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# SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF 2-(2'-SUBSTITUTED - BENZYLIDENE - HYDRAZINO -ACETYL) – MERCAPTO -5-METHYL - 1, 3, 4-THIADIAZOLES AND 2 -[2'- {4 - SUBSTITUTED -ARYL - 3 - CHLORO - 2 - OXO –AZETIDINE } –ACETYL-AMINO-MERCAPTO] - 5-METHYL - 1, 3, 4 -THIADIAZOLES

**RAJIV DUA*AND S.K. SRIVASTAVA**

Synthetic Organic Medicinal Chemistry Laboratory, Department of Chemistry,
Dr. H.S. Gour Central University, Sagar (M.P), India.

***Corresponding author*** duanobleheights@gmail.com

## ABSTRACT

As a part of systematic investigation of synthesis and biological activity of several new 2-(2'-substituted-benzylidene-hydrazino-acetyl)-mercapto-5-methyl-1,3,4-thiadiazoles, **3(a-n)** and 2-[2'-{4-substituted-aryl-3-chloro-2-oxo-azetidine}-acetyl-amino-mercapto]-5-methyl-1,3,4-thiadiazoles, **4(a-n)** have been synthesized from 2-(2'-hydrazino-acetyl)-mercapto-5-methyl-1,3,4-thiadiazoles, **2** using 5-methyl-1,3,4-thiadiazole-2-thiol as the starting material. All the synthesized products were evaluated for their antibacterial activity against *Bacillus substilis, Escherichia coli. Klebsiella pneumoniae* and *Streptococcus aureus* bacteria and antifungal activity against *Aspergillus niger, Aspergillus flavus, Fusarium oxisporium* and *Trichoderma viride* fungi respectively. The structures of all the synthesized compounds have been determined by their spectral and microanalytical data.

## KEYWORDS

5-Methyl-1,3,4-thiadiazole-2-thiol, benzylidenes, 2-oxo-azetidines, antimicrobial activity.

## INTRODUCTION

Various 2-mercapto/substituted mercapto-1,3,4-thiadiazole derivatives have recently received significant importance because of their diverse biological properties.[1] 1, 3, 4-thiadiazoles possesses various biological activities such as antibacterial[2], antifungal[3], carbonic anhydrase inhibitor[4], antidiabetic[5], plant growth regulator and agrochemicals.[6] β-lactam drugs are most widely prescribed antibiotics use in medicine[7]. 2-azetidinone derivative possess wide therapeutic activity like antifungal,[8] anticonvulsant,[9] antitumour[10], cholesterol absorption inhibitior[11] and enzyme inhibition activities.[12,13] Some new N-sulphonyl-phenyl-amino-3-chloro-4-phenyl-azetidine-2-one derivatives were found to be more potent antibacterial against E.coli. We have synthesized several new thiadiazolo-azetidinones derivatives in order to study their biodynamic behavior. The present paper reports the synthesis of 2-(2'-benzylidene-hydrazino-acetyl-mercapto)-5-methyl-methyl-1,3,4-thiadiazoles, **3(a-n)** and 2-[2'-{4-substituted-aryl-3-chloro-2-oxo-azetidine}-acetylamino-mercapto]-5- 1,3,4-thiadiazoles **4(a-n)** by appropriate methods. All the synthesized compounds have been screened for their antibacterial activity against *B.*

*substilis, E. coli, S. aureus* and *K. pneumoniae* bacteria and *antifungal activity against A. niger, A. flavus, F. oxisporium* and *T. viride* fungi respectively.

## MATERIALS AND METHODS

Melting points were taken in open capillaries and are uncorrected. Purity of compounds was monitored on silica gel 'G' coated TLC plates. IR spectra were recorded on a Schimadzu 8201 PC spectrophotometer in KBr and $^1$HNMR spectra on a Brucker DRX 300 spectrometer in $CDCl_3$ at 300 MHz using TMS as an internal standard. The reagent grade chemicals were purchased from the commercial sources and purified by either distillation or recrystallization before use.

**2-(2'-chloroacetyl)-mercapto-5-methyl-1,3,4-thiadiazole,1:** The equimolar solution of 5-methyl-1,3,4-thiadiazole-2-thiol (0.40 mole, 52.88 gm) and chloroacetyl chloride (0.40 mole, 39.38 gm) in methanol (350 ml) was refluxed on a water bath for about 3 hrs , cooled, filtered, washed with ice-cooled water and purified over the column of silica gel using chloroform: acetone (7:3 v/v) mixture as eluant. The eluate was concentrated to give a product which was recrystallized from chloroform to give **compound 1**. Yield 82%, m.p. 196-198ºC. Anal. calcd. for $C_5H_5N_2OS_2Cl$ : C, 28.77, H, 2.39, N, 13.42 %; found C, 28.73, H, 2.37, N, 13.39% ; IR: 2868 (-$CH_3$), 1632, 1194, 1129, 1071, 695 (thiadiazole ring), 1659 (>C=O), 2962 (-$CH_2$), 772 (-$CH_2$Cl);$^1$HNMR: 2.45 (s, 3H, -$CH_3$), 4.41(s, 2H, -$CH_2$).

**2-(2'-hydrazino-acetyl)-mercapto-5-methyl-1,3,4-thiadiazole,2:** The **compound-1** (0.20 mole, 41.7 g) and hydrazine hydrate (0.20 mole, 9.8 gm) in methanol (300 ml) was refluxed on a water bath for about 5 hours. It was cooled, filtered to get a product which was purified over the column of silica gel using chloroform as eluant. The eluate was concentrated to give a product which was recrystallized with ethanol to give **compound 2**. Yield 79%, m.p. 185-87ºC. Anal. calcd. for $C_5H_8N_4OS_2$ : C, 29.45, H, 3.92, N, 27.45%; found C, 29.38, H, 3.87, N, 27.39%; IR : 3451, 3356, 3272,3170 (-$NHNH_2$), 2867 (-$CH_3$), 1634, 1196, 1126, 1064, 691 (Thiadiazole ring), 1656 (>C=O), 2965 (-$CH_2$) ; $^1$HNMR : 8.34 (s, 1H, -NH), 4.83 (s, 1H, -$NH_2$), 4.39 (s, 2H, -$CH_2$), 2.43 (s, 3H, -$CH_3$).

**2-(2'-substituted-benzylidene-hydrazino-acetyl)-mercapto-5-methyl-1, 3, 4-thiadiazole, 3(a-n):** A mixture of **compound 2** (0.014 mole, 2.85 g) and benzaldehyde (0.014 mole, 1.48g ) in methanol (40 ml) with 3-4 drops of acetic acid was refluxed on a water bath for about 2 hours. The solvent was distilled off under reduced pressure and solid thus obtained was purified over the column of silica gel using chloroform: methanol (7:3 v/v) mixture as eluant. The eluate was concentrated to give a product which was recrystallized from ethanol to give **compound 3a**. Yield 85%, m.p. 117-19ºC. Anal. calcd. for $C_{12}H_{12}N_4OS_2$ : C, 49.31, H, 4.10, N, 19.17%; found C, 49.29, H, 4.07, N, 19.14%; IR : 3352(-NH), 2864 (-$CH_3$), 2965 (-$CH_2$), 1661 (>C=O), 1550 (-N=CH), 1628, 1197, 1130, 1069, 690 (thiadiazole ring), 3039, 1593, 741 (aromatic ring).; $^1$HNMR : 2.46 (s, 3H, -$CH_3$), 4.38 (s, 2H, -$CH_2$), 8.31 (s, 1H, -NH), 7.31-7.85 (m, 5H, Ar-H), 4.76 (s, 1H, -N=CH).

Likewise other compounds **3(b-n)** were synthesized by treating the **compound 2** with selected aromatic aldehydes. The characterization data of the compounds **3(b-n)** are given in **Table – 1.**

**Table 1**

*Characterization data of compounds **3(b-n) and 4(b-n)***

| Comp. | Ar | Yield % | m.p. (°C) | Molecular formula | Found % (Calcd %) | | |
|---|---|---|---|---|---|---|---|
| | | | | | C | H | N |
| 3b | 2-$ClC_6H_4$ | 78 | 218-20 | $C_{12}H_{11}N_4OS_2Cl$ | 44.07 (44.10) | 3.33 (3.36) | 17.13 (17.15) |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3c | 3-$ClC_6H_4$ | 82 | 226-28 | $C_{12}H_{11}N_4OS_2Cl$ | 44.03 (44.10) | 3.29 (3.36) | 17.09 (17.15) |
| 3d | 4-$ClC_6H_4$ | 84 | 223-225 | $C_{12}H_{11}N_4OS_2Cl$ | 44.04 (44.10) | 3.31 (3.36) | 17.07 (17.15) |
| 3e | 2-$BrC_6H_4$ | 91 | 175-77 | $C_{12}H_{11}N_4OS_2Br$ | 38.78 (38.81) | 2.92 (2.98) | 14.99 (15.09) |
| 3f | 3-$BrC_6H_4$ | 89 | 168-70 | $C_{12}H_{11}N_4OS_2Br$ | 38.75 (38.81) | 2.93 (2.98) | 14.95 (15.09) |
| 3g | 4-$BrC_6H_4$ | 92 | 171-73 | $C_{12}H_{11}N_4OS_2Br$ | 38.77 (38.81) | 2.96 (2.98) | 14.96 (15.09) |
| 3h | 2-$NO_2C_6H_4$ | 81 | 158-60 | $C_{12}H_{11}N_5O_3S_2$ | 42.69 (42.72) | 3.23 (3.26) | 20.74 (20.77) |
| 3i | 3-$NO_2C_6H_4$ | 76 | 146-48 | $C_{12}H_{11}N_5O_3S_2$ | 42.70 (42.72) | 3.22 (3.26) | 20.73 (20.77) |
| 3j | 4-$NO_2C_6H_4$ | 79 | 161-63 | $C_{12}H_{11}N_5O_3S_2$ | 42.68 (42.72) | 3.24 (3.26) | 20.75 (20.77) |
| 3k | 2-$OCH_3C_6H_4$ | 70 | 198-200 | $C_{13}H_{14}N_4O_2S_2$ | 48.41 (48.44) | 4.32 (4.34) | 17.38 (17.39) |
| 3l | 3-$OCH_3C_6H_4$ | 69 | 189-91 | $C_{13}H_{14}N_4O_2S_2$ | 48.43 (48.44) | 4.31 (4.34) | 17.37 (17.39) |
| 3m | 4-$OCH_3C_6H_4$ | 72 | 194-96 | $C_{13}H_{14}N_4O_2S_2$ | 48.42 (48.44) | 4.33 (4.34) | 17.36 (17.39) |
| 3n | 4,4'$(CH_3)_2NC_6H_4$ | 87 | 96-98 | $C_{14}H_{17}N_5OS_2$ | 50.09 (50.14) | 5.02 (5.07) | 20.86 (20.89) |
| 4b | 2-$ClC_6H_4$ | 62 | 134-36 | $C_{14}H_{12}N_4O_2S_2Cl_2$ | 41.65 (41.68) | 2.95 (2.97) | 13.87 (13.89) |
| 4c | 3-$ClC_6H_4$ | 65 | 131-33 | $C_{14}H_{12}N_4O_2S_2Cl_2$ | 41.63 (41.68) | 2.93 (2.97) | 13.86 (13.89) |
| 4d | 4-$ClC_6H_4$ | 64 | 137-39 | $C_{14}H_{12}N_4O_2S_2Cl_2$ | 41.65 (41.68) | 2.95 (2.97) | 13.85 (13.89) |
| 4e | 2-$BrC_6H_4$ | 74 | 153-155 | $C_{14}H_{12}N_4O_2S_2BrCl$ | 34.11 (34.14) | 2.40 (2.43) | 11.35 (11.38) |
| 4f | 3-$BrC_6H_4$ | 69 | 159-61 | $C_{14}H_{12}N_4O_2S_2BrCl$ | 34.12 (34.14) | 2.41 (2.43) | 11.36 (11.38) |
| 4g | 4-$BrC_6H_4$ | 70 | 163-165 | $C_{14}H_{12}N_4O_2S_2BrCl$ | 34.10 (34.14) | 2.39 (2.43) | 11.34 (11.38) |
| 4h | 2-$NO_2C_6H_4$ | 70 | 176-78 | $C_{14}H_{12}N_5O_4S_2Cl$ | 40.59 (40.62) | 2.87 (2.90) | 16.90 (16.92) |
| 4i | 3-$NO_2C_6H_4$ | 68 | 178-80 | $C_{14}H_{12}N_5O_4S_2Cl$ | 40.58 | 2.88 | 16.89 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | (40.62) | (2.90) | (16.92) |
| 4j | 4-$NO_2C_6H_4$ | 64 | 171-73 | $C_{14}H_{12}N_5O_4S_2Cl$ | 40.56 (40.62) | 2.86 (2.90) | 16.88 (16.92) |
| 4k | 2-$OCH_3C_6H_4$ | 78 | 206-08 | $C_{15}H_{15}N_4O_2S_2Cl$ | 47.00 (47.05) | 3.91 (3.92) | 14.62 (14.64) |
| 4l | 3-$OCH_3C_6H_4$ | 81 | 202-04 | $C_{15}H_{15}N_4O_2S_2Cl$ | 47.02 (47.05) | 3.89 (3.92) | 14.61 (14.64) |
| 4m | 4-$OCH_3C_6H_4$ | 80 | 210-12 | $C_{15}H_{15}N_4O_2S_2Cl$ | 46.99 (47.05) | 3.88 (3.92) | 14.60 (14.64) |
| 4n | 4,4'$(CH_3)_2NC_6H4$ | 92 | 184-86 | $C_{16}H_{18}N_4O_2S_2Cl$ | 48.27 (48.30) | 4.49 (4.52) | 14.06 (14.08) |

**2-[2'-{4-substituted-aryl-3-chloro-2-oxo-azetidine}-acetyl-amino-mercapto]-5-methyl-1,3,4-thiadiazole, 4(b-n):**The **compound 3a** (0.004 mole, 1.16 g) and triethylamine (0.004 mole, 0.69 g) in ethanol (30 ml) with chloroacetyl chloride (0.004 mole, 0.39 g) was first stirred for about 2 hours, followed by refluxing on a water bath for about 12 hours. The solvent was distilled off under reduced pressure and the solid thus obtained was purified over the column of silica gel using $CHCl_3$: $CH_3OH$ (8:2 v/v) as eluant. The eluate was concentrated to give a product which was recrystallized from ethanol to give **compound 4a.** Yield 72%, m.p. 168-70ºC. Anal. calcd. for $C_{14}H_{13}N_4O_2S_2Cl$ : C, 50.45, H, 3.90, N, 16.81%; found C, 50.42, H, 3.86, N, 16.79 %; ; IR : 3362 (-NH), 2863 (-$CH_3$), 2963 (-$CH_2$), 1661 (>C=O), 1630, 1196, 1132, 1068, 642 (thiadiazole ring), 1773 (>C=O, cyclic), 768 (-CHCl), 3012, 1592, 746 (aromatic ring ); $^1$HNMR : 8.36 (s, 1H, -NH), 7.26-7.74 (m, 5H, Ar-H), 5.14(d, J=5.0 Hz,1H,-CHCl), 4.32 (s, 2H, -$CH_2$). 4.11 (d, J=5.0 Hz, 1H, -NCHAr), 2.43 (s, 3H, -$CH_3$).

Other compounds **4(b-n)** were synthesized in the similar manner using compounds **3(b-n)**. Characterization data are presented in **Table-1**.

## RESULTS AND DISCUSSION

5-Methyl-1,3,4-thiadiazole-2-thiol on reaction with chloroacetyl chloride yielded 2-(2'-chloroacetyl)-mercapto-5-methyl-1,3,4-thiadiazole, **1** which on amination with hydrazine hydrate afforded 2-(2'-hydrazino-acetyl)-mercapto-5-methyl-1,3,4-thiadiazole, **2**. The compound **2** on condensation with various selected aromatic aldehydes yielded 2-(2'-benzylidene-hydrazino-acetyl)-mercapto-5-methyl-1,3,4-thiadiazoles, **3(b-n).** The compounds **3(b-n)** on treatment with chloroacetyl chloride in the **presence** of triethyl amine afforded 2-[2'-{4-substitute-aryl-3-chloro-2-oxo-azetidine}-acetyl-amino-mercapto]-5-methyl-1,3,4-thiadiazoles, **4(b-n)** Their structures have been elucidated on the basis of their spectral and microanalytical data. The steps involved in the synthesis are shown in the **scheme-1**.

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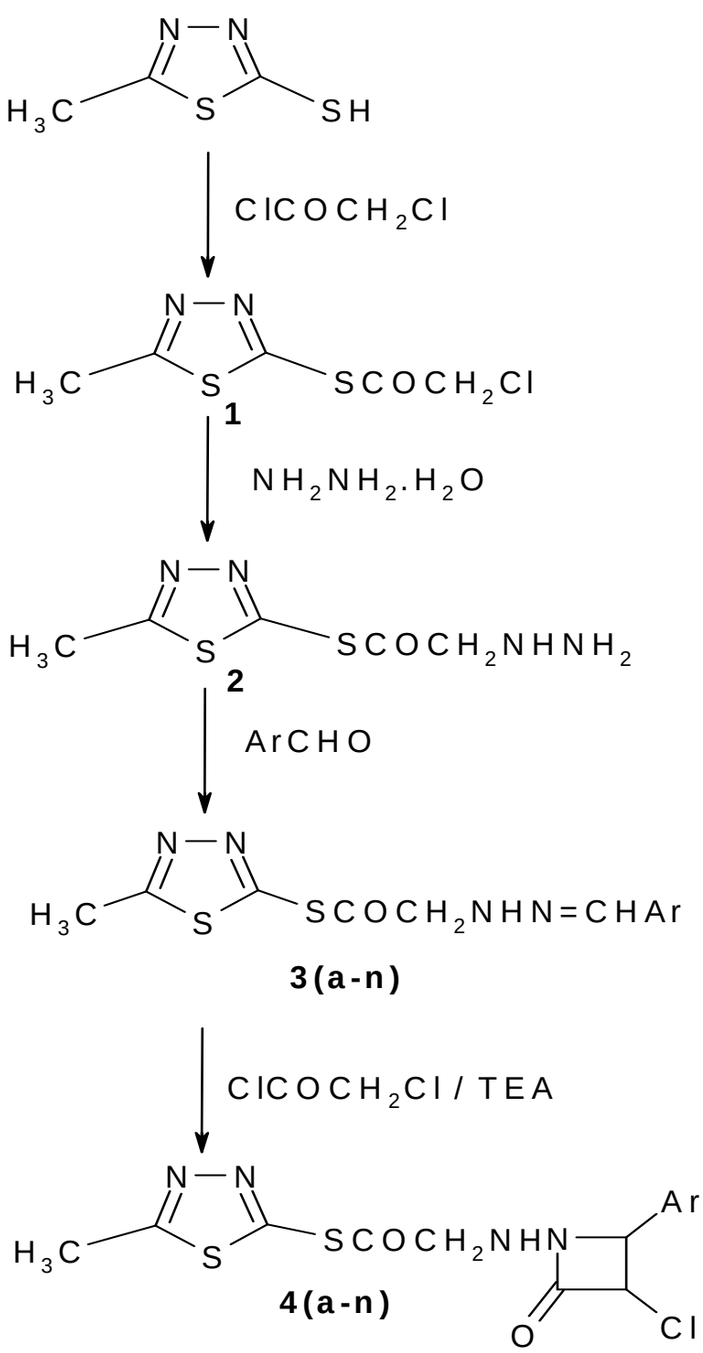
**Scheme -1**




**Ar = Substituted aryl groups**

## BIOLOGICAL ACTIVITY

All the synthesized compounds **1,2, 3(b-n)** and **4(b-n)** have been screened *in vitro* for their antibacterial activity at two concentrations (50 and 100 ppm) against *B. substilis* (Bs), *E. coli* (Ec), *S. aureus* (Sa) and *K. pneumoneae* (Kp) and antifungal activity against *A. niger* (An), *A. flavus* (Af), *F. oxisporium* (Fo) and *T. viride* (Tv) at two concentrations (50 and 100 ppm) by filter paper disc method.[14,15] Standard antibacterial Streptomycin and fungicide Griseofulvin were also screened under the similar conditions for comparison. The following compounds were found active against the tested bacteria : 3h,3j (Ec, Sa), 3b,3d (Ec), 3e,3f (Bs, Kp), 3g ( Ec), 3i (Bs, Kp, Sa), 4h, 4j,4b, 4c, 4d, 4e, 4f, 4g (Bs, Ec, Kp, Sa), 4i (Ec, Kp, Sa), 4h (Kp), 4k (Ec), 4n (Kp, Sa) and Fungi : 3e, 3g (An ,Af), 3d (Fo), 3h, 3j (Af ,Fo), 3k (An) 4f (Af, Tv),4c ,4d ,4g(An),4n (Fo), 4b(Tv),4i (Af, Fo),4j (An, Af), 4h (Fo, Tv), 4k (An, Af),4l,4n (An) respectively. On the basis of structural activity relationship it has been observed that among the substituents present on the phenyl ring, halo derivatives were found to be highly active.

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