7.38-7.94 (m, 5H, benzene), 8.44 (s, 1H, H-6 pyrazine), 9.41 (s, 1H, H-3 pyrazine), 10.70 (s, 1H, N<u>H</u>); ^{13}C NMR (DMSO): 13.88 (<u>C</u>H$_3$), 21.48 (<u>C</u>H$_3$), 126.52 (C-3, 5 benzene), 128.43 (C-2,6 benzene), 129.76 (C-4 benzene), 137.66 (C-1 benzene), 141.80 (C-2 pyrazine), 142.78 (C-6 pyrazine), 142.93 (C-3 pyrazine), 155.79 (C-5 pyrazine), 157.47 (<u>C</u>H), 159.14 (<u>C</u>O); EIMS: *m/z* (%) 254 [M^+] (28), 239 (42), 161 (39), 133 (85), 121 (19), 103 (54), 94 (100), 77 (44), 43 (76).

(E)-N'-(2-Hydroxybenzylidene)-5-methylpyrazine-2-carbohydrazide (3b)

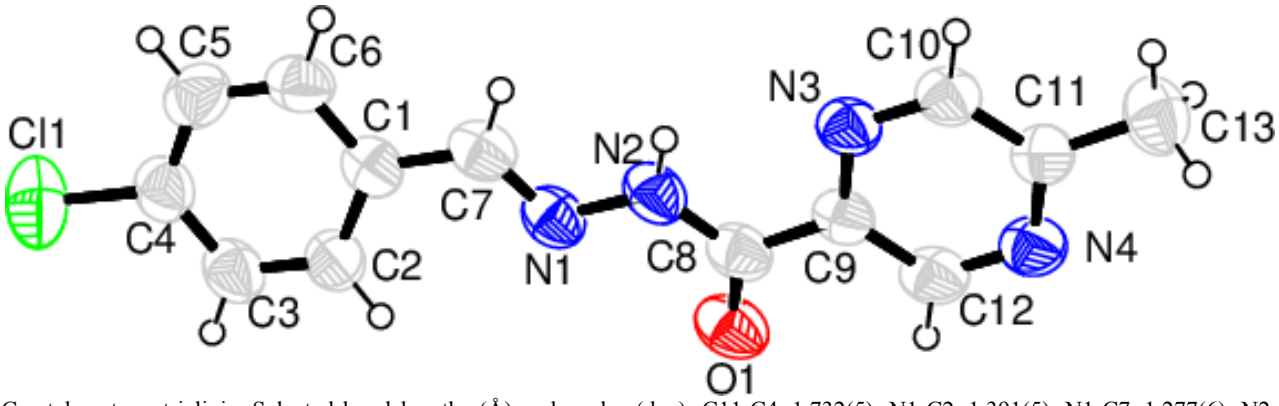
Creamy coloured crystals; yield: 0.8g, 3.0 mmol, 83%; R_f: 0.40 (30% acetone in petroleum ether); solubility: soluble in chloroform, acetonitrile, acetone; mp: 207-208°C (217-218°C) (Carron, *et al.*, 1987); FT-IR (cm^{-1}): 3242, 3059, 1674, 1614, 1512, 1481, 1269, 879, 748; ^{1}H NMR ($CDCl_3$): δ 2.71 (s, 3H, C<u>H</u>$_3$), 6.91-7.38 (m, 4H, benzene), 8.43 (s, 1H, C<u>H</u>), 8.52 (s, 1H, H-6 pyrazine), 9.35 (s, 1H, H-3 pyrazine), 10.62 (s, 1H, N<u>H</u>), 11.01 (s, 1H, O<u>H</u>); ^{13}C NMR ($CDCl_3$): δ 22.06 (<u>C</u>H$_3$), 117.24 (C-3 benzene), 117.36 (C-1 benzene), 119.45 (C-5 benzene), 131.10 (C-6 benzene), 132.25 (C-4 benzene), 140.62 (C-2 pyrazine), 142.37 (<u>C</u>H), 143.84 (C-6 pyrazine), 151.70 (C-3 pyrazine), 158,17 (C-5 pyrazine), 158.70 (C-2 phenyl), 159.42 (<u>C</u>O); EIMS: *m/z* (%) 256 [M^+] (33), 163 (3), 138 (44), 119 (39), 94 (100), 43 (42).

(E)-N'-(1-(2-Hydroxyphenyl)ethylidene)-5-methylpyrazine-2-carbohydrazide (3c)

Off-white crystals; yield: 0.6g, 2.4 mmol, 65%; R_f: 0.51 (10% methanol in chloroform); solubility: soluble in chloroform, acetone, dimethyl sulfoxide; mp: 223-224°C; FT-IR (cm^{-1}): 3358, 1693, 1608, 1583, 1492, 1475, 1238, 752; ^{1}H NMR ($CDCl_3$): *δ* 2.46 (s, 3H, C<u>H</u>$_3$), 2.70 (s, 3H, C<u>H</u>$_3$), 6.85-7.49 (m, 4H, benzene), 8.42 (s, 1H, H-6 pyrazine), 9.31 (s, 1H, H-3 pyrazine), 10.63 (s, 1H, N<u>H</u>), 12.67 (s, 1H, O<u>H</u>); ^{13}C NMR ($CDCl_3$): *δ* 12.55 (<u>C</u>H$_3$), 22.07 (<u>C</u>H$_3$), 118.27 (C-3 benzene), 118.69 (C-1 benzene), 118.83 (C-5 benzene), 127.80 (C-6 benzene), 131.76 (C-4 benzene), 140.69 (C-2 pyrazine), 142.42 (C-6 pyrazine), 143.80 (C-3 pyrazine), 156.49 (C-5 pyrazine), 158.12 (N=<u>C</u>), 158.45 (C-2 benzene), 159.44 (<u>C</u>O). EIMS: *m/z* (%) 270 [M^+] (83), 253 (100), 177 (23), 149 (31), 121 (50), 94 (85).



Scheme 1: Synthesis of hydrazones (3a-h)



Crystal system: triclinic. Selected bond lengths (Å) and angles (deg): C11-C4=1.732(5), N1-C2=1.391(5), N1-C7=1.277(6), N2-C8=1.361(6), N3-C9=1.342(5), O1-C8=1.226(5), C1-C7=1.466(6), C8-C9=1.484(7), C11-C13=1.504(6); N2-N1-C7=114.1(4), N1-N2-C8=121.1(4), N2-C1-C7=124.0(5), C3-C4-C11=120.7(5), N1-C7-C1=120.7(5), N2-C8-O1=123.6(5), N2-C8-C9=114.2(5), O1-C8-C9=122.2(5), N4-C11-C13=118.4(4).

Fig. 1: Crystal structure of (*E*)-*N*'-(4-chlorobenzylidene)-5-methylpyrazine-2-carbohydrazide (3d).

(E)-N'-(4-Chlorobenzylidene)-5-methylpyrazine-2-carbohydrazide (3d)

Creamy coloured crystals; yield: 0.8g, 2.8 mmol, 79%; R_f: 0.55 (30% acetone in petroleum ether); solubility: soluble in methanol, dimethyl sulfoxide; mp: 244-245°C (90-91°C) (Carron, *et al.*, 1987); FT-IR (cm^{-1}): 3296, 3014, 1678, 1579, 1521, 823, 779; ^{1}H NMR ($CDCl_3$): δ 2.71 (s, 3H, C<u>H</u>$_3$), 7.42 (d, 2H, *J*=8.4 Hz, H-2,6 benzene), 7.78 (d, 2H, *J*=8.4 Hz, H-3,5 benzene), 8.32 (s, 1H, C<u>H</u>), 8.43 (s, 1H, H-6 pyrazine), 9.39 (s, 1H, H-3 pyrazine), 10.70 (s, 1H, NH); ^{13}C NMR (DMSO): δ 21.45 (<u>C</u>H$_3$), 128.83 (2C, C-3,5 benzene), 128.98 (2C, C-2,6 benzene), 133.14 (C-1 benzene), 134.71 (C-4 benzene), 141.76 (C-2 pyrazine), 143.12 (<u>C</u>H), 148.18 (C-6 pyrazine), 157.40 (C-5 pyrazine), 159.69 (<u>C</u>O); EIMS: *m/z* (%) 74 [M^+] (3), 181 (2), 137 (60), 121 (12), 94 (100), 44 (9).

(E)-N'-(3-Bromobenzylidene)-5-methylpyrazine-2-carbohydrazide (3e)

Off-white crystals; yield: 0.6g, 2.0 mmol, 56%; R_f: 0.46 (30% acetone in petroleum ether); solubility: soluble in chloroform, acetone, dimethyl sulfoxide; mp: 215-216°C; FT-IR (cm^{-1}): 3257, 3043, 1674, 1606, 1579, 1533, 1479, 1328, 1263, 1153, 1056, 1031, 781, 680; ^{1}H NMR ($CDCl_3$) δ 2.71 (s, 3H, C<u>H</u>$_3$), 7.28-8.02 (m, 4H benzene), 8.30 (s, 1H, C<u>H</u>), 8.44 (s, 1H, H-6 pyrazine), 9.39 (s, 1H, H-3 pyrazine), 10.73 (s, 1H, N<u>H</u>); ^{13}C NMR ($CDCl_3$): δ 22.05 (<u>C</u>H$_3$), 123.00 (C-3 benzene), 126.60 (C-6 benzene), 130.26 (C-5 benzene), 130.33 (C-2 benzene), 133.61 (C-4 benzene), 135.47 (C-1 benzene), 140.86 (C-2 pyrazine), 142.29 (<u>C</u>H), 143.97 (C-6 pyrazine), 147.61 (C-3 pyrazine), 158.05 (C-5 pyrazine), 159.21 (<u>C</u>O); EIMS: *m/z* (%) 318 [M^+] (7), 197 (9), 183 (14), 169 (5), 157 (7), 137 (99), 121 (41), 94 (100),41 (6).

(E)-N'-(4-Bromobenzylidene)-5-methylpyrazine-2-carbohydrazide (3f)

Off-white crystals; yield: 0.9g, 2.9 mmol, 79%; R_f: 0.40 (30% acetone in petroleum ether); solubility: soluble in

Table 1: Elemental analyses data of the synthesized compounds

Comp.	Molecular Formula	Molecular Weight	Calculated (%)			Found (%)		
			C	H	N	C	H	N
1	$C_7H_8N_2O_2$	152.15	55.26	5.30	18.41	55.57	4.98	18.64
2[a]	$C_6H_8N_4O$	152.15	47.36	5.30	36.82	47.52	5.68	36.62
3a	$C_{14}H_{14}N_4O$	254.29	66.13	5.55	22.03	65.93	5.31	21.89
3b	$C_{13}H_{12}N_4O_2$	256.26	60.93	4.72	21.86	60.98	4.54	21.41
3c	$C_{14}H_{14}N_4O_2$	270.29	62.21	5.22	20.73	61.98	5.37	20.25
3d	$C_{13}H_{11}ClN_4O$	274.71	56.84	4.04	20.40	56.91	3.87	20.50
3e	$C_{13}H_{11}BrN_4O$	318.16	48.92	3.47	17.55	48.66	3.18	17.25
3f	$C_{13}H_{11}BrN_4O$	319.16	48.92	3.47	17.55	48.95	3.53	17.58
3g	$C_{13}H_{10}C_{l2}N_4O$	309.15	50.51	3.26	18.12	50.47	3.14	18.37
3h	$C_{17}H_{14}N_4O_2$	306.32	66.66	4.61	18.29	66.43	4.44	18.10

[a]Carron, *et al.*, 1987.

Table 2: Urease inhibition activities of the compounds synthesized

Compound	Conc. (mM)	% Inhibition	$IC_{50} \pm$ SEM (μM)
1	0.5	27.8	-
2	0.5	70.2	232.6 ± 2.7
3a	0.5	39.3	-
3b	0.5	34.3	-
3c	0.5	3.1	-
3d	0.5	34.0	-
3e	0.5	34.4	-
3f	0.5	18.1	-
3g	0.5	34.4	-
3h	0.5	37.8	-
Thiourea	0.5	96.9	21.8 ± 1.6

Table 3: Antimicrobial activities of the synthesized compounds

Microbe	Zone of inhibition (mm)										Standard (Tazocine)
	1	2	3a	3b	3c	3d	3e	3f	3g	3h	
E. Faecalis	22	14	-	07	03	04	06	03	06	09	28
E. Coli	07	-	-	11	05	-	-	-	-	21	32
P. Mirabilis	03	-	-	-	-	-	03	-	03	-	30
S. Aureus	-	06	-	08	-	-	-	04	-	06	31
P. Aeruginosa	02	02	-	-	-	-	03	-	03	-	22
K. Pneumoniae	04	02	-	-	-	05	-	-	-	05	20
B. Cereus	-	04	-	-	03	-	-	-	-	07	18
C. Freundii	06	03	0	04	-	03	-	-	-	-	20
A. Niger	04	05	-	-	-	-	02	-	02	04	18
A. Flavus	03	-	-	05	-	-	-	-	-	03	14

chloroform, acetone, dimethyl sulfoxide; mp: 253-254°C; FT-IR (cm^{-1}): 3294, 3093, 1678, 1612, 1589, 1519, 1481, 1375, 1151, 1062, 1031, 1010, 821, 603; ^{1}H NMR (CDCl3): δ 2.72 (s, 3H, C<u>H</u>$_3$), 7.58 (d, 2H, *J*=8.7 Hz, H-2,6 benzene), 7.71 (d, 2H, *J*=8.7 Hz, H-3,5 benzene), 8.31 (s, 1H, C<u>H</u>), 8.44 (s, 1H, H-6 pyrazine), 9.40 (s, 1H, H-3 pyrazine), 10.70 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): 22.04 (<u>C</u>H$_3$), 125.20 (C-4 benzene), 129.24 (2C, C-2,6 benzene), 132.04 (2C, C-3,5 benzene), 132.36 (C-1 benzene), 142.27 (C-2 pyrazine), 143.95 (N=<u>C</u>H), 148.07 (C-6 pyrazine), 142.29 (<u>C</u>H), 147.61 (C-3 pyrazine), 158.01 (C-5 pyrazine), 159.15 (<u>C</u>O); EIMS: *m/z* (%) 319 [M^+] (2), 183 ((9), 137 (28), 94 (100), 44 (31).

(E)-N'-(2,6-Dichlorobenzylidene)-5-methylpyrazine-2-carbohydrazide (3g)

Off-white crystals; yield: 0.7g, 2.3 mmol, 64%; R_f: 0.48 (30% acetone in petroleum ether); solubility: soluble in chloroform, acetonitrile, dimethyl sulfoxide; mp: 223-224°C; FT-IR (cm^{-1}): 3219, 3053, 1662, 1592, 1529, 1427,

Table 4: Antioxidant activities of the synthesized compounds

Compound	Conc. (mM)	IC_{50}+/- SEM (μM)	% RSA (Radical Scavenging Activity)
1	0.5	-	5.029
2	0.5	336.67 +/- 2.83	52.379
3a	0.5	-	-
3b	0.5	-	3.9
3c	0.5	-	1.3
3d	0.5	-	-
3e	0.5	-	3.14
3f	0.5	-	-
3g	0.5	-	-
3h	0.5	-	17.279
Gallic acid	0.5	23.436 +/- 0.43	93.13

1271, 1060, 1035, 777, 524, 518, 432; ^{1}H NMR (DMSO): δ 2.63 (s, 3H, C<u>H</u>$_3$), 7.42-7.58 (m, 3H, benzene), 8.68 (s, 1H, C<u>H</u>), 8.84 (s, 1H, H-6 pyrazine), 9.12 (s, 1H, H-3 pyrazine), 12.55 (s, 1H, N<u>H</u>); ^{13}C NMR (DMSO): δ 21.45 (<u>C</u>H$_3$), 129.03 (2C, C-3, 5 benzene), 130.66 (C-1 benzene), 131.30 (C-4 benzene), 133.91 (2C, C-2,6 benzene), 141.67 (C-2 pyrazine), 142.82 (<u>C</u>H), 143.26 (C-6 pyrazine), 144.91 (C-3 pyrazine), 157.48 (C-5 pyrazine), 159.94 (<u>C</u>O); EIMS: *m/z* (%) 309 (5), 272 (46), 171 (9), 137 (99), 121 (57), 109 (7), 94 (100), 41 (8).

(E)-N'-((2-Hydroxynaphthalen-1-yl) methylene)-5-methylpyrazine-2-carbohydrazide (3h)

Yellow crystals; yield: 1.0g, 3.2 mmol, 88%; R_f: 0.44 (30% acetone in petroleum ether); solubility: soluble in chloroform, acetonitrile, dimethyl sulfoxide; mp: 223-224°C; FT-IR (cm^{-1}): 3304, 3259, 3201, 3051, 1676, 1622, 1585, 1529, 1465, 1390, 1311, 1242, 1178, 1031; ^{1}H NMR ($CDCl_3$): δ 2.71 (s, 3H, C<u>H</u>$_3$), 7.23-8.05 (m, 6H naphthalene), 8.45 (s, 1H, C<u>H</u>), 9.36 (s, 1H, H-6 pyrazine), 9.41 (s, 1H, H-3 pyrazine), 10.74 (s, 1H, N<u>H</u>), 12.36 (s, 1H, O<u>H</u>); ^{13}C NMR ($CDCl_3$): δ 22.07 (<u>C</u>H$_3$), 107.76 (C-1 naphthalene), 119.46 (C-3 naphthalene), 119.76 (C-6 naphthalene), 123.62 (C-8 naphthalene), 127.66 (C-7 naphthalene), 128.13 (C-5 naphthalene), 129.23 (C-9 naphthalene), 132.03 (C-4 naphthalene), 133.59 (C-10 naphthalene), 140.66 (C-2 pyrazine), 142.39 (<u>C</u>H), 143.80 (C-6 pyrazine), 148.16 (C-3 pyrazine), 158.12 (C-5 pyrazine), 158.59 (C-2 naphthalene), 159.40 (CO); EIMS: *m/z* (%) 306 [M$^+$] (15), 289 (3), 169 (100), 141 (17), 94 (31).

RESULTS

Hydrazones (3a-h) were synthesized starting from 5-methylpyrazine carboxylic acid. The 5-methylpyrazine carboxylic acid was converted to the methyl ester by treating it with catalytic sulphuric acid in methanol. The ester resulted in hydrazide on reacting with hydrazine hydrate, which in turn resulted in the target hydrazones while reacting with different aromatic carbonyl compounds. Elemental analysis (table 1) and other spectroscopic data (IR, MS, NMR) confirmed the successful synthesis of all these compounds.

The synthesized compounds were evaluated for urease inhibition, antimicrobial and antioxidant activities. The results are tabulated in table 2, 3 and 4, respectively. Thiourea, tazocine and gallic acid were used as standards in these activities. In urease inhibition activity, the hydrazide 2 was found to be the most active with an inhibition activity of 70.2% and an IC_{50} value of 232.6 ± 2.7 μM. In antimicrobial activities, all the synthesized compounds showed non-significant activities. The antioxidant activities revealed hydrazide **2** as the most active antioxidant compound among the series but at a highly toxic level of concentration (336.67± 2.83 μM).

DISCUSSION

The 5-methylpyrazine carbohydrazide based hydrazones under investigation were synthesized following scheme 1.

The 5-methylpyrazine carboxylic acid was treated with CH_3OH in the presence sulphuric acid (catalytic) giving its methyl ester (1). In the IR spectrum, the appearance of the peaks for C-O stretching vibrations at 1294 and 1031 cm^{-1} indicated the formation of the ester. In ^{1}H NMR spectrum, the appearance of the signal for three methyl hydrogens of the ester group at 3.95 ppm confirmed the successful synthesis. The ester methyl carbon resonated at 52.93 ppm in ^{13}C NMR spectrum. In EIMS, the molecular ion peak was observed at *m/z* 152 whereas base peak was present at *m/z* 94. Elemental analysis showed that the calculated and observed values were in good agreement.

The ester on treatment with hydrazine hydrate gave 5-methylpyrazine-2-carbohydrazide (2). The IR spectrum indicated the formation of hydrazide 2 by the presence of N-H stretchings at 3302 and 3224 cm^{-1}. The carbonyl peak shifted to a lower frequency of 1647 cm^{-1} in the product as compared to the ester carbonyl in the starting

material (1716 cm^{-1}), also indicating the successful conversion of ester to hydrazide. The structure of hydrazide was further confirmed by EIMS studies where the molecular ion peak was found at *m/z* 152. The elemental analysis data proved the purity of the product.

Hydrazones (3a-h) were synthesized by condensation of hydrazide (2) with equimolar amounts of different aromatic carbonyl compounds in methanol under reflux. The IR spectra indicated the formation of hydrazones by the appearance of new peaks for the C=N bond in the range of 1579 to 1622 cm^{-1}. The N-H absorption appeared as a single peak in the region 3219-3358cm^{-1}. The carbonyl peak in most of the hydrazones shifted to a higher frequency (1662-1697cm^{-1}) as compared to the hydrazide. Aromatic signals appeared in the range 1450-1600cm^{-1}. Hydrazones with halogens (chloro and bromo groups) exhibited C-X peaks in the region 1030 to 1100 cm^{-1}. In ^{1}H NMR spectra, the appearance of the only N-H proton in the range of 10.62 to 12.55 ppm confirmed the successful synthesis. A singlet due to *H*C=N proton of the products 3b and 3d-h from 8.30 to 8.68 ppm and a singlet for three methyl protons in the fragment H_3CC=N in products 3a and 3c at 2.44 and 2.46ppm, respectively, also confirmed the synthesis. The aromatic protons of the phenyl and naphthyl rings were observed from 6.85 to 8.05 ppm while they resonated from 8.42 to 9.41 ppm for the pyrazine ring in these hydrazones. The -OH proton in compounds 3b, 3c and 3h resonated as a deshielded broad singlet at 11.01, 12.67 and 12.36 ppm, respectively. In ^{13}C NMR spectra, the specific peak for C=N moiety in all the hydrazones gave very useful proof for the confirmation of successful synthesis. The peak was observed in the range 142.29 to 158.12ppm. The additional peaks in the aromatic region of the ^{13}C NMR spectra from 107.76 to 137.66 ppm also confirmed the successful synthesis of these compounds. The carbonyl carbon resonated in the narrow range of 159.14 to 160.46 ppm. EIMS aided further in confirmation of these hydrazones. The molecular ion peak was observed for all the hydrazones apart from their specific fragmentation pattern, thus confirming their successful synthesis. The synthesis was unambiguously confirmed by the single crystal X-ray studies of (*E*)-*N*'-(4-chlorobenzylidene)-5-methylpyrazine-2-carbohydrazide (3d) (fig. 1).

Urease inhibition activity of the target molecules was performed following a reported procedure (Khan, *et al*., 2010; Akhtar, *et al*., 2010) using thiourea as the standard with an IC_{50} value of 21.8±1.6μM and an inhibition activity of 96.9% (table 2). The synthesized compounds showed non-significant urease inhibition activities. IC_{50} value was calculated for those compounds showing activity greater than 50%. The hydrazide 2 was found to be the most active with an inhibition activity of 70.2% and an IC_{50} value of 232.6±2.7μM. All the target molecules were also evaluated against different bacterial and fungal strains using agar well diffusion method (table 3). Tazocine was used as a standard. All the compounds showed non-significant activities. The free radical scavenging (antioxidant) activity of all the synthesized compounds was also evaluated using a reported procedure (Lee, *et al*., 1998). Gallic acid was used as the standard with an IC_{50} value of 23.44±0.43 μM and a radical scavenging activity of 93.13% (table 4). The most active compound was found to be the hydrazide 2 (52.38%) again but at a highly toxic level of concentration (336.67 ±2.83μM).

CONCLUSIONS

A series of pyrazine carbohydrazide based hydrazones was synthesized while starting from 5-methylpyrazine-2-carboxylic acid. The structures of the hydrazones were confirmed by spectroanalytical techniques and unambiguously confirmed by single crystal X-ray analysis of compound 3d. Urease inhibition, antioxidant and antimicrobial activities of the synthesized compounds were evaluated. Hydrazide **(2)** was found more active than the hydrazones in urease inhibition and antioxidant activities but at a highly toxic level of concentration. All the hydrazones showed non-significant urease inhibition and antioxidant activities. Most of the compounds were also found inactive against different microbial strains.

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