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Synthesis, Characterization and Urease Inhibiting Derivatives of 5-(3,4-Methylenedioxyphenyl)-1,3,4-Oxadiazol-2-thiol

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In the present work, the urease inhibition activity of 1,3,4-oxadiazole bearing molecules was evaluated and were found to be potential inhibitors. 3,4-(Methylenedioxy)benzoic acid (**1**) was employed to synthesize 5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazol-2-thiol (**4**) *via* a series of steps. It was further stepped to yield S-substituted-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole derivatives (**6a-h**) on reaction with alkyl/aralkyl halides (**5a-h**) in DMF using LiH as an activator. All the synthesized compounds were well supported by IR, ^{1}H NMR and EIMS spectral analysis. The enzyme inhibition activity against urease enzyme showed these molecules as potent inhibitors of this enzyme.

Keywords: 3,4-(Methylenedioxy)benzoic acid, 1,3,4-Oxadiazole, Urease, ^{1}H NMR and EIMS.

INTRODUCTION

Because of bacterial drug resistance, the scientists are attempting to inaugurate new potent compounds which may be employed for the cure of various diseases[1]. Most of the researches are working on heterocyclic compounds in this regard[2]. Oxadiazole nucleus has attributed much attention because to their pharmacological importance especially 1,3,4-oxadiazoles[3-6]. Benzodioxole moiety bears a crucial importance among the potential drugs. Many anticancer, antidepressant *etc*. drugs possess this moiety in their structures[7-8].

Urease (EC 3.5.1.5) is urea amidohydrolase and has an important role in pathogenic processes taking place in humans and animals. It has the main part in the cause of urolithiasis, pyelonephritis, urinary catheter incrustation, peptic ulceration, kidney stone and hepatic encephalopathy[9-11].

The need of the hour is to introduce potent molecules that may be used as remedy for various complaints. Relating to our research projects[12-13], the S-substitution of 5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazol-2-thiol bearing benzodioxole moiety was carried out with an aim of preparing new potent urease inhibitors with low toxicity.

EXPERIMENTAL

Chemicals were labeled by Alfa Aesar and Sigma-Aldrich with extra purification and analytical grade solvents were purchased from local suppliers. Melting points were computed on a Griffin and George apparatus employing open capillary tube and were uncorrected. Purity of the synthesized molecules was assessed by thin layer chromatography (TLC) using pre-coated silica gel G-25-UV$_{254}$ plates with solvent system (*n*-C_6H_{14}:$CH_3COOC_2H_5$) with single spot under UV at 254 nm. The I.R. spectra were figured on a Jasco-320-A spectrophotometer with KBr pellet and the unit used for ($\bar{\nu}$) is cm^{-1}. ^{1}H NMR spectra were figured on a Bruker spectrometers operating at 300/400 MHz in $CDCl_3$. Chemical shifts are given in ppm with TMS as reference standard. Mass spectra (EIMS) were figured on a JMS-HX-110 spectrometer, with a data system.

Synthesis of ethyl 3,4-(methylene-dioxy)benzoate (2): 3,4-(Methylenedioxy)benzoic acid (**1**, 5 g) was homogenized in 99 % ethanol (20 mL) in a 250 mL round bottom flask. 2.5 mL conc. H_2SO_4 was added and the mixture was refluxed for 3 h. TLC was developed for confirmation of reaction completion. After completion, the ester was extracted by solvent

extraction using 50 mL $CHCl_3$ from a separating funnel after the addition of 150 mL distilled water. Before the separation of organic layer, the aq. Na_2CO_3 solution was poured to neutralize the mixture up to pH of 9-10. This step converted untreated organic and left sulphuric acid into salts washed away by aqueous layer. Chloroform was distilled off to collect yellowish transparent ester **2**. Yellowish transparent liquid; Yield: 90 %; m.f.: $C_{10}H_{10}O_4$; Molecular mass: 194; IR (KBr, ν_{max}, cm^{-1}): 3006 (aromatic C-H stretching), 1710 (C=O ester stretching), 1605 (Ar C=C stretching), 1110 (C-O ester stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.64 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6'), 7.44 (d, *J* = 1.2 Hz, 1H, H-2'), 6.82 (d, *J* = 8.0 Hz, 1H, H-5'), 6.01 (s, 2H, H-7'), 4.32 (q, *J* = 7.2 Hz, 2H, -OCH_2CH_3), 1.37 (t, *J* = 7.2 Hz, 3H, -OCH_2CH_3); EIMS (*m/z*): 194 $[M]^{\bullet+}$, 165 $[C_8H_5O_4]^+$, 149 $[C_8H_5O_3]^+$, 121 $[C_7H_5O_2]^+$, 29 $[C_2H_5]^+$.

Synthesis of 3,4-(methylenedioxy)-benzohydrazide (3): The molecule **2** (0.1 mol) was diluted in 250 mL round bottom flask with 100 mL ethanol and followed by addition of 10 mL 80 % $N_2H_4.H_2O$. Refluxing was continued for 6 h. After monitoring by TLC, ice cold distilled H_2O was poured to acquire the precipitates (**3**) which were filtered and washed off with distilled H_2O. White amorphous solid; Yield: 97 %; m.p: 170 °C; m.f.: $C_8H_8N_2O_3$; Molecular mass: 180; IR (KBr, ν_{max}, cm^{-1}): 3250 (NH stretching), 3010 (aromatic C-H stretching), 1730 (C=O amide stretching), 1610 (Ar C=C stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.66 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6'), 7.45 (d, *J* = 1.2 Hz, 1H, H-2'), 6.87 (d, *J* = 8.0 Hz, 1H, H-5'), 6.06 (s, 2H, H-7'); EIMS (*m/z*): 180 $[M]^{\bullet+}$, 164 $[C_8H_6NO_3]^+$, 149 $[C_8H_5O_3]^+$, 121 $[C_7H_5O_2]^+$.

Synthesis of 5-(3,4-methylene-dioxyphenyl)-1,3,4-oxadiazol-2-thiol (4): The product **3** (0.1 mol) was shaken well with 100 mL absolute ethanol in 250 mL round bottom flask and set to reflux after the addition of CS_2 (0.3 mol) and KOH (0.2 mol) for 5 h. After the final TLC, 300 mL distilled water was added along with dil. HCl to make pH of 3-4 to acquire the precipitates of **4**. The addition of acid is crucial to change back the salt form of 5-(3,4-methylene-dioxyphenyl)-1,3,4-oxadiazol-2-thiol into acidic one but limited amount because excess reduces the amount of product. The precipitates were filtered, washed with dist. H_2O and re-crystallized from CH_3OH. White amorphous solid; Yield: 83 %; m.p: 238 °C; m.f.: $C_9H_6N_2O_3S$; Molecular mass: 222; IR (KBr, ν_{max}, cm^{-1}): 3011 (aromatic C-H stretching), 1613 (Ar C=C stretching), 1575 (C=N stretching), 1109 (C-O-C stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.52 (dd, *J* = 8.4, 1.2 Hz, 1H, H-6'), 7.44 (s, 1H, H-2'), 6.89 (d, *J* = 8.4 Hz, 1H, H-5'), 6.06 (s, 2H, H-7'); EIMS (*m/z*): 236 $[M]^{\bullet+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\bullet+}$, 147 $[C_8H_5NO_2]^{\bullet+}$, 121 $[C_7H_5O_2]^+$.

General procedure for the synthesis of S-substituted derivatives (6a-h): The compound **4** (0.2 g, 0.009 mol) was homogenized in 15 mL DMF in a 100 mL round bottom flask and then LiH (0.004 g) was added along with stirring. The electrophiles *i.e*. alkyl/aralkyl halides (**5a-h**, 0.009 mol) were poured into homogeneous solution after 0.25 h and further stirred for 4-6 h. After single spot by TLC, ice cold dist. water was introduced into reaction contents along with aq. Na_2CO_3 up to pH of 9-10 and the precipitates (**6a-h**) were acquired. The mixture is basified to get rid of the untreated **4**. Precipitates were filtered, washed with distilled H_2O and dried.

2-(2-Bromoethyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6a): Creamy white amorphous solid; Yield: 90 %; m.p: 122 °C; m.f.: $C_{11}H_9N_2O_3SBr$; Molecular mass: 329; IR (KBr, ν_{max}, cm^{-1}): 3014 (aromatic C-H stretching), 1616 (Ar C=C stretching), 1579 (C=N stretching), 1107 (C-O-C stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.53 (dd, *J* = 8.0, 1.2 Hz, 1H, H-6'), 7.43 (d, *J* = 1.2 Hz, 1H, H-2'), 6.89 (d, *J* = 8.0 Hz, 1H, H-5'), 6.04 (s, 2H, H-7'), 3.76 (t, *J* = 7.2 Hz, 2H, H-2''), 3.68 (t, *J* = 7.2 Hz, 2H, H-1''); EIMS (*m/z*): 331 $[M + 2]^{\bullet+}$, 329 $[M]^{\bullet+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\bullet+}$, 147 $[C_8H_5NO_2]^{\bullet+}$, 121 $[C_7H_5O_2]^+$, 108 $[C_2H_4Br]^+$, 28 $[C_2H_4]^+$.

2-(2-Chloroethyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6b): White amorphous solid; Yield: 90 %; m.p: 130 °C; m.f.: $C_{11}H_9N_2O_3SCl$; Molecular mass: 284; IR (KBr, ν_{max}, cm^{-1}): 3285 (aromatic C-H stretching), 1630 (Ar C=C stretching), 1576 (C=N stretching), 1109 (C-O-C stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.53 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6'), 7.43 (d, *J* = 1.6 Hz, 1H, H-2'), 6.87 (d, *J* = 8.0 Hz, 1H, H-5'), 6.04 (d, *J* = 2.4 Hz, 2H, H-7'), 3.93 (t, *J* = 7.2 Hz, 2H, H-2''), 3.61 (t, *J* = 7.2 Hz, 2H, H-1''); EIMS (*m/z*): 286 $[M + 2]^{\bullet+}$, 284 $[M]^{\bullet+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\bullet+}$, 147 $[C_8H_5NO_2]^{\bullet+}$, 121 $[C_7H_5O_2]^+$, 63 $[C_2H_4Cl]^+$, 28 $[C_2H_4]^+$.

2-(Ethoxycarbonyl)methylthio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6c): White amorphous solid; Yield: 95 %; m.p: 116 °C; m.f.: $C_{13}H_{12}N_2O_5S$; Molecular mass: 308; IR (KBr, ν_{max}, cm^{-1}): 3020 (aromatic C-H stretching), 1715 (C=O stretching), 1617 (Ar C=C stretching), 1576 (C=N stretching), 1106 (C-O-C stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.52 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6'), 7.42 (d, *J* = 1.2 Hz, 1H, H-2'), 6.89 (d, *J* = 8.0 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.26 (q, *J* = 7.2 Hz, 2H, H-3''), 4.06 (s, 2H, H-1''), 1.29 (t, *J* = 7.2 Hz, 3H, H-4''); EIMS (*m/z*): 308 $[M]^{\bullet+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\bullet+}$, 147 $[C_8H_5NO_2]^{\bullet+}$, 121 $[C_7H_5O_2]^+$, 87 $[C_4H_7O_2]^+$, 42 $[C_2H_2O]^+$.

2-Benzylthio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6d): White amorphous solid; Yield: 86 %; m.p: 130 °C; m.f.: $C_{16}H_{12}N_2O_3S$; Molecular mass: 312; IR (KBr, ν_{max}, cm^{-1}): 3285 (aromatic C-H stretching), 1630 (Ar C=C stretching), 1588 (C=N stretching), 1109 (C-O-C stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.51 (dd, *J* = 7.2, 1.2 Hz, 1H, H-6'), 7.44 (brd.s, 1H, H-2'), 7.41 (d, *J* = 7.2 Hz, 2H, H-2'', H-6''), 7.34-7.30 (m, 3H, H-3'' to H-5''), 6.88 (d, *J* = 7.2 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.48 (s, 2H, H-7''); EIMS (*m/z*): 312 $[M]^{\bullet+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\bullet+}$, 147 $[C_8H_5NO_2]^{\bullet+}$, 121 $[C_7H_5O_2]^+$, 122 $[C_7H_6S]^+$, 91 $[C_7H_7]^{\bullet+}$, 77 $[C_6H_5]^{\bullet+}$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^{\bullet+}$.

2-(2-Methylbenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazol (6e): White amorphous solid; Yield: 84 %; m.p: 106 °C; m.f.: $C_{17}H_{14}N_2O_3S$; Molecular mass: 326; IR (KBr, ν_{max}, cm^{-1}): 3294 (aromatic C-H stretching), 1627 (Ar C=C stretching), 1591 (C=N stretching), 1108 (C-O-C stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.52 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6'), 7.43 (d, *J* = 1.6 Hz, 1H, H-2'), 7.38 (d, *J* = 7.2 Hz, 1H, H-6''), 7.19-7.12 (m, 2H, H-4'', H-5''), 6.89 (d, *J* = 8.0 Hz, 1H, H-5'), 6.06 (d, *J* = 6.4 Hz, 1H, H-3''), 6.03 (s,

2H, H-7'), 4.52 (s, 2H, H-7''), 2.43 (s, 3H, CH_3-2''); EIMS (*m/z*): 326 $[M]^{\bullet+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\bullet+}$, 147 $[C_8H_5NO_2]^{\bullet+}$, 137 $[C_8H_9S]^+$, 121 $[C_7H_5O_2]^+$, 106 $[C_8H_9]^+$, 90 $[C_7H_6]^{\bullet+}$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^{\bullet+}$, 51 $[C_4H_3]^+$.

2-(2-Bromobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6f): White amorphous solid; Yield: 82 %; m.p: 120 °C; m.f.: $C_{16}H_{11}N_2O_3SBr$; Molecular mass: 391; IR (KBr, ν_{max}, cm^{-1}): 3329 (aromatic C-H stretching), 1633 (Ar C=C stretching), 1580 (C=N stretching), 1108 (C-O-C stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.62 (dd, *J* = 7.6, 1.6 Hz, 1H, H-3''), 7.58 (d, *J* = 8.0 Hz, 1H, H-6'), 7.50 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6''), 7.41 (d, *J* = 1.6 Hz, 1H, H-2'), 7.18-7.12 (m, 2H, H-4'', H-5''), 6.88 (d, *J* = 8.0 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.60 (s, 2H, H-7''); EIMS (*m/z*): 393 $[M + 2]^{\bullet+}$, 391 $[M]^{\bullet+}$, 221 $[C_9H_5N_2O_3S]^+$, 202 $[C_7H_6SBr]^+$, 189 $[C_9H_5N_2O_3]^+$, 171 $[C_7H_7Br]^{\bullet+}$, 163 $[C_8H_5NO_3]^{\bullet+}$, 157 $[C_6H_5Br]^{\bullet+}$, 147 $[C_8H_5NO_2]^{\bullet+}$, 121 $[C_7H_5O_2]^+$, 90 $[C_7H_6]^+$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^{\bullet+}$, 51 $[C_4H_3]^+$.

2-(3-Bromobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6g): White amorphous solid; Yield: 85 %; m.p: 110 °C; m.f.: $C_{16}H_{11}N_2O_3SBr$; Molecular mass: 391; IR (KBr, ν_{max}, cm^{-1}): 3290 (aromatic C-H stretching), 1628 (Ar C=C stretching), 1587 (C=N stretching), 1107 (C-O-C stretching); ^1H NMR ($CDCl_3$, 300 MHz, δ/ppm): 7.59 (s, 1H, H-2''), 7.51 (dd, *J* = 8.1, 1.5 Hz, 1H, H-6'), 7.41 (brd.s, 1H, H-2'), 7.39-7.37 (m, 2H, H-4'', H-6''), 7.18 (t, *J* = 7.8 Hz, 1H, H-5''), 6.89 (d, *J* = 7.1 Hz, 1H, H-5'), 6.06 (brd.s, 2H, H-7'), 4.43 (s, 2H, H-7''); EIMS (*m/z*): 393 $[M + 2]^{\bullet+}$, 391 $[M]^{\bullet+}$, 221 $[C_9H_5N_2O_3S]^+$, 202 $[C_7H_6SBr]^+$, 189 $[C_9H_5N_2O_3]^+$, 171 $[C_7H_7Br]^{\bullet+}$, 163 $[C_8H_5NO_3]^{\bullet+}$, 157 $[C_6H_5Br]^{\bullet+}$, 147 $[C_8H_5NO_2]^{\bullet+}$, 121 $[C_7H_5O_2]^+$, 90 $[C_7H_6]^+$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^{\bullet+}$, 51 $[C_4H_3]^+$.

2-(4-Bromobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6h): White amorphous solid; Yield: 93 %; m.p: 125 °C; m.f.: $C_{16}H_{11}N_2O_3SBr$; Molecular mass: 391; IR (KBr, ν_{max}, cm^{-1}): 3450 (aromatic C-H stretching), 1627 (Ar C=C stretching), 1578 (C=N stretching), 1109 (C-O-C stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.50 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6'), 7.44 (d, *J* = 8.4 Hz, 2H, H-3'', H-5''), 7.40 (d, *J* = 1.6 Hz, 1H, H-2'), 7.32 (d, *J* = 8.4 Hz, 2H, H-2'', H-6''), 6.88 (d, *J* = 8.0 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.42 (s, 2H, H-7''); EIMS (*m/z*): 393 $[M + 2]^{\bullet+}$, 391 $[M]^{\bullet+}$, 221 $[C_9H_5N_2O_3S]^+$, 202 $[C_7H_6SBr]^+$, 189 $[C_9H_5N_2O_3]^+$, 171 $[C_7H_7Br]^{\bullet+}$, 163 $[C_8H_5NO_3]^{\bullet+}$, 157 $[C_6H_5Br]^{\bullet+}$, 147 $[C_8H_5NO_2]^{\bullet+}$, 121 $[C_7H_5O_2]^+$, 90 $[C_7H_6]^+$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^{\bullet+}$, 51 $[C_4H_3]^+$.

Urease inhibition assay

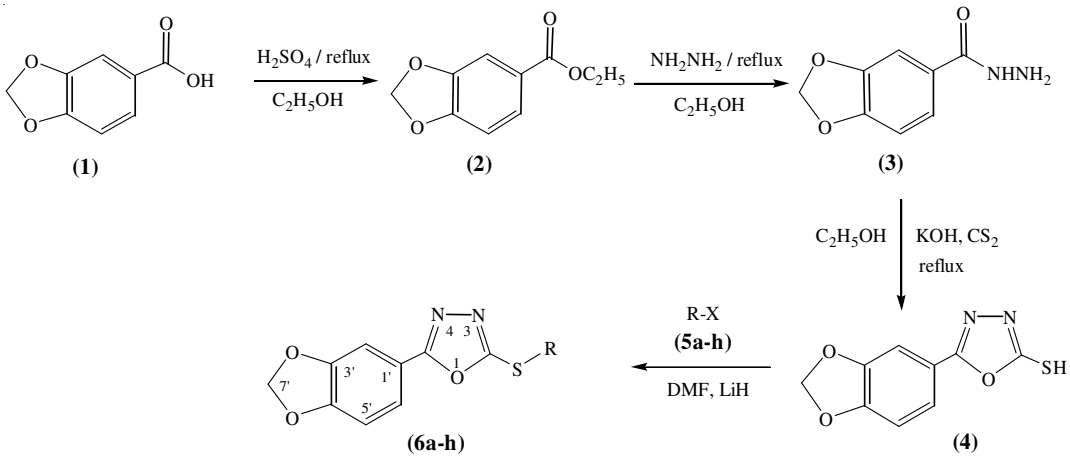
The urease enzyme inhibition assay is the modified form of Berthelot assay[14].

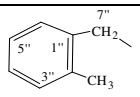
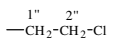
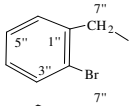
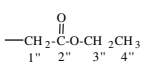
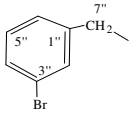
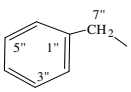
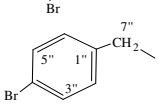
Statistical analysis

The results were compiled in triplicate and presented as mean ± sem. The statistical analysis was computed by ME 2010.

RESULTS AND DISCUSSION

Eight 1,3,4-oxadiazole derivatives (**6a-h**), were proficiently synthesized in good yields as given in **Scheme-I**. The



Compound	R	Compound	R
6a		**6e**	
6b		**6f**	
6c		**6g**	
6d		**6h**	

Scheme-I

reaction specifications and the structural evaluating data are given earlier.

Our prompting accusative for taking this task was to synthesize new 1,3,4-oxadiazole bearing compounds but with potential against urease enzyme. The research work comprised of synthesis of 5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazol-2-thiol (**4**) from 3,4-(methylenedioxy)benzoic acid (**1**) through three steps. Primary the ethyl ester, **2** of **1** was synthesized by refluxing with ethanol for 3 h using conc. H_2SO_4 as catalyst. Secondary, the corresponding hydrazide, **3** of **2** was yielded after a reflux of 6 h with 80 % $N_2H_4.H_2O$ in ethanol. Tertiary, **3** was intermolecularly cyclized to **4** *via* a reflux of 5 h in ethanol using CS_2 as second reactant and KOH to basify the medium. The target 1,3,4-oxadiazole derivatives (**6a-h**) were yielded by stirring different alkyl/aralkyl halides (**5a-h**) with **4** in N,N-dimethyl formamide (DMF) in the presence of LiH for 4-6 h (**Scheme-I**). The structures of the yielded compounds were well confirmed by IR, ^{1}H NMR and EIMS spectral data.

Compound **6a** was synthesized as creamy white amorphous solid in a good yield of 90 % with m.p. 122 ºC. The molecular formula $C_{11}H_9N_2O_3SBr$ was set up by EI-MS with $[M]^{\bullet+}$ peak at 329 (*m/z*) and also by the protons resonating in ^{1}H NMR spectrum. The IR spectrum supported the 1,3,4-oxadiazole structure by showing stretching bands at 1591-1576 cm^{-1} for (C=N) and at 1109-1106 cm^{-1} for (C-O-C). The distinct peak in EI-MS at 221 (*m/z*) was owing to 5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazol-2-thio cation. The ^{1}H NMR spectrum showed four signals, one doublet doublet, one doublet with small coupling, one doublet with large coupling and one singlet at δ 7.53 (dd, *J* = 8.0, 1.2 Hz, 1H, H-6'), 7.43 (d, *J* = 1.2 Hz, 1H, H-2'), 6.89 (d, *J* = 8.0 Hz, 1H, H-5') and 6.04 (s, 2H, H-7') were attributed to methylenedioxyphenyl ring; and two triplets at δ 3.76 (t, *J* = 7.2 Hz, 2H, H-2") and 3.68 (t, *J* = 7.2 Hz, 2H, H-1") owed to bromoethyl group in the molecule. All this demonstrated the structure of **6a** as 2-(2-bromoethyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole. The structures of other molecules were also corroborated by spectral data given in experimental section. The mass fragmentation pattern of 2-(2-bromobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (**6f**) is sketched in Fig. 1.

Urease enzyme inhibition activity (*in vitro*): *in vitro* urease inhibition activity results for all the synthesized molecules are tabulated as %age inhibition and IC_{50} values (Table-1). All the compounds were also screened against urease enzyme and showed varying degree of enzyme inhibition from moderate to excellent as evident from Table-1. The synthesized compounds **6a**, **6b**, **6e**, **6g** and **6h** showed relatively better inhibition potential. The molecule **6g** exhibited almost the same inhibition activity as that of the reference standard. It showed the IC_{50} value of 30.10 ± 0.65 µM with respect to thiourea, the reference standard with IC_{50} value of 21.28 ± 0.11 µM. The promising activity of **6g** was probably because of the presence of *meta*-substituted bromoaralkyl group (the halogenated group). The molecule **6e** displayed two times less activity relative to reference as by IC_{50} values. Two synthesized compounds, **6a** and **6h** depicted same inhibitory potential; three times less than that of the reference standard. The structural

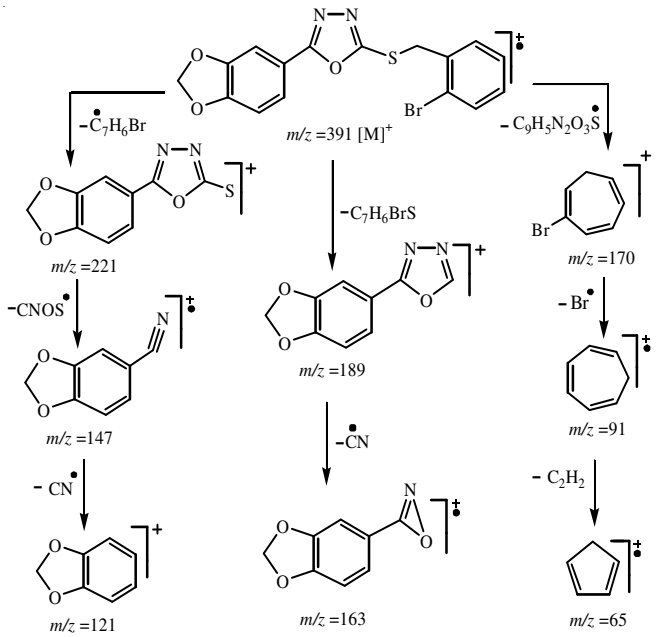


Fig. 1. Mass fragmentation pattern of 2-(2-bromobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole **(6f)**

TABLE-1
UREASE ENZYME INHIBITION STUDY OF THE SYNTHESIZED COMPOUNDS

Compound	Urease enzyme	
	%age Inhibition	IC_{50} (µM)
6a	70.90 ± 0.67	68.20 ± 0.91
6b	97.0 ± 0.79	313.49 ± 0.56
6c	6.50 ± 0.19	-
6d	27.90 ± 0.46	-
6e	61.36 ± 0.97	46.20 ± 0.85
6f	12.40 ± 0.51	-
6g	96.10 ± 0.34	30.10 ± 0.65
6h	95.90 ± 0.29	68.50 ± 1.40
Thiourea	98.61 ± 0.77	21.28 ± 0.11

Note: IC_{50} values (concentration at which there is 50 % enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA)

overview of these potent compounds rendered them as halogen bearing molecules. The order of the enzyme inhibition activity of these molecules was found to be as:

6g > 6e > 6a > 6h > 6b

Conclusion

The synthesized products were developed in better yields with ground support of spectroscopic data. The basic aim of this synthetic work was to search out potent inhibitors of urease enzyme and the attempt remained fruitful to much extent. Out of eight synthesized molecules, five were found to be potential inhibitor of urease enzyme with relatively very low IC_{50} values. These molecules because of anti-urease potential, may assist in drug designing to the pharmaceutical industries.

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