# Synthesis and Antibacterial Activity of 1-(2,4-Dichlorobenzoyl)-4-substituted Thiosemicarbazides, 1,2,4-Triazoles and Their Methyl Derivatives



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A series of 1-(2,4-dichlorobenzoyl)thiosemicarbazides, *s*-triazoles and their methyl derivatives have been synthesised by condensation of 2,4-dichlorobenzoyl hydrazine with aryl isothiocyanates. Subsequent ring closure of the substituted thiosemicarbazides yielded the *s*-triazoles, and reaction with methyl iodide resulted methyl derivatives. All the compounds were subjected to *in vitro* testing against two gram-positive and two gram-negative bacteria. Antibacterial activity was found to be moderate to good in most of the compounds.

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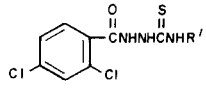
In view of the recent findings that 1,2,4-triazoles nucleus is associated with diverse pharmacological activities such as analgesic, antiasthmatic, diuretic, antihypertensive anticholinergic, antibacterial, antifungal an antiinflammatory properties [1-3], it was thought judicious to study the antibacterial activity *in vitro* of the synthesised new 2,4-dichlorobenzoylthiosemicarbazides and the triazoles with the 2,4-dichlorophenyl moiety.

Chemistry.

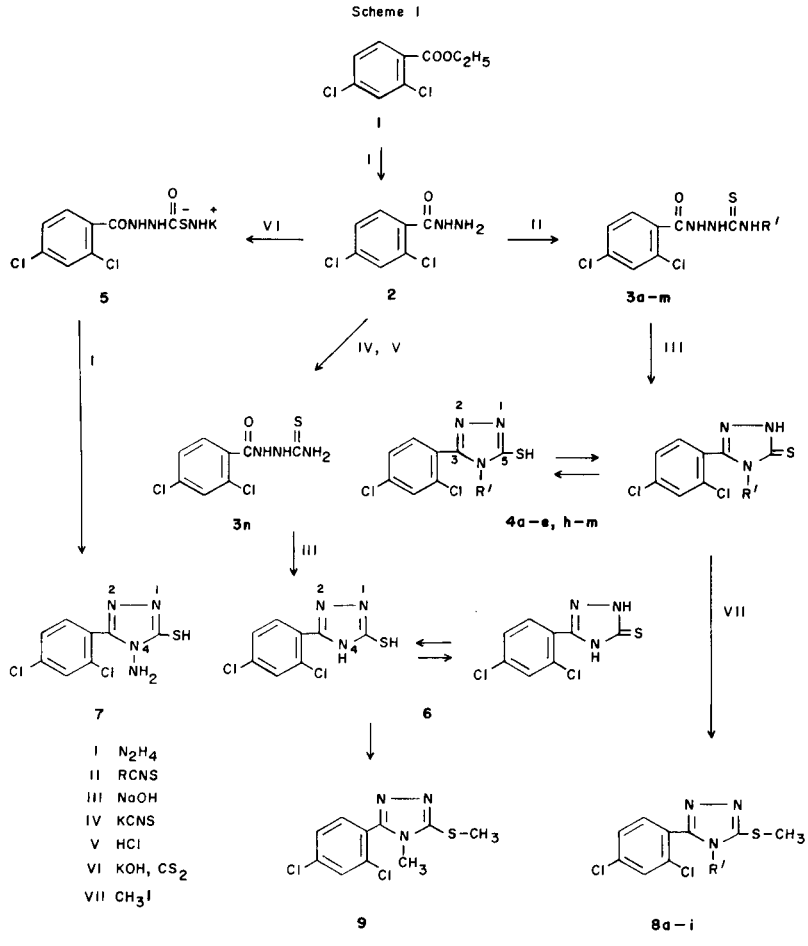
Following the method of Bernstein [4] 1-(2,4-dichloro-

Table 1

1-(2,4-Dichlorobenzoyl)-4-arylthiosemicarbazides **3a-n**




| Compound No. | R' | Mp (°C) | Yield % | Molecular Formula | Microanalyses % Calcd./(Found) C | H | N |
|---|---|---|---|---|---|---|---|
| **3a** | $-C_6H_5$ | 160-161 | 85 | $C_{14}H_{11}Cl_2N_3OS$ | 49.41 | 3.24 | 12.35 |
| | | | | | (49.38 | 3.20 | 12.40) |
| **3b** | $-C_6H_4Br$ (*p*) | 173 | 80 | $C_{14}H_{10}Cl_2BrN_3OS$ | 40.00 | 2.38 | 10.00 |
| | | | | | (40.06 | 2.40 | 10.05) |
| **3c** | $-C_6H_4Cl$(*p*) | 170 | 82 | $C_{14}H_{10}Cl_3N_3OS$ | 44.85 | 2.67 | 11.21 |
| | | | | | (44.79 | 2.60 | 11.25) |
| **3d** | $-C_6H_4Cl$(*o*) | 162 | 90 | $C_{14}H_{10}Cl_3N_3OS$ | 44.85 | 2.67 | 11.21 |
| | | | | | (44.80 | 2.61 | 11.20) |
| **3e** | $-C_6H_4Cl$(*m*) | 164 | 89 | $C_{14}H_{10}Cl_3N_3OS$ | 44.85 | 2.67 | 11.21 |
| | | | | | (44.79 | 2.62 | 11.22) |
| **3f** | $-C_6H_4(NO_2)$(*o*) | 153 | 70 | $C_{14}H_{10}Cl_2N_4O_3S$ | 43.63 | 2.59 | 14.54 |
| | | | | | (43.60 | 2.64 | 14.50) |
| **3g** | $-C_6H_4(NO_2)$(*p*) | 138 | 70 | $C_{14}H_{10}Cl_2N_4O_3S$ | 43.63 | 2.59 | 14.54 |
| | | | | | (43.69 | 2.51 | 14.48) |
| **3h** | $-C_6H_4(NO_2)$(*m*) | 175 | 85 | $C_{14}H_{10}Cl_2N_4O_3S$ | 43.63 | 2.59 | 14.54 |
| | | | | | (43.58 | 2.50 | 14.47) |
| **3i** | $-C_6H_4(CH_3)$(*o*) | 145 | 87 | $C_{15}H_{13}Cl_2N_3OS$ | 50.84 | 3.67 | 11.86 |
| | | | | | (50.81 | 3.60 | 11.88) |
| **3j** | $-C_6H_4(CH_3)$(*p*) | 177 | 90 | $C_{15}H_{13}Cl_2N_3OS$ | 50.84 | 3.67 | 11.86 |
| | | | | | (50.80 | 3.62 | 11.79) |
| **3k** | $-C_6H_4(OCH_3)$(*o*) | 151 | 92 | $C_{15}H_{13}Cl_2N_3O_2S$ | 48.64 | 3.51 | 11.35 |
| | | | | | (48.60 | 3.49 | 11.30) |
| **3l** | $-C_6H_4(OCH_3)$(*p*) | 158 | 89 | $C_{15}H_{13}Cl_2N_3O_2S$ | 48.64 | 3.51 | 11.35 |
| | | | | | (48.58 | 3.56 | 11.40) |
| **3m** | $-CH_2C_6H_5$ | 142 | 85 | $C_{15}H_{13}Cl_2N_3OS$ | 50.84 | 3.67 | 11.86 |
| | | | | | (50.80 | 3.59 | 11.80) |
| **3n** | H | 204 | 88 | $C_8H_7Cl_2N_3OS$ | 36.36 | 2.65 | 15.90 |
| | | | | | (36.40 | 2.62 | 15.82) |




benzoyl)hydrazine (**2**) was obtained by refluxing the ester (**1**) and hydrazine hydrate (99%) in absolute ethanol. Condensation of this hydrazine **2** with suitable aryl isothiocyanates resulted in the formation of 1-(2,4-dichlorobenzoyl)-4-arylthiosemicarbazides **3a-n**. These thiosemicarbazides on oxidative cyclisation [5] with 1*N* sodium hydroxide solution under reflux resulted in their corresponding 3-(2,4-dichlorophenyl)-4-aryl-1,2,4-triazole-5-thiols **4a-e, h-m**. These thiols when treated with methyl iodide in presence of sodium acetate [3] in acetone formed the methylthiotriazoles **8a-i**.

Further the same hydrazine **2** when reacted with potassium thiocyanate and hydrochloric acid [6] resulted in the 1-(2,4-dichlorobenzoyl)thiosemicarbazide (**3n**) which on oxidative cyclisation gave the triazole **6**. Triazole **6** on methylation forms *S*- and *N*-dimethyltriazole **9**.

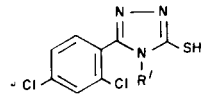
Hydrazine **2** when treated with carbon disulphide and potassium hydroxide formed the potassium salt of the thiosemicarbazide **5** which on treatment with hydrazine hydrate (99%) yielded the 3-(2,4-dichlorophenyl)-4-amino-1,2,4-triazole-5-thiol (**7**). The structures of these newly synthesised compounds were established on the basis of elemental analysis, ir, nmr, and mass spectral data.

Antibacterial Activity.

Applying the agar plate diffusion technique [7] all of the newly synthesised compounds were screened *in vitro* for antibacterial activity against *Bacillus Subtilis, Bacillus Cereus, Esch. Coli* and *Pseudomonas solanarium*. In this method a standard 5 mm diameter sterilised filter paper disc impregnated with the compound (1 mg/ml of acetone) was placed on an agar plate seeded with the test organism. The plates were incubated for 24 hours at 37°. The zone of inhibition of bacterial growth around the disc was observed. The screening results given in Table 4 indicated that all the compounds exhibited antibacterial activities against one or the other type of bacteria. The antibacterial activities of the thiosemicarbazides are comparable to those of their cyclised product triazoles. Only in a few cases the triazoles showed greater inhibition. Almost all the compounds showed more inhibition against *Esch Coli*, whereas inhibition towards *Pseudomonas solanarium* was less. The 4-aminotriazole **7**, showed the highest inhibitory effect against all the test organisms.

Table 2

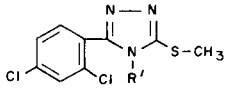
3-(2,4-Dichlorophenyl)-4-aryl-4*H*-1,2,4-triazole-5-thiols **4a-e, h-m, 6** and **7**



| Compound No. | R' | Mp (°C) | Yield % | Molecular Formula | Microanalyses % Calcd./(Found) C | H | N |
|---|---|---|---|---|---|---|---|
| **4a** | -$C_6H_5$ | 210 | 95 | $C_{14}H_9Cl_2N_3S$ | 52.18 | 2.82 | 13.04 |
| | | | | | (52.44 | 2.88 | 13.04) |
| **4b** | -$C_6H_4Br(p)$ | 266 | 89 | $C_{14}H_8Cl_2BrN_3S$ | 41.93 | 2.02 | 10.53 |
| | | | | | (41.89 | 1.99 | 10.47) |
| **4c** | -$C_6H_4Cl(p)$ | 276 | 94 | $C_{14}H_8Cl_3N_3S$ | 47.13 | 2.24 | 11.72 |
| | | | | | (47.05 | 2.24 | 11.76) |
| **4d** | -$C_6H_4Cl(o)$ | 237 | 95 | $C_{14}H_8Cl_3N_3S$ | 47.13 | 2.24 | 11.72 |
| | | | | | (47.08 | 2.20 | 11.80) |
| **4e** | -$C_6H_4Cl(m)$ | 198 | 90 | $C_{14}H_8Cl_3N_3S$ | 47.13 | 2.24 | 11.72 |
| | | | | | (47.06 | 2.28 | 11.77) |
| **4h** | -$C_6H_4(NO_2)(m)$ | 268 | 80 | $C_{14}H_8Cl_2N_4O_2S$ | 45.77 | 2.17 | 15.25 |
| | | | | | (45.71 | 2.31 | 15.30) |
| **4i** | -$C_6H_4(CH_3)(o)$ | 238 | 86 | $C_{15}H_{11}Cl_2N_3S$ | 53.57 | 3.27 | 12.51 |
| | | | | | (53.50 | 3.24 | 12.48) |
| **4j** | -$C_6H_4(CH_3)(p)$ | 201 | 87 | $C_{15}H_{11}Cl_2N_3S$ | 53.57 | 3.27 | 12.51 |
| | | | | | (53.51 | 3.22 | 12.47) |
| **4k** | -$C_6H_4(OCH_3)(o)$ | 260 | 90 | $C_{15}H_{11}Cl_2N_3OS$ | 51.13 | 3.12 | 11.93 |
| | | | | | (51.20 | 3.09 | 11.88) |
| **4l** | -$C_6H_4(OCH_3)(p)$ | 206 | 86 | $C_{15}H_{11}Cl_2N_3OS$ | 51.13 | 3.12 | 11.93 |
| | | | | | (51.22 | 3.16 | 11.90) |
| **4m** | -$CH_2C_6H_5$ | 199-200 | 84 | $C_{15}H_{11}Cl_2N_3S$ | 53.57 | 3.27 | 12.50 |
| | | | | | (53.62 | 3.30 | 12.42) |
| **6** | H | 289-290 | 85 | $C_8H_5Cl_2N_3S$ | 39.02 | 2.03 | 17.07 |
| | | | | | (38.96 | 2.10 | 17.16) |
| **7** | -$NH_2$ | 210 | 80 | $C_8H_6Cl_2N_4S$ | 36.78 | 2.29 | 21.45 |
| | | | | | (36.71 | 2.32 | 21.50) |

Table 3

3-(2,4-Dichlorophenyl)-4-aryl-5-methylthio-4*H*-1,2,4-triazoles **8a-i** and **9**



| Compound No. | R' | Mp (°C) | Yield % | Molecular Formula | Microanalyses % Calcd./(Found) C | H | N |
|---|---|---|---|---|---|---|---|
| **8a** | -$C_6H_5$ | 140-141 | 82 | $C_{15}H_{11}Cl_2N_3S$ | 53.57 | 3.27 | 12.50 |
| | | | | | (53.50 | 3.30 | 12.48) |
| **8b** | -$C_6H_4Br(p)$ | 147-148 | 80 | $C_{15}H_{10}Cl_2BrN_3S$ | 43.37 | 2.40 | 10.12 |
| | | | | | (43.40 | 2.38 | 10.14) |
| **8c** | -$C_6H_4Cl(p)$ | 129-130 | 78 | $C_{15}H_{10}Cl_3N_3S$ | 48.51 | 2.69 | 11.32 |
| | | | | | (48.56 | 2.70 | 11.39) |
| **8d** | -$C_6H_4Cl(o)$ | 120 | 80 | $C_{15}H_{10}Cl_3N_3S$ | 48.51 | 2.69 | 11.32 |
| | | | | | (48.58 | 2.72 | 11.40) |
| **8e** | -$C_6H_4Cl(m)$ | 132 | 75 | $C_{15}H_{10}Cl_3N_3S$ | 48.51 | 2.69 | 11.32 |
| | | | | | (48.56 | 2.73 | 11.40) |
| **8f** | -$C_6H_4(CH_3)(o)$ | 149-150 | 81 | $C_{16}H_{13}Cl_2N_3S$ | 54.85 | 3.71 | 12.00 |
| | | | | | (54.80 | 3.69 | 12.05) |
| **8g** | -$C_6H_4(CH_3)(p)$ | 135 | 82 | $C_{16}H_{13}Cl_2N_3S$ | 54.85 | 3.71 | 12.00 |
| | | | | | (54.89 | 3.68 | 12.06) |
| **8h** | -$C_6H_4(OCH_3)(o)$ | 151 | 83 | $C_{16}H_{13}Cl_2N_3OS$ | 52.45 | 3.55 | 11.47 |
| | | | | | (52.40 | 3.51 | 11.50) |
| **8i** | -$C_6H_4(OCH_3)(p)$ | 105 | 68 | $C_{16}H_{13}Cl_2N_3OS$ | 52.45 | 3.55 | 11.47 |
| | | | | | (52.42 | 3.49 | 11.53) |
| **9** | -$CH_3$ | 114 | 72 | $C_{10}H_9Cl_2N_3S$ | 43.79 | 3.28 | 15.32 |
| | | | | | (43.81 | 3.22 | 15.30) |

Table 4

Antibacterial Activity of the Thiosemicarbazides, Triazoles and Their Methyl Derivatives

| Compound No. [a] | *B. Cereus* | *B. Subtilis* | *Esch. Coli* | *P. Salanarium* |
|---|---|---|---|---|
| **3a** | + | + | + | – |
| **3b** | + | + | – | – |
| **3c** | + | + | + + | + |
| **3d** | + | + | + | + |
| **3e** | + + | + + | + | + |
| **3g** | + | + + | + | – |
| **3i** | – | – | + | – |
| **3j** | – | – | – | – |
| **3k** | – | – | + + | + |
| **3l** | – | – | + + | – |
| **3m** | + | + | + + | + |
| **4a** | + + | + | + + | – |
| **4b** | + | + | + | – |
| **4c** | + + | + | + + | + |
| **4d** | + + | + + | + | + + |
| **4e** | + + | + | + + | – |
| **4h** | + | + | + | + |
| **4i** | – | – | + + | – |
| **4j** | + | – | – | + |
| **4k** | + | – | + | – |
| **4l** | – | + + | + + | + |
| **4m** | – | – | + + | + |
| **6** | + + | – | + + | – |
| **7** | + + + | + + + | + + + | + + + |
| **8a** | + | + | – | – |
| **8b** | – | + | – | – |
| **8c** | + | + | + | + |
| **8d** | – | – | – | – |
| **8e** | + | + | + | – |
| **8f** | + | – | – | – |
| **8g** | + | – | + | – |
| **8h** | + | + | – | – |
| **8i** | + | + | + | – |
| **9** | – | – | + | – |

[a] Numbering is the same as in Tables 1, 2, and 3. Zone of inhibition: + = 5 - 7 mm; + + = 8 - 14 mm; + + + = 15 - 20 mm; ' – ' = No inhibition.

## EXPERIMENTAL

General Procedure.

The melting points were determined on a Buchi oil-heated apparatus and are uncorrected. Infrared spectra ($\nu$ max $cm^{-1}$) were recorded on a Perkin-Elmer 237B spectrophotometer in potassium bromide discs. The nmr spectra were recorded on a Varian T60 instrument at 60 MHz and on a Varian EM-390 instrument at 90 MHz using TMS as internal reference. Mass spectra were recorded on an AEIMS-30 instrument and on a Varian MAT III instrument at 70 eV.

2,4-Dichlorobenzoylhydrazine (**2**) was prepared following the procedure mentioned in the literature [4], yield (60%), mp 163° (lit 163-164°).

1-(2,4-Dichlorobenzoyl)-4-arylthiosemicarbazides **3a-m**.

An equimolecular quantity of 2,4-dichlorobenzoylhydrazine (4.10 g, 0.02 mole) and phenyl isothiocyanate (3.24 g, 0.02 mole) in 40 ml of absolute ethanol was refluxed for 6 hours. On cooling to room temperature fine crystals of 1-(2,4-dichlorobenzoyl)-4-phenylthiosemicarbazides appeared. This was filtered and recrystallised from ethanol. Other thiosemicarbazides of this series were prepared in a similar way using appropriate aryl isothiocyanates, yield 85%, mp 160-161°; ir (potassium bromide): $cm^{-1}$ 3280 (NH), 1660 (C=O); 1350 (C=S); nmr (deuterioacetone): TMS = 0 ppm, 6.2-8.00 (m, 8H, Ar-H), 9.00-9.5 (bs, NH).

*Anal.* Calcd. for $C_{14}H_{11}Cl_2N_3OS$: C, 49.41; H, 3.24; N, 12.35. Found: C, 49.38; H, 3.20; N, 12.40.

1-(2,4-Dichlorobenzoyl)thiosemicarbazide (**3n**).

This was prepared by refluxing a suspension of **2** (2.05 g, 0.01 mole), potassium thiocyanate (1.94 g, 0.02 mole), hydrochloric acid (10 ml) and water (200 ml) for 3 hours. The white solid that appeared on cooling was filtered, dried and recrystallised from ethanol, yield 88%, mp 204°; ir (potassium bromide): $cm^{-1}$ 3280 (NH), 1670 (CONH), 1360 (C=S); nmr (deuteriodimethyl sulphoxide): TMS = 0 ppm, 4.6 (s, 2H), 9-9.5 (bs, NH).

*Anal.* Calcd. for $C_8H_7Cl_2N_3OS$: C, 36.36; H, 2.65; N, 15.90. Found: C, 36.40; H, 2.62; N, 15.82.

3-(2,4-Dichlorophenyl)-4-aryl-1,2,4-triazole-5-thiols **4a-e, h-m**.

1-(2,4-Dichlorobenzoyl)-4-arylthiosemicarbazide (2.38 g, 0.07 mole) was refluxed in sodium hydroxide solution (4%, 25 ml) for 3 hours. The resulting solution was treated with charcoal, filtered and cooled. The filtrate was acidified with hydrochloric acid to *p*H 5-6. The solid which appeared was filtered, dried and recrystallised from dilute ethanol, mp 210°, yield 95%; ir (potassium bromide): $cm^{-1}$ 2575 (weak, SH), 1605 (C=N), 1325 (C=S).

*Anal.* Calcd. for $C_{14}H_9Cl_2N_3S$: C, 52.20; H, 2.82; N, 13.11. Found: C, 52.17; H, 2.79; N, 13.04.

3-(2,4-Dichlorophenyl)-1*H*-1,2,4-triazole-5-thiol (**6**).

Compound **3n** (1.85 g, 0.07 mole) was refluxed in 25 ml of 4% sodium hydroxide solution for 3 hours. The resulting solution after charcoal treatment and filtration was acidified with hydrochloric acid to *p*H 5-6. The solid that appeared was filtered, dried and recrystallised from dilute ethanol, mp 289-290°, yield 85%; ir (potassium bromide): $cm^{-1}$ 2580 (weak, SH), 1375 (sharp, C=S), 1605 (C=N).

3-(2,4-Dichlorophenyl)-4-amino-1,2,4-triazole-5-thiol (**7**).

Following the method of Reid and Heindel [8] a solution of potassium hydroxide (8.40 g, 0.15 mole), 2,4-dichlorobenzoylhydrazine (**2**) (20.5 g, 0.10 mole), carbon disulphide (11.4 g, 0.15 mole) in absolute ethanol (350 ml) was slowly refluxed for 10 hours. This was cooled to room temperature and diluted with 200 ml of dry ether. The precipitate that appeared was filtered, washed with 2 × 50 ml of ether and vacuum dried at 65°. The ir spectra of this salt showed bands ($\nu$ $cm^{-1}$) at 3300, 1625, 1275, 1230 and 1650. To a suspension of 20 mmoles of this potassium salt **5**, hydrazine hydrate (40 mmoles) and water (4 ml) were added and refluxed with stirring for about 1 hour, until the evolution of hydrogen sulphide had ceased to evolve. Dilution with water (100 ml) and acidification with hydrochloric acid allowed a white solid to separate. This was filtered, washed with 2 × 30 ml of cold water and recrystallised from ethanol-water, mp 210°, yield 75%; ir (potassium bromide): $cm^{-1}$ 3290, 2920, 1625, 1565, 1485; nmr (deuterioteridimethyl sulphoxide): TMS = 0 ppm, 5.5 (s, 2H, $NH_2$), and 13.46 (s, 1H, SH).

*Anal.* Calcd. for $C_8H_6Cl_2N_4S$: C, 36.78; H, 2.29; N, 21.45. Found: C, 36.71; H, 2.32; N, 21.50.

3-(2,4-Dichlorophenyl)-4-aryl-5-methylthio-1,2,4-triazoles **8a-i**, **9**.

To a suspension of **4a** (0.321 g, 1 mmole), fused sodium acetate (0.2 g) and methyl iodide (1 mmole) were added. The mixture was refluxed for 4 hours, cooled to room temperature and poured on crushed ice, scratched with a glass rod and kept overnight in a refrigerator. The white solid which appeared was filtered and recrystallised from methanol. A few other triazoles of this series were *S*-methylated in a similar manner. The *S*-methylation was confirmed by the singlet at 2.66 ppm and for compound **9** two singlets at $\delta$ 2.66 ppm and $\delta$ 3.76 ppm.

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