Synthesis and Antibacterial Activity of 1,3,4-Oxadiazole and 1,2,4-Triazole Derivatives of Salicylic Acid and its Synthetic Intermediates

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ABSTRACT

Eight compounds 2–9 **have been synthesized starting from salicylic acid, two of them (**7 **and** 9**) are novel. The four final products namely: 5-(2-hydroxy phenyl)-1,3,4-oxadiazole-2-thione** 4**, 3-(2-hydroxy phenyl)-1H-1,2,4-triazole-5-thiol** 6**, 3-(2-hydroxy phenyl)-4-amino-1,2,4-triazole-5-thiol** 8 **and 3-(2-hydroxy phenyl)-1-amino-1,2,4-triazole-5-thiol** 9 **have been prepared using known reactions. The structures of intermediates and final products were determined by spectroscopic IR, UV, ^{1}H-NMR & MS-methods in addition to elemental analysis. Antibacterial activities of compounds** 1–6 **and** 8 **were investigated *in vitro* against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* and the results are reported herein.**

KEYWORDS

1,3,4-Oxadiazole-2-thione; 1H-1,2,4-triazole-5-thiol; 4-amino-1,2,4-triazole-5-thiol; salicylic acid; antibacterial activity.

1. Introduction

Salicylic acid is widely used as antirheumatic and anti-inflammatory agents[1] while compounds with the thiourea –NH(CS)NH- function show antibacterial and antiviral activities.[2] The 1,3,4-oxadiazoles and 1,2,4-triazoles are known to have a wide scope of biological and industrial activities. Among the biological applications reported for 1,3,4-oxadiazole derivatives are muscle relaxant,[3] analgesic,[3] hypnotic,[3] sedative,[3] CNS depressing,[4] tranquilizing,[4] anticancer[4] and antituberculostatic.[5] Furthermore the 1,3,4-oxadiazole derivatives have some industrial applications in the fields of dyes,[6] photosensivity,[3,6] electrical materials[6] and liquid crystals[3,6].

Similarly 1,2,4-triazole derivatives have considerable biological (antibacterial,[7] antifungal[7] and antitumor[8]) activities and some industrial uses in the fields of photography[9] and corrosion inhibitors.[10] In this paper we report the synthesis of different heterocycles with the salicylic acid moiety represented by compounds **4**, **6**, **8** and **9**.

The thermal rearrangement between **8** and **9** is discussed below. The antibacterial activities of the starting materials, the synthetic intermediates and the products were tested and are reported below.

2. Results and Discussion

2.1. Synthesis

The final products **4, 6**, **8** and **9** have been synthesized by a common pathway as summarized in Scheme 1. The methyl salicylate **2** was synthesized in 90 % yield, and the IR spectrum showed an absorption at 1678 cm^{-1} for the CO-ester group which is in accordance with the literature.[11]

The hydrazide **3** which is used as the starting material for the common synthesis was obtained in 90 % yield by heating the ester **2** with hydrazine hydrate 64 %. The product exhibited characteristic IR bands at 3400 cm^{-1} for OH and 3265 cm^{-1} for NH and 1631 cm^{-1} for CO-N stretching.

For the preparation of the oxadiazole **4**, the hydrazide **3** was heated with an alcoholic solution of KOH and CS_2 under reflux conditions followed by acidification with HCl to give a brownish crystalline product **4** in a very good yield (95 %). The mass spectrum showed a molecular ion at 194.2 (M^+) and the elemental analysis corresponded with structure **4**. The IR spectrum showed an absorption at 1676 cm^{-1} for C=N which suggested that compound **4** existed as the thione tautomer **4a** rather than the thiol form **4b** which normaly exhibits the C=N stretching at lower region, i.e. in about 1638 cm^{-1} due to maximum conjugation.[12] Further support for **4a** came from the ^{1}H-NMR spectrum which exhibited upfield singlets at 13.4 ppm and 10.54 ppm each integrated for one proton which was designated to the NH and OH protons[9,13] while the SH proton which is normally present at 3.5–6.5 ppm[14] was absent. Other signals associated with the aromatic protons appeared to match the expected signals (see the experimental section).

The synthesis of the triazole **6** was achieved by two steps from the hydrazide **3**, first by treatment of **3** with ammonium thiocyanate and HCl for 15 hours under reflux conditions to give the thiosemicarbazide derivative **5** as a crystalline product in a very good yield (94 %). The IR spectrum in CCl_4 solution showed a broad absorption in the region 3300–3100 cm^{-1} due to free and bonded OH and NH. The peak at 1660 cm^{-1} was assigned to CO-N and the peak at 1241 cm^{-1} was assigned to C=S.[14,15] The mass spectrum showed the molecular ion fragments at m/z 138 for salicylamide, 120 for 2-hydroxy benzoyl and 91 for N-aminothiourea. The second step was achieved by heating **5** in ethanolic KOH under reflux conditions followed by removal of the ethanol by vacuum distillation. A solid product was extracted with ethyl acetate from the excess aqueous KOH layer. The extract yielded the triazole **6** in 79 % after evaporation of the organic solvent. The IR spectra in THF solution showed charac-

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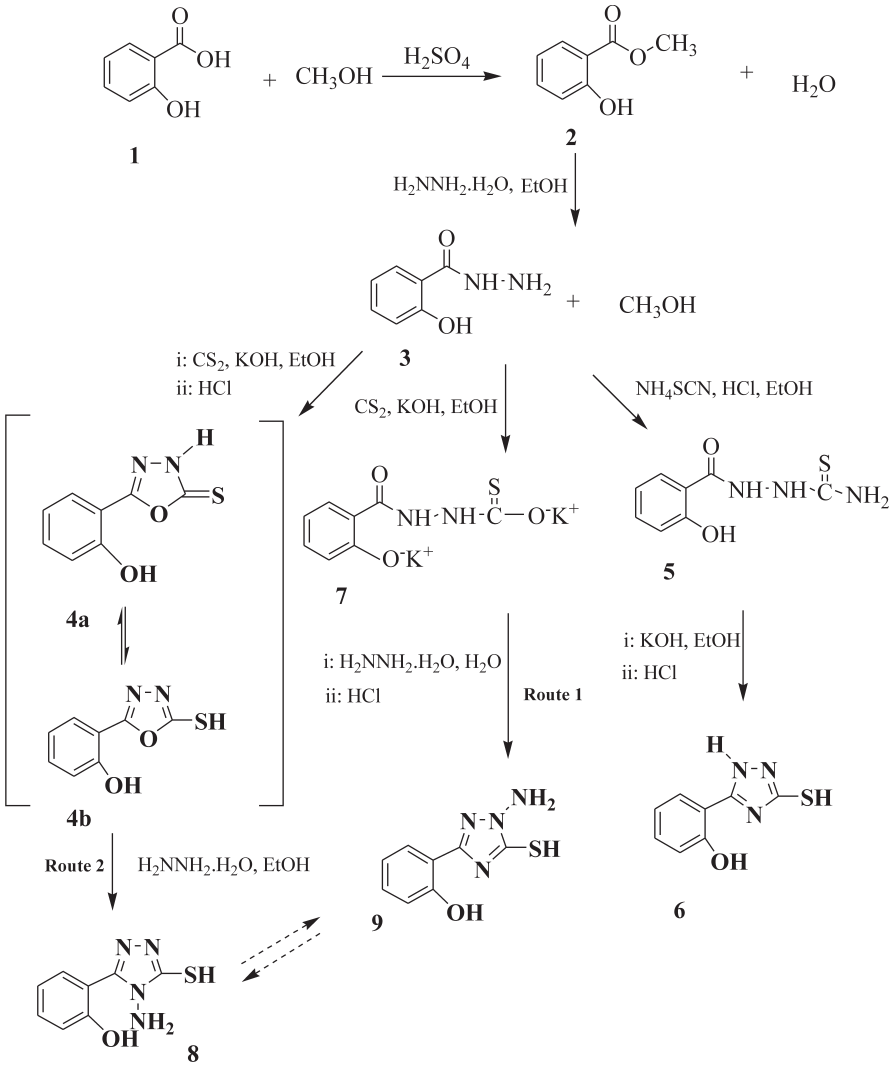


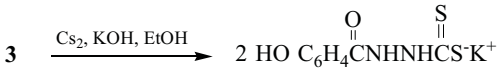
Scheme 1
Synthesis of different heterocycles with the salicylic acid moiety.

teristic absorptions at 3300–3200 cm^{-1}, 2861–2780 cm^{-1} for SH and 1671 cm^{-1} for C=N although some tautomerism to thione might have taken place.[13]

The ^{1}H–NMR spectrum showed signals at 12.95 ppm and 10.05 ppm for NH and OH, respectively.

4-Amino triazole **8** was obtained by heating the oxadiazole **4** and hydrazine hydrate under reflux conditions for 8 h. The product formed crystalline fibres and had a slightly higher melting point 144 °C. The ^{1}H–NMR spectrum showed signals at 13.2 ppm (NH_2), 10.05 ppm (OH) and signals belonging to the phenylene protons at 8.04, 7.25, 6.9, 6.8 and 3.6 ppm for SH. The IR, MS spectrum and elemental analysis were in accordance with the structure for **8**.

The same compound **8** was also prepared according to the method by Dimova[2] and Zhang[16]. This involved the treatment of the hydrazide–**3** with KOH/CS_2 in ethanol and they reported the formation of the dithio carbazinic acid–**10**.



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However, in our hands we isolated the novel 2N-(2-potassium oxy benzoyl)-potassium thiocarbazinic acid–**7** (2-K^+ $^-$O-C_6H_4 $CONHNHCSO^-$ K^+) as a brownish crystalline with melting point 196–197 °C in a yield of 68 %.

Neither of these authors gave any physical evidence for the formation of substance **10**. The infrared spectrum of the product we observed (**7**) showed characteristic absorption bands at 3465 cm^{-1} (broad) for free and bonded N-H, at 1648 cm^{-1} for CO-N and at 1447 cm^{-1} for C=S. The mass spectrum showed a molecular ion (M^+) at m/z 285.3 and a fragmentation ion at m/z 201 which relates to 2-K^+ $^-$O-C_6H_4-$CONHNHCS^+$. The elemental analysis of this compound correlated well with the formula $C_8H_6N_2O_3SK_2$ (see the experimental section).

Treatment of compound **7** with hydrazine hydrate is expected to give **9**, the product was a crystalline solid which showed a lower melting point (87–88 °C) than the 4-amino-triazole (**8**) which was prepared from the oxadiazole **4** as described above (Scheme 1). The IR, UV and elemental analysis for the product **9** were similar to that of **8**, but some differences were observed in the ^{1}H NMR spectrum. The NH signal showed at 13.8 , a signal at 12.25 for OH and the phenylene protons at 7.75, 7.45, 6.95, 6.85 ppm were observed. On the basis of the mass spectrum which showed a molecular ion (M^+) at m/z 208.1 and other spectral and elemental analysis data the structure of 1-amino-triazole **9** was proposed. Formation of compound **9** might have resulted from the thermal rearrangement of the NH_2 group in position N-4 of **8** to position N-1 of **9**, as a similar phenomenon was reported before in the literature[17,18]. Molecular models of **9** suggest that the molecule prefers to exist in a coplanar form,

whereas **8** prefers the non-coplanar form. These differences are obviously affecting the polarity and the π electron distribution between the two forms **8** and **9** and hence affecting their melting points and the NMR spectra.

2.2. Biological Tests

The filter paper disk method (NCCLS)[19,20] was employed in duplicate for the *in vitro* study of antibacterial effects against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and Gram-positive bacteria *Staphylococcus aureus* using Ampicellin and Gentamycin as references. The inhibitory effects of compounds **1–6** and **8** against these bacteria are summarized in Table 1 and are shown in Figs 1–3.

The screening results indicate that all the examined compounds, generally exhibit moderate activities against Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* as compared with Ampicellin and Gentamycin. The triazole **6** has a better inhibition effect than the Gentamycin against *S. aureus* since the latter is known to show some resistance to Gentamycin.[21,22] On the other hand the Gram-negative bacteria *P. aeruginosa* was only affected by the triazole **6** and the salicylic acid **1**.

The inhibitory activities of the tested compounds on the Gram-negative and Gram-positive bacteria are arranged from higher to lower activities as follows.

Compounds **2** and **6** have a moderately active effect on the Gram-negative bacteria *E. coli*, while the compounds **5**, **8** and **3** as well as **1** exhibited slight activity against the same bacteria.

Compounds **6**, **8**, **1**, **2**, **4a** and **5** showed moderate to slight activity against the Gram-positive bacteria *S. aureus*.

Compounds **4a** and **1** showed moderate to slight activity against the Gram-negative bacteria *P. aeruginosa*, while the compounds **2**, **3**, **4a**, **5** and **8** exhibited no effect against the same bacteria.

To confirm the above test, the minimum inhibition concentrations were determined in liquid medium for the active substances for three times and the averages are shown in Table 2.

Table 1 Inhibition zones in (mm).

Compound*	*E. coli*	*S. aureus*	*P. aeruginosa*
Ampicellin	10	10	10
Gentamycin	10	08	12
1	6	7.5	6
2	9.5	6.5	0
3	6	0	0
4a	8.5	6	0
5	8	6	0
6	9	9	9
8	7	7.5	0

* Concentration 10 mg mL^{-1}.
Key to the inhibition zones activities.
Highly active = inhibition zone >12 mm.
Moderately active = inhibition zone 9–12 mm.
Slightly active = inhibition zone 6–9 mm.
Inactive = inhibition zone <6 mm.

3. Experimental

3.1. General

The melting points were measured with a BÜCHI 540 melting point apparatus and are uncorrected. The IR spectra were recorded using KBr discs and a JASCO V-530 spectrophotometer and in the IR spectra solutions were obtained with a GENESIS II FTIR spectrophotometer. The UV spectra were recorded on a ZUZI Split-Beam UV-Vis 4418PC (4418SPC) spectrophotometer. The ^{1}H NMR (250 MHz) spectra were recorded in DMSO-d_6 and the Mass spectra were recorded on a MAT 312 mass spectrometer using glycerol as matrix.

Microorganisms in this study were supplied by the university hospital of Oran and identified in the laboratory of applied microbiology, University of Oran Es Senia. The Mueller Hinton medium was supplied by Difco.

3.2. Synthesis of Compounds

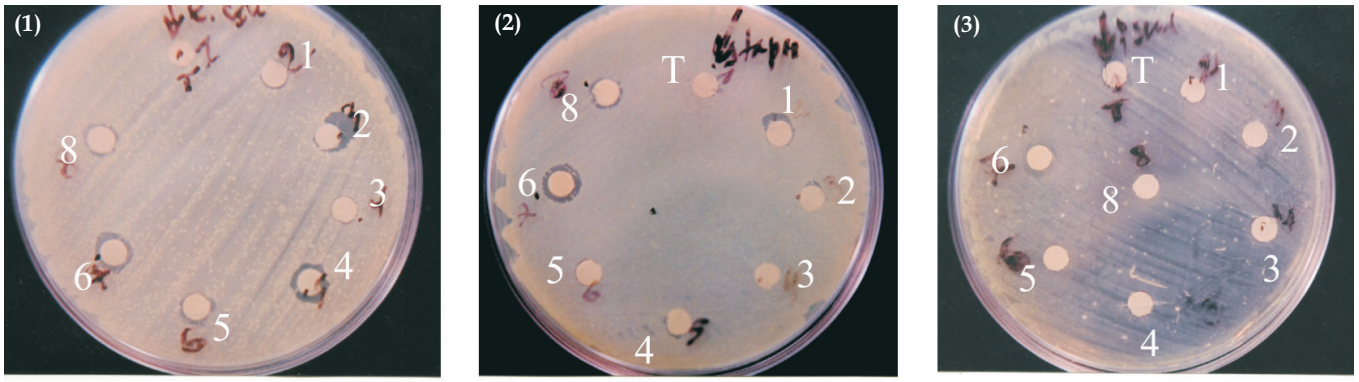
Methyl-2-hydroxybenzoate **2** *(Methylsalicylate)*

To a mixture of salicylic acid **1** (20.7 g, 0.15 mole) in methanol (90 mL), conc., H_2SO_4 (16 mL) was added dropwise with stirring. The mixture was refluxed on a water bath at 80 °C for 5 h.

TLC eluted with ethanol/benzene 1:4 showed $R_f = 0.55$ for the acid **1** and $R_f = 0.74$ for the ester **2**. Ice water (100 mL) was added at the end of the reaction with stirring. The aqueous mixture was extracted two times with n-hexane (25 mL). The combined organic layers were washed with 5 % aqueous $NaHCO_3$ (150 mL) until the pH reached 7 and then washed with 50 mL of water. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to dryness to give a colourless oil; methyl salicylate 2 (20.6 g, yield 90 %); ν_{max}/ cm^{-1} (liquid film) 3187 (OH), 1678 (CO). Lit 3190 (OH)[11], 1675 (CO)[11]. UV (methanol), λ_{max} 235 nm. Lg ε 3.52. Lit. λ_{max} 237. Lg ε 4.0[11].

Salicylic Hydrazide **3**

Methylsalicylate **2** (5.0 g, 0.032 mole), ethanol (20 mL) and hydrazine hydrate 64 % (6 mL) were mixed together and heated under reflux at 80 °C for 8 h. TLC eluted with ethanol/benzene 1:4 showed the development of a new spot at $R_f = 0.51$. Ethanol was evaporated under reduced pressure and a white solid product was recrystallized from H_2O/MeOH to give salicylic



Figures 1–3 Antibiogrammes of (**1**) *E.scherichia coli.*, (**2**) *Staphylococcus aureus*, and (**3**) *Pseudomonas aeruginosa.*

Table 2 Inhibition of microorganisms by compounds **1–6** and **8** at different concentrations.

Compound:	**1**			**2**			**3**			**4a**			**5**			**6**			**8**		
	Concentration‡ $\mu g\ mL^{-1}$			Concentration‡ $\mu g\ mL^{-1}$			Concentration‡ $\mu g\ mL^{-1}$			Concentration‡ $\mu g\ mL^{-1}$			Concentration‡ $\mu g\ mL^{-1}$			Concentration‡ $\mu g\ mL^{-1}$			Concentration‡ $\mu g\ mL^{-1}$		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
i†	+	–	–	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	–	–
ii	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–
iii	+	+	–	+	–	–	–	–	–	+	–	–	+	–	–	+	+	+	+	+	–

† Microorganisms: i, *Escherichia coli;* ii, *Pseudomonas aeruginosa;* iii, *Staphylococcus aureus.*
‡ Concentrations ($\mu g\ mL^{-1}$): a = 640 ; b = 320 ; c= 160.
Notes: the sign (+) for microorganism inhibitors and (–) for microorganisms intact.

hydrazide **3** (4.5 g, yield 90 %), m.p. 147 °C, lit. 147–150 °C[23], ν_{max}/ cm^{-1} (KBr) 3400 (OH), 3265 (NH), 1591 (CO-N), 1364 (Aromatic); UV (methanol) λ_{max} 240, 300 nm. Lg ε 3.66, 3.55, respectively.

5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2-thione **4a**

Salicylic hydrazide **3** (1.5 g., 0.01 mole) and ethanol (200 mL) were added to a solution of KOH (0.84 g, 0.015 mole) in ethanol (20 mL) and CS_2 (20 mL). The reaction mixture was heated under reflux at 80 °C for 9 h. The mixture became orange in colour. TLC eluted with ethanol/benzene 2:4 showed a product as R_f = 0.3. Excess ethanol was removed under vacuum and the remainder of the solution was acidified with dil. HCl (10 %) to pH 5. A brownish solid was filtered off and washed with ethyl acetate to dissolve the organic product. The washing solution upon standing overnight at room temperature gave brown fibres which were recrystallized from $CHCl_3$/EtOH to give **4** (1.85g, 95 % yield), m.p. 167–168 °C; ν_{max}/ cm^{-1} (CCl_4) 1676 (C=N), 1304–1251, 1157 (C-O-C); U.V. (methanol) λ_{max} 235, 290, 300 nm. Lg ε 3.62, 3.54, 3.57, respectively; δ_H (250 MHz, DMSO-d6) 13.4(1H,s), 10.54 (1H, s), 7.61 (1H, d), 7.31 (1H t), 6.97 (1H d), 6.92 (1H, t); MS, 194.2 M^+. (Found: C, 49.21; H, 02.98; N, 14.39 %. Calc. for $C_8H_6N_2O_2S$ (194.21); C, 49,48; H, 03.09; N, 14.43 %.

Thiosemicarbazide Salicylic Acid **5**

Salicylic hydrazide **3** (1g, 0.066 mole) was dissolved in ethanol with stirring. Ammonium thiocyanate (1.6 g, 0.021 mole) and HCl (26 mL, 31 %) were added and the reaction mixture was heated under reflux on a water bath for 15 h. TLC eluted with ethanol/benzene 2:4 showed the development of a new spot, R_f = 0.55. Excess solvent was evaporated to almost dryness and the crystalline solid was filtered off and recrystallized from toluene/petroleum-ether 60–80 to give **5** (1.3 g, yield 94 %), m.p. 144 °C; ν_{max}/ cm^{-1} (CCl_4) 3300–3100 (br. OH, NH, stretching), 1660 (CO-N), 1610 (C=C), 1241 (C=S); U.V.(methanol) λ_{max} 235, 300 nm, Lg ε 3.49, 3.41. δ_H (250 MHz, DMSO-d6) 13.8 (4H,m),10.05(1H,s), 7.7 (1H, d), 7.4 (1H, t), 6.9 (1H, d), 6.8 (1H, t). MS, 138 M^+ (salicylamide), 120 M^+ (2-oxy-benzoyl), 91 M^+(N-aminothio urea) (Found: C, 45.12; H, 4.09; N, 19.70 %. Calc. for $C_8H_9N_3O_2S$ (211.24), C, 45.50; H, 4.27; N, 19.9 %.

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

Thiosemicarbazide salicylic acid **5** (2.1 g, 0.01 mol) was dissolved in ethanol (200 mL). An ethanolic solution of KOH (0.85 g, KOH, 0.015 mol) 20 mL was added and heated under reflux at 80 °C for 4 h, to give one TLC spot (ethanol/benzene 2:4) at R_f = 0.11. Excess ethanol was evaporated to dryness and the bulk of the solid was dissolved in ethyl acetate, filtered and evaporated to dryness to give a solid which was recrystallized from petroleum-ether/CH_2Cl_2 (1/3) to give brownish fibres **6** (1.5 g, yield 79 %), m.p. 135 °C; ν_{max}/ cm^{-1} (THF) 3300–3200 (OH and NH), 2861–2780 (SH) and 1671 (C=N); U.V. (methanol) λ_{max} 235, 300. Lg ε 3.55, 3.49, respectively. δ_H (250 MHz, DMSO-d6) 12.95 (1H, s),10.05(1H,s), 7.75 (1H, d), 7.42 (1H, t), 6.95 (1H, d), 6.9 (1H, t),4.2(1H,s). MS, 191.8 M^+. (Found: C, 49.32; H, 03.52; N, 21.51 %. Calc. for $C_8H_7N_3OS$ (193.23); C, 49.74; H, 03.63; N, 21.76 %).

2N-(2-Potassium oxybenzyl)-potassium Thiocarbazinic Acid **7**

A mixture of salicylic hydrazide **3** (1.5 g, 0.01 mole) in ethanol 230 mL, alcoholic solution of KOH (8.4 g, 0.15 mole) in ethanol 15 mL and CS_2 (9 mL 0.15 mol) were added dropwise and heated under reflux on a water bath at 80 °C for 10 h. The ethanol was partially evaporated to 100 mL. The reaction mixture was cooled to room temperature, ether (200 mL) was added and a brownish precipitate was formed. The product was filtered off and washed twice with ether (50 mL), dried at room temperature to give a solid mass which was dissolved partially in warm ethylacetate and filtered off. The filtrate was evaporated down to dryness to give brownish crystalline **7**, recrystallized from chloroform/ethanol (1/1) to give the pure product (1.5 g, yield 59 %), m.p. 196–197 °C. ν_{max}/cm^{-1} (THF) 3465 (NH), 1648 (CON); 1238 (C=S); U.V, λ_{max} (methanol), 300, 290, 245 nm, Lg ε, 3.53, 3.50, 3.54, respectively. MS, 201 (2-KO-C_6H_4-CONHNHCS) and 285.3 (7); (Found: C, 37.51, H, 2.92, N, 09.26 %. Calc. for $C_8H_6N_2O_3SK_2$ (248); C, 33.33; H, 02.08; N, 09.7 %).

3-(2-Hydroxy phenyl)-4-amino-1H-1,2,4-triazole-5-thiol **8**

The oxadiazole 4 (0.97 g, 0.005 mole) was dissolved in 80 mL ethanol and hydrazine hydrate 64 % (10 mL) was added and the reaction mixture was heated under reflux on an water bath at 90 °C for 8 h. TLC (ethanol/benzene 2:4) gave a spot at R_f = 0.4. Excess ethanol was evaporated and the remaining solid/liquid mixture was filtered off and washed with ethyl acetate to give yellowish-brown fibres (**8**) which was recrystallized from chloroform/ethanol (2/1) to give yellowish-brown crystals (0.75 g, yield 72 %), m.p. 144 °C; ν_{max}/ cm^{-1} (THF) 3500–3400 (OH, NH); 2681 (SH); UV.(methanol) λ_{max}, 240, 285 nm, Lg ε 3.5, 3.30. δ_H (250 MHz, DMSO-d6) 13.2(2H,s), 10.05(1H,s), 8.04 (1H, d), 7.25 (1H, t), 6.9 (1H, d), 6.8 (1H, t),4.2(1H,s). MS 208,3; (Found: C, 45.82; H, 3.7; N, 26.63 %. Calc. for $C_8H_8N_4OS$ (208.24); C, 46.15; H, 3.84; N, 26.92 %).

3-(2-Hydroxy phenyl)-1-amino-1H-1,2,4-triazole-5-thiol **9**

Compound **7** (1.1 g, 0.004 mole) dissolved in water (8 mL) and hydrazine hydrate 64 % (4 mL) were heated under reflux on an oil bath at 110 °C for 6 h. TLC (ethanol/benzene 2:4) gave a spot at R_f = 0.31. The reaction mixture was cooled to room temperature, iced-water 100 mL was added and the solution was made acidic with 10 % HCl. A precipitate formed and was filtered off. The filtrate was extracted three times with 30 mL of ethyl acetate. The extracts were combined and dried over anhydrous Na_2SO_4. The

filtrate was evaporated to dryness to give a brown solid **9**, which was recrystallized from chloroform/ethanol (2/1) to give of product, (0.61 g, yield 68 %), mp. 87–88 °C. ν_{max} / cm^{-1} (THF) 3500–3400 (OH, NH), 2677 (SH); U.V. (methanol) λ_{max}, 230 nm. Lg ε 3.48. δ_H (250 MHz, DMSO-d6) 13.85(2H,s), 10.25 (2H, s), 7.75 (1H, t), 7.45 (1H, t), 6.95 (1H, d), 6.85 (1H, t), 3.9(1H,s). MS, 208.1 M^+; (Found: C, 45.83; H, 03.72; N, 26.63 %. Calc. for $C_8H_8N_4OS$ (208.24); C, 46.15; H, 03.84; N, 26.92 %).

3.3. Antibacterial Tests

The filter paper disc method was performed in duplicate using fresh Mueller Hinton agar medium. This agar medium was inoculated with 0.5 mL of cultures containing about 10^6 CFU/mL. Filter paper discs (5 mm diameter) saturated with solutions of each compound (concentrations 10 mg mL^{-1} ethanol) was placed on the indicated agar mediums. The incubation time was 24 h at 37 °C. The blank test disc with ethanol was used. Inhibitory activity was evaluated by measuring the diameter of clear zone observed around the disc (in mm).

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