Synthesis, Characterization and Biological activity of Some Novel Sulphur Bridged Pyrazoles

Singaravel Mohan[a,b *], Sarkkarai Ananthan[b], Kambikudi Raman Murugan[a]

[a] Research & Development Centre, Orchid Chemicals & Pharmaceuticals Ltd, Sholinganallur, Chennai-600 119, Tamil Nadu, India.
[b] Department of Chemistry, Presidency College, Chennai-600 005,
[*] Author to whom correspondence should be addressed;
Email: singaravelmohan@yahoo.co.in

Abstract: Mercaptoheterocyclic compounds on treatment with ethyl bromoacetate in the presence of base afforded thioacetate derivative of mercaptoheterocyclic compounds. These on subsequent treatment with hydrazine hydrate yielded acylated hydrazine derivative of mercaptoheterocyclic compounds. Reaction of these acylated hydrazine derivatives of mercaptoheterocyclic compounds with ketene dithioacetal derivative afforded sulphur bridged pyrazole derivatives.

Keywords: Mercaptoheterocycles, ethyl bromoacetate, hydrazine hydrate, ketene dithioacetal, pyrazoles

Introduction:

In recent years pyrazole derivatives have received significant attention owing to their diverse range of biological properties particularly being antifungal, antitubercular, antibacterial, antiviral, anticancer and antioxidant[1-4]. Heterocycles bearing nitrogen, sulphur, oxygen and thiazole moieties constitute the core structure of a number of biologically interesting compounds. Some of them are tetrazoles, fused thiazoles, thiadiazoles, oxadiazoles, triazoles, which are structural subunits of several biologically active compounds[5-7].

Scope of the present work:

Substitution of the heterocyclic moieties on the pyrazole ring is anticipated to have potential biological activity. Ketene dithioacetals are important and versatile reagents, especially, for the synthesis of substituted pyrazole derivatives through condensation reaction of hydrazine derivatives[8,9]. The generality of this reaction is extended for various mercaptoheterocyclic compounds. In the present communication synthesis, characterization and the biological activity of various pyrazoles substituted with heterocyclic moieties are reported.

Materials and Methods:

General

Melting points were determined on a Buchi Melting point B-540 instrument and are uncorrected. The purity of the compounds was analyzed by thin layer chromatography (pre-coated silica gel, Merck). The mass spectra were recorded in PE-SCIEX API-3000 LC/MS/MS with Turbo ion spray. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d6 on a Bruker Avance 400 MHz Spectrometer with multinuclear BBO Probe and TMS as an internal standard.

General procedure for synthesis of thioacetate derivative of mercaptoheterocyclic compounds (2a-f)

Anhydrous sodium carbonate (55 mmol) was added to a solution of mercaptoheterocyclic compound (***1a-f;*** 100 mmol) in acetone (50 mL). To the reaction mixture, ethyl bromoacetate (100 mmol) was added slowly at room temperature under stirring. The progress of the reaction was monitored by thin layer chromatography using a mixture of ethyl acetate and n-hexane (3:7) as eluent. The by-product sodium bromide was removed by filtration. The mother liquor containing the product was concentrated under vacuum to remove acetone and the residual

acetone was removed using methanol to afford ***2a-f***. The residue was used for next step with out purification.

Ethyl (1,3-benzothiazol-2-ylthio)acetate (2a)

2-mercapto-1,3-benzothiazole **1a** was reacted with ethyl bromoacetate in the presence of anhydrous sodium carbonate in acetone in a similar manner as disclosed in the general procedure for **2a-f**. It resulted in the formation of ethyl (1,3-benzothiazol-2-ylthio)acetate **2a**. m/z 254.4 $(M+H)^+$

Ethyl [(1-methyl-1H-tetrazol-5-yl)thio]acetate (2b)

1-methyl-1H-tetrazole-5-thiol **1b** is reacted with ethyl bromoacetate in the presence of anhydrous sodium carbonate in acetone in a similar manner as disclosed in the general procedure for **2a-f**. It resulted in the formation of ethyl [(1-methyl-1H-tetrazol-5-yl)thio]acetate **2b**. m/z 203.4 $(M+H)^+$

Ethyl [(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetate (2c)

5-methyl-1,3,4-thiadiazole-2-thiol **1c** is reacted with ethyl bromoacetate in the presence of anhydrous sodium carbonate in acetone in a similar manner as disclosed in the general procedure for **2a-f**. It resulted in the formation of ethyl [(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetate **2c**. m/z 219.4 $(M+H)^+$

Ethyl [(5-phenyl-1,3,4-oxadiazol-2-yl)thio]acetate (2d)

5-phenyl-1,3,4-oxadiazole-2-thiol **1d** is reacted with ethyl bromoacetate in the presence of anhydrous sodium carbonate in acetone in a similar manner as disclosed in the general procedure for **2a-f**. It resulted in the formation of ethyl [(5-phenyl-1,3,4-oxadiazol-2-yl)thio]acetate **2d**. m/z 264.2 $(M+H)^+$

Ethyl (1,3,4-thiadiazol-2-ylthio)acetate (2e)

1,3,4-thiadiazole-2-thiol **1e** is reacted with ethyl bromoacetate in the presence of anhydrous sodium carbonate in acetone in a similar manner as disclosed in the general procedure for **2a-f**. It resulted in the formation of ethyl [(5-phenyl-1,3,4-oxadiazol-2-yl)thio]acetate **2d**. m/z 205.5 $(M+H)^+$

Ethyl [(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio]acetate (2f)

3-mercapto-2-methyl-1,2-dihydro-1,2,4-triazine-5,6-dione **1f** is reacted with ethyl bromoacetate in the presence of anhydrous sodium carbonate in acetone in a similar manner as disclosed in the general procedure for **2a-f**. It resulted in the formation of ethyl [(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio]acetate **2f**. m/z 245.3 $(M+H)^+$

General procedure for synthesis of acylated hydrazine derivative of mercaptoheterocyclic compounds (3a-f)

The above obtained residue (approximately 25-30g; ***2a-f)*** was dissolved in methanol (30 mL). To the clear solution 100 % hydrazine hydrate (200 mmol) was added and heated under reflux. The progress of the reaction was monitored by thin layer chromatography using a mixture of chloroform and methanol (9:1) as eluent. The reaction mass was cooled to 0-5° C to crystallize the product. On filtration and washing with chilled methanol it afforded acylated hydrazine derivative of mercaptoheterocyclic compounds (***3a-f***).

2-(1,3-Benzothiazol-2-ylthio)acetohydrazide (3a)

The thioacetate derivative of mercaptoheterocyclic compound **2a** when reacted with 100 % hydrazine hydrate in methanol as per the general procedure for **3a-f** resulted in the formation of 2-(1,3-benzothiazol-2-ylthio)acetohydrazide **3a**. m/z 240.2 $(M+H)^+$; ^{1}H NMR 4.10 (2H, s, SCH_2), 4.32 (2H, s, NH_2), 7.34-8.03 (4H, m, Ar), 9.43 (1H, s, NH)

2-[(1-Methyl-1H-tetrazol-5-yl)thio]acetohydrazide (3b)

The thioacetate derivative of mercaptoheterocyclic compound **2b** when reacted with hydrazine hydrate in methanol as per the general procedure for **3a-f** resulted in the formation of 2-[(1-methyl-1H-tetrazol-5-yl)thio]acetohydrazide **3b**. m/z 189.4 $(M+H)^+$; ^{1}H NMR 3.96 (3H, s, NCH_3), 3.98 (2H, s, SCH_2), 4.33 (2H, s, NH_2), 9.37 (1H, s, NH)

2-[(5-Methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide (3c)

The thioacetate derivative of mercaptoheterocyclic compound **2c** when reacted with 100 % hydrazine hydrate in methanol as per the general procedure for **3a-f** resulted in the formation of 2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide **3c**. m/z 205.2 $(M+H)^+$; ^{1}H NMR 2.68 (3H, s, CH_3), 3.96 (2H, s, SCH_2), 4.37 (2H, s, NH_2), 9.37 (1H, s, NH)

2-[(5-Phenyl-1,3,4-oxadiazol-2-yl)thio]acetohydrazide (3d)

The thioacetate derivative of mercaptoheterocyclic compound **2d** when reacted with hydrazine hydrate in methanol as per the general procedure for **3a-f** resulted in the formation of 2-[(5-phenyl-1,3,4-oxadiazol-2-yl)thio]acetohydrazide **3d**. m/z 251.2 $(M+H)^+$; ^{1}H NMR 4.04 (2H, s, SCH_2), 4.38 (2H, s, NH_2), 7.52-7.98 (5H, m, Ar), 9.44 (1H, s, NH)

2-(1,3,4-Thiadiazol-2-ylthio)acetohydrazide (3e)

The thioacetate derivative of mercaptoheterocyclic compound **2e** when reacted with hydrazine hydrate in methanol as per the general procedure for **3a-f** resulted in the formation of 2-(1,3,4-thiadiazol-2-ylthio)acetohydrazide **3e**. m/z 191.3 $(M+H)^+$; ^{1}H NMR 4.02 (2H, s, SCH_2), 4.34 (2H, s, NH_2), 9.39 (1H, s, NH), 9.52 (1H, s, CH)

2-[(2-Methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio]acetohydrazide (3f)

The thioacetate derivative of mercaptoheterocyclic compound **2f** when reacted with hydrazine hydrate in methanol as per the general procedure for **3a-f** resulted in the formation of 2-[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio]acetohydrazide **3f**. m/z 232.3 $(M+H)^+$; ^{1}H NMR 3.61 (3H, s, NCH_3), 3.97 (2H, s, SCH_2), 4.43 (2H, s, NH_2), 10.33 (1H, s, $\underline{NH}NH_2$), 11.75 (1H, s, $CO\underline{NH}$)

General procedure for synthesis of pyrazole derivative using acyl hydrazine derivatives of mercaptoheterocyclic compounds. (5a-10a)

A mixture of acylated hydrazine derivative of mercaptoheterocyclic compound (***3a-f***; 100 mmol) and ketene dithioacetal derivative (***4a***; 150 mmol) in methanol (70 mL) was heated under reflux till completion of reaction. The reaction was monitored by thin layer chromatography using mixture of chloroform and methanol (8:2) as eluent. The reaction mass was cooled to 0-5° C to crystallize the product. On filtration and washing with chilled methanol it afforded pyrazole derivatives substituted with mercaptoheterocycles (***5a-10a***).

Ethyl 5-amino-1-[(1,3-benzothiazol-2-ylthio)acetyl]-3-(methylthio)-1H-pyrazole-4-carboxylate (5a)

The acetohydrazide compound **3a** and ethyl 2-cyano-3,3-bis(methylthio)acrylate in methanol were reacted in a similar manner as disclosed in the general procedure for **5a-10a.** It afforded ethyl 5-amino-1-[(1,3-benzothiazol-2-ylthio)acetyl]-3-(methylthio)-1H-pyrazole-4-carboxylate (Yield 73.23 %). m/z 409.0 $(M+H)^+$; mp 143.7 °C; ^{1}H NMR 1.27 (3H, t, $CH_2\underline{CH_3}$), 2.46 (3H, s, SCH_3), 4.21 (2H, q, $\underline{CH_2}CH_3$), 4.98 (2H, s, SCH_2), 7.45 (2H, s, NH_2), 7.30-8.00 (4H, m, Ar); Anal. Calcd. for $C_{16}H_{16}N_4O_3S_3$: C, 47.04; H, 3.95; N, 13.71. Found: C, 47.22; H, 3.86; N, 13.86.

Ethyl 5-amino-1-{[(1-methyl-1H-tetrazol-5-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carboxylate (6a)

The acetohydrazide compound **3b** and ethyl 2-cyano-3,3-bis(methylthio)acrylate in methanol were reacted in a similar manner as disclosed in the general procedure for **5a-10a.** It afforded ethyl 5-amino-1-{[(1-methyl-1H-

tetrazol-5-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carboxylate (Yield 60.56 %). m/z 358.0 $(M+H)^+$; mp 151.8 °C; ^{1}H NMR 1.26 (3H, t, $CH_2\underline{CH_3}$), 2.43 (3H, s, SCH_3), 4.00 (3H, s, NCH_3), 4.24 (2H, q, $\underline{CH_2}CH_3$), 4.87 (2H, s, SCH_2), 7.45 (2H, s, NH_2).

Ethyl 5-amino-1-{[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carboxylate (7a)

The acetohydrazide compound **3c** and ethyl 2-cyano-3,3-bis(methylthio)acrylate in methanol were reacted in a similar manner as disclosed in the general procedure for **5a-10a.** It afforded ethyl 5-amino-1-{[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carboxylate (Yield 71.11 %). m/z 374.0 $(M+H)^+$; mp 168.9 °C; ^{1}H NMR 1.27 (3H, t, $CH_2\underline{CH_3}$), 2.44 (3H, s, SCH_3), 2.67 (3H, s, C-CH_3), 4.22 (2H, q, $\underline{CH_2}CH_3$), 4.88 (2H, s, SCH_2), 7.45 (2H, s, NH_2).

Ethyl 5-amino-3-(methylthio)-1-{[(5-phenyl-1,3,4-oxadiazol-2-yl)thio]acetyl}-1H-pyrazole-4-carboxylate (8a)

The acetohydrazide compound **3d** and ethyl 2-cyano-3,3-bis(methylthio)acrylate in methanol were reacted in a similar manner as disclosed in the general procedure for **5a-10a.** It afforded ethyl 5-amino-3-(methylthio)-1-{[(5-phenyl-1,3,4-oxadiazol-2-yl)thio]acetyl}-1H-pyrazole-4-carboxylate (Yield 74.50 %). m/z 420.1 $(M+H)^+$; mp 194.9 °C; ^{1}H NMR 1.26 (3H, t, $CH_2\underline{CH_3}$), 2.44 (3H, s, SCH_3), 4.22 (2H, q, $\underline{CH_2}CH_3$), 4.92 (2H, s, SCH_2), 7.56 (2H, s, NH_2), 7.60-7.95 (5H, m, Ar).

Ethyl 5-amino-3-(methylthio)-1-[(1,3,4-thiadiazol-2-ylthio)acetyl]-1H-pyrazole-4-carboxylate (9a)

The acetohydrazide compound **3e** and ethyl 2-cyano-3,3-bis(methylthio)acrylate in methanol were reacted in a similar manner as disclosed in the general procedure for **5a-10a.** It afforded ethyl 5-amino-3-(methylthio)-1-[(1,3,4-thiadiazol-2-ylthio)acetyl]-1H-pyrazole-4-carboxylate (Yield 63.53 %). m/z 360.1 $(M+H)^+$; mp 153.0 °C; ^{1}H NMR 1.27 (3H, t, $CH_2\underline{CH_3}$), 2.27 (3H, s, SCH_3), 4.21 (2H, q, $\underline{CH_2}CH_3$), 4.94 (2H, s, SCH_2), 7.46 (2H, s, NH_2), 9.50 (1H, s, CH).

Ethyl 5-amino-1-{[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carboxylate (10a):

The acetohydrazide compound **3f** and ethyl 2-cyano-3,3-bis(methylthio)acrylate in methanol were reacted in a similar manner as disclosed in the general procedure for **5a-10a.** It afforded ethyl 5-amino-1-{[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carboxylate (Yield 63.52 %). m/z 401.0 $(M+H)^+$; mp 198.1 °C; ^{1}H NMR 1.22 (3H, t, $CH_2\underline{CH_3}$), 2.32 (3H, s, SCH_3), 3.68 (3H, s, NCH_3), 4.19 (2H, q, $\underline{CH_2}CH_3$), 4.76 (2H, s, SCH_2), 7.44 (2H, s, NH_2), 11.93 (1H, s, NH).

General procedure for synthesis of pyrazole derivative using acyl hydrazine derivatives of mercaptoheterocyclic compounds. (5b-10b)

A mixture of acylated hydrazine derivative of mercaptoheterocyclic compounds (***3 a-f***; 100 mmol) and [bis(methylthio)methylene]malononitrile (***4b***; 500 mmol) in methanol (70 mL) was heated under reflux till completion of reaction. The reaction was monitored by thin layer chromatography using mixture of chloroform and methanol (8:2) as eluent. The reaction mass was cooled to 0-5° C to crystallize the product. On filtration and washing with chilled methanol it afforded pyrazole derivatives substituted with mercaptoheterocycles (***5b-10b***).

5-Amino-1-[(1,3-benzothiazol-2-ylthio)acetyl]-3-(methylthio)-1H-pyrazole-4-carbonitrile (5b)

The acetohydrazide compound **3a** and [bis(methylthio)methylene]malononitrile in methanol were reacted in a similar manner as disclosed in the general procedure for **5b-10b.** It afforded 5-amino-1-[(1,3-benzothiazol-2-ylthio)acetyl]-3-(methylthio)-1H-pyrazole-4-carbonitrile (Yield 86.07 %). m/z 362.2 $(M+H)^+$; mp 206.8 °C (dec); ^{1}H NMR 2.50 (3H, s, SCH_3), 4.96 (2H, s, SCH_2), 7.35-8.03 (4H, m, Ar), 8.15 (2H, s, NH_2).

5-Amino-1-{[(1-methyl-1H-tetrazol-5-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carbonitrile (6b)

The acetohydrazide compound **3b** and [bis(methylthio)methylene]malononitrile in methanol were reacted in

a similar manner as disclosed in the general procedure for **5b-10b.** It afforded 5-amino-1-{[(1-methyl-1H-tetrazol-5-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carbonitrile (Yield 72.77 %). m/z 311.1 $(M+H)^+$; mp 192.7 °C; ^{1}H NMR 2.50 (3H, s, SCH_3), 4.00 (3H, s, N CH_3), 4.85 (2H, s, SCH_2), 8.14 (2H, s, NH_2).

5-Amino-1-{[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carbonitrile (7b)

The acetohydrazide compound **3c** and [bis(methylthio)methylene]malononitrile in methanol were reacted in a similar manner as disclosed in the general procedure for **5b-10b.** It afforded 5-amino-1-{[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carbonitrile (Yield 81.38 %). m/z 326.9 $(M+H)^+$; mp 184.8 °C (dec); ^{1}H NMR 2.50 (3H, s, SCH_3), 2.67 (3H, s, C-CH_3), 4.86 (2H, s, SCH_2), 8.14 (2H, s, NH_2).

5-Amino-3-(methylthio)-1-{[(5-phenyl-1,3,4-oxadiazol-2-yl)thio]acetyl}-1H-pyrazole-4-carbonitrile (8b)

The acetohydrazide compound **3d** and [bis(methylthio)methylene]malononitrile in methanol were reacted in a similar manner as disclosed in the general procedure for **5b-10b.** It afforded 5-amino-3-(methylthio)-1-{[(5-phenyl-1,3,4-oxadiazol-2-yl)thio]acetyl}-1H-pyrazole-4-carbonitrile (Yield 80.55 %). m/z 373.0 $(M+H)^+$; mp 201.0 °C; ^{1}H NMR 2.55 (3H, s, SCH_3), 4.91 (2H, s, SCH_2), 7.57-7.96 (5H, m, Ar), 8.18 (2H, s, NH_2).

5-Amino-3-(methylthio)-1-[(1,3,4-thiadiazol-2-ylthio)acetyl]-1H-pyrazole-4-carbonitrile (9b)

The acetohydrazide compound **3e** and [bis(methylthio)methylene]malononitrile in methanol were reacted in a similar manner as disclosed in the general procedure for **5b-10b.** It afforded 5-amino-3-(methylthio)-1-[(1,3,4-thiadiazol-2-ylthio)acetyl]-1H-pyrazole-4-carbonitrile (Yield 79.19 %). m/z 312.9 $(M+H)^+$; mp 184.2 °C (dec); ^{1}H NMR 2.49 (3H, s, SCH_3), 4.92 (2H, s, SCH_2), 8.15 (2H, s, NH_2) 9.52 (1H, s, C-H).

5-Amino-1-{[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carbonitrile (10b)

The acetohydrazide compound **3f** and [bis(methylthio)methylene]malononitrile in methanol were reacted in a similar manner as disclosed in the general procedure for **5b-10b.** It afforded 5-amino-1-{[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio]acetyl}-3-(methylthio)-1H-pyrazole-4-carbonitrile (Yield 78.52 %). m/z 354.2 $(M+H)^+$; mp 192.5 °C (dec); ^{1}H NMR 2.50 (3H, s, SCH_3), 3.68 (3H, s, N CH_3), 4.72 (2H, s, SCH_2), 8.13 (2H, s, NH_2) 11.86 (1H, s, NH).

Results and Discussion:

Various hydrazine derivatives of mercaptoheterocyclic compounds were prepared by two steps. First step of the reaction involved the condensation of mercaptoheterocyclic compounds with ethyl bromoacetate in the presence of anhydrous sodium carbonate to form thioacetate derivative of mercaptoheterocyclic compounds[10]. This, on subsequent reaction with excess hydrazine hydrate afforded acylated hydrazine derivative containing the mercaptoheterocycles[11]. The reaction was monitored by thin layer chromatography (TLC) using a mixture of ethyl acetate and hexane (3:7) as eluent. This is shown in **scheme-1**. The acylated hydrazine derivatives were confirmed by mass spectroscopy, ^{1}H NMR and ^{13}C NMR spectroscopy.

These mercaptoheterocyclic compounds (Represented in **Figure-1**) are structural subunits of several biologically active compounds. The reaction of acylated hydrazine derivative of mercaptoheterocyclic compound (**3a-f;** represented in **Table-1**) with ketene dithioacetal derivative (**4a-b;** represented in **Table-2**)[12] in methanol under reflux condition resulted in the formation of pyrazole derivatives (**5a-10a** and **5b-10b;** represented in **Table-3**). The progress of the reaction was monitored by thin layer chromatography using a mixture of chloroform and methanol (8:2) as eluent. After completion of reaction, the reaction mass was cooled to 0-5 °C. The crystallized product was isolated by filtration and washed with chilled methanol. In some of the reactions, the product crystallized even at reflux temperature. The isolated product was confirmed by mass spectroscopy, ^{1}H NMR and ^{13}C NMR spectroscopy. This is shown in **scheme 2**.

In Scheme-1, the Het-SH represents mercaptoheterocyclic compounds. The mercaptoheterocyclic compounds used in the present study are selected from compounds represented in Figure-1.

Biological activity of Pyrazole derivatives:

The synthesized pyrazole derivatives **5-10(a-b)** were tested for antibacterial activity against both gram positive and gram negative bacteria such as *Staphylococcus aureus, Bacillus subtilis* from gram positive organisms and *Escherichia Coli, Pseudomonas aeruginosa* from gram negative organisms as well as for antifungal activity against *Candida albicans* by agar well diffusion method in 1000, 100 and 10 µg concentration per mL of dimethyl sulphoxide. During this study, Amikacin, Ampicillin, Cefepime, Cephalexin, Linezolid, Tetracycline, and Fluconazole were used as the reference standards.

Among the various pyrazoles prepared above, the pyrazole derivative, **8b** showed activity against *Bacillus subtilis* from gram positive organisms and *Escherichia Coli* from gram negative organism with 12.40 mm and 11.25 mm of zone of inhibition respectively with the concentration of 1000 µg/mL. Further the same pyrazole derivative also showed antifungal activity in the concentration of 1000 and 100 µg/mL against *C. Abicans* with 40.50 mm and 23.26 mm zone of inhibition respectively. The pyrazole derivative, **10b** showed activity against *Bacillus subtilis* from gram positive with 9.22 mm of zone of inhibition with the concentration of 1000 µg/mL. Remaining pyrazole derivatives prepared as above showed no zone of inhibition with *Staphylococcus aureus, Bacillus subtilis* from gram positive organisms and *Escherichia Coli, Pseudomonas aeruginosa* from gram negative organisms as well as for antifungal activity against *Candida albicans*.

Conclusion:

Various pyrazole derivatives were prepared from substituted mercaptoheterocyclic compounds. The compounds were confirmed by various analytical techniques and these compounds were tested for both gram positive and gram negative bacteria for their antimicrobial activity. The ketene dithioacetal derivatives (**4a-b**) used for the synthesis of pyrazole derivatives were prepared according to the procedure reported in the literature[12].

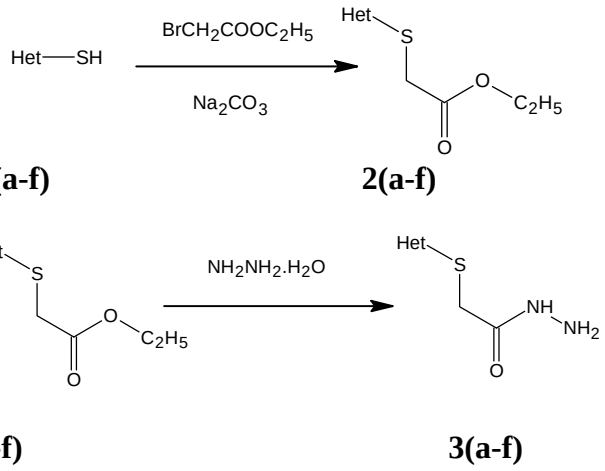
Acknowledgements

We are thankful to the management of Orchid Chemicals and Pharmaceutical Ltd for the excellent support to perform the research project. We are also thankful for the kind support from the Staff of Orchid Chemicals and Pharmaceuticals Ltd in recording the spectral data and studying the biological activity of the synthesized pyrazole derivatives.

References

1. Abunada, N. M.; Hassaneen, H. M.; Kandile, N. G.; Miqdad, O. A. *Molecules,* **2008,** *13,* 1501.
2. Ezawa, M.; Garvey, D. S.; Janero, D. R.; Khanapure, S. P.; Letts, L. G.; Martino, A.; Ranatunge, R. R.; Schwalb, D. J.; Young, D. V. *Letters in Drug Design & Discovery,* **2005,** *2,* 40.
3. Suleyman, H.; Emin Buyukokuroglu, M. *Biol. Pharm. Bull.* **2001,** *24 (10),* 1133.
4. Abadi, A. H.; Haleem Eissa, A. A.; Hassan, G. S. *Chem. Pharm. Bull.,* **2003,** *51 (7),* 838.
5. Yadav, R.; Srivastava, S. D.; Srivastava, S. K.; *Indian J. Chem.* **2005,** *44B,* 1262.
6. Cacic, M.; Trkovnik, M.; Cacic, F.; Has-Schon, E. *Molecules,* **2006,** *11,* 134.
7. Barsoum, F. F.; Hosni, H. M.; Girgis, A. S. *Bioorg. & Med. Chem.* **2006,** *14*, 3929.
8. Tominaga, Y.; Honkawa, Y.; Hara, M.; Hosomi, A. *J. Heterocyclic Chem.* **1990,** *27,* 775.
9. Abu Elmaati, T. M.; El-Taweel, F. M. *J. Heterocyclic Chem.,* **2004,** *41,* 109.
10. Desai, K. G.; Desai, K. R. *Indian J. Chem.* **2005,** *44B,* 2093.
11. Yadav, R.; Srivastava, S. D.; Srivastava, S. K.; *J. Indian Chem. Soc.* **2004,** *81,* 342.
12. Masataka, Y.; Tsuneo, I.; *Synthesis,* **1984,** 797.

Scheme-1: Synthesis of acylated hydrazine derivatives of mercaptoheterocyclic compounds.



Scheme-2: Synthesis of sulphur bridged pyrazole derivatives.

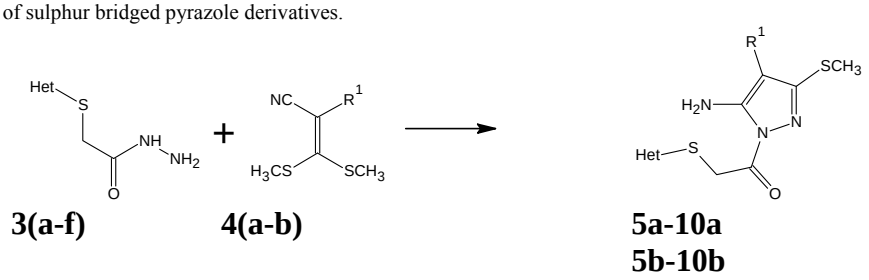


Figure-1: Mercaptoheterocyclic compounds used in the present study.

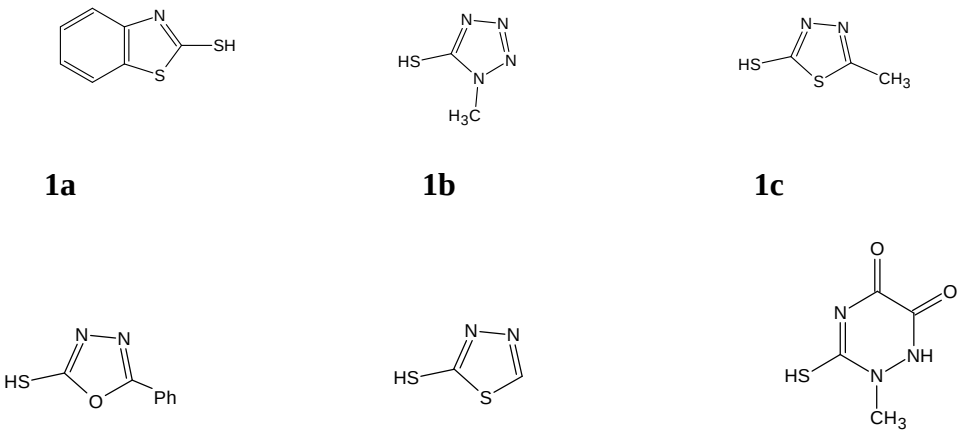


Table-1: Representation of Het in 2a-f and 3a-f.

Compound No	Het
2a and 3a	2-mercapto-1,3-benzothiazolyl (**1a**)
2b and 3b	5-mercapto-1-methyltetrazolyl (**1b**)
2c and 3c	2-mercapto-5-methylthiadiazolyl (**1c**)
2d and 3d	5-phenyl-2-mercapto-oxadiazolyl (**1d**)
2e and 3e	2-mercaptothiadiazolyl (**1e**)
2f and 3f	3-mercapto-2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazinyl (**1f**)

Table-2: Representation of R^1 in 4a-b.

Compound No	R^1
4a	$COOC_2H_5$
4b	CN

Table-3: Representation of R^1 and Het in 5a-10a and 5b-10b.

Compound No	R^1	Het
5a	$COOC_2H_5$	2-mercapto-1,3-benzothiazolyl (**1a**)
6a	$COOC_2H_5$	5-mercapto-1-methyltetrazolyl (**1b**)
7a	$COOC_2H_5$	2-mercapto-5-methylthiadiazolyl (**1c**)
8a	$COOC_2H_5$	5-phenyl-2-mercapto-oxadiazolyl (**1d**)
9a	$COOC_2H_5$	2-mercaptothiadiazolyl (**1e**)
10a	$COOC_2H_5$	3-mercapto-2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazinyl (**1f**)
5b	CN	2-mercapto-1,3-benzothiazolyl (**1a**)
6b	CN	5-mercapto-1-methyltetrazolyl (**1b**)
7b	CN	2-mercapto-5-methylthiadiazole (**1c**)
8b	CN	5-phenyl-2-mercapto-oxadiazolyl (**1d**)
9b	CN	2-mercaptothiadiazolyl (**1e**)
10b	CN	3-mercapto-2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazinyl (**1f**)