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Synthesis and Antimicrobial activity Of Some New Thiosemicarbazones

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ABSTRACT

Some new [5-mercapto-3-(p-nitrophenyl)-4-amino substituted-1,2,4-triazole] thiosemicarbazones were synthesized in microwave and there structures were confirmed by I.R. and 1H NMR and their antimicrobial screening against *E. Coli* , *Staphylococcus aureus* and *Candida albicans* were established by zone of inhibition.

Key words: thiosemicarbazones, zones of inhibition, antimicrobial activity.

1. INTRODUCTION

In recent years much attention has been focused on the synthesis of 1,2,4-triazoles and their derivatives because of their wide spectrum biological properties. A large number of Nitrogen containing heterocycles display antifungal, antibacterial, anticonvulsant, insecticidal, anti-inflammatory, tuberculotherapeutic and anti-HIV activity[1-6].The earlier studies have also indicated that the thiol/mercapto grouping at position 5 of 1,2,4-triazoles enhances biological properties[7-10]. It was thought of interest to synthesize some substituted 5-mercapto-3(4-nitrophenyl)-4-amino substituted-1,2,4-triazole compounds for their antibacterial and antifungal activity.

2. MATERIALS AND METHODS

All the chemicals and solvents were obtained from commercial sources like S. D. Fine Chem. Research Lab.Ind., Lancaster (Germany) and purified using standard procedure whenever required. Melting points were measured in open capillary tube on VEEGO (VMP-D) melting point apparatus and are uncorrected. IR spectra were recorded in KBr pellets on a SHIMADZU FTIR 8400S infrared spectrophotometer. The ^{1}H-NMR spectra were determined in DMSO-d_6 at 300 MHz on a JEOL FT NMR spectrophotometer using TMS as an internal standard. The progress of the reaction and the purity of compounds were monitored by TLC using silica gel plates. Microwave assisted reaction were carried out in a "CATALYST SYSTEM" microwave oven.

Micro Wave Assisted Synthesis of Ethyl-p-nitrobenzoate (1) (Figure 1)

In a 250 ml Microwave flask, a mixture of 25 gm (0.149mole) pure p-nitrobenzoic acid, 66.8 gm (80ml,1.45 mole) of absolute ethanol and 11.92 gm (6.49ml) of concentrated sulphuric acid were taken. A few porcelain chips were added and the reaction mixture was refluxed in Microwave for 2.5 hours at 7-8 power. The excess of ethanol was removed under reduced pressure and the reaction mixture was cooled. Sufficient saturated solution of sodium bicarbonate was added to render the reaction mixture free from acid. Ethyl p-nitrobenzoate was separated out as solid. It was filtered out on buchner funnel and was dried in air and was collected.

Molecular formula $C_9H_9NO_4$,, Molecular weight 195, Yield (%) 75.36%, m.p. 56-59 °C

IR (KBr, cm^{-1}): 1716.53($COOC_2H_5$), TLC: Ethyl acetate: Hexane (3:2)

Microwave Assisted Synthesis of P-nitrobenzocarbohydrazide (2)

A mixture of Ethyl p-nitrobenzoate 26.00gm (0.13 mole) and hydrazine hydrate 9.90gm, (9.62ml, 0.19mole) was refluxed with 60.00ml absolute Ethanol for 3-4 hours in Micro Wave Oven at 6-7 power. Then Ethanol was distilled off, the reaction mixture was cooled, yellow crystals of p-nitrobenzocarbohydrazide was precipitated out, it was filtered on buchner funnel, dried and collected.

Molecular formula $C_7H_7N_3O_3$, Molecular weight181, Yield(%)73.45%, m.p.210^{0}C

IR(KBr, cm^{-1}): 1678 (CONH),1518 ($C=NO_2$), TLC: Ethyl acetate: Hexane (3:2)

Synthesis of Potassium Salt of P-nitrobenzocarbohydrazide (3)

590ml absolute Ethanol was taken in a 1000ml round bottom flask, 40.50gm (0.72mole) potassium hydroxide was added in it and was stirred to dissolve potassium hydroxide. In this mixture 72.00gm (0.39 mole) p-nitrobenzocarbohydrazide and 54.96gm (43.62ml, 0.72mole) carbon disulphide was also added. This mixture was agitated for 12-18 hour. It was diluted with 149ml dry Ether and the product was filtered on buchner funnel and vacuum dried at 65-70^{0}C. The salt prepared as described above was obtained in qantitative yield and were used without further purification.

Fig 1. Scheme of Synthesis

Preparation of 5-mercapto- 4-amino- 3-(p-nitrophenyl)-1,2,4-Triazole (4)

105.00gm (0.35mole) Potassium Salt was dissolved in sufficient water in 1000ml round bottom flask. To this solution, 99% Hydrazine Hydrate 26.25gm (25.48ml, 0.52mole) was added. The reaction mixture was refluxed on a water bath until the evolution of H_2S gas ceased. It was then diluted with cold water (200-300ml) and was carefully acidified with concentrated hydrochloric acid. Thus yellowish white solid was precipitated out, it was filtered on buchner funnel, washed with cold water, dried and recrystallised from ethanol.

Molecular formula $C_8H_7N_5SO_2$, Molecular weight 237, Yield 56.59%, m.p. 245 °C

IR(KBr,cm^{-1}): 3350(NH_2), 3093,2956.67,835.12 (-CH),1514.02 ($C-NO_2$), 1606.59 (C=N),1352.01 (C=S),1170 (C-N-C), TLC: Ethyl acetate: Hexane (3:2)

Synthesis of 5-mercapto-3-(p-nirophenyl)-4H-1,2,4-triazole-4-yl thiosemicarbazide (5)

Sodium hydroxide 1.1gm (0.027 mole) and carbon disulphide (0.021mole, 1.2 ml) was added to a solution of 5-mercapto-4-amino-3(p-nitrophenyl)-1,2,4-triazole 2.00gm (0.0084mole) in DMF (20ml). The mixture was stirred at 0-10^{0}C for 1:45 hour, this stirred mixture was added Hydrazine hydrate 1.2ml (0.024 mole) and stirring was continued at 70-80 ^{0}C for 1:45 hour, more on adding crushed ice, the greenish white solid crystals separated out which was recrystallized from ethanol-water (4:1).

TLC: Ethyl acetate: Hexane (3:2)

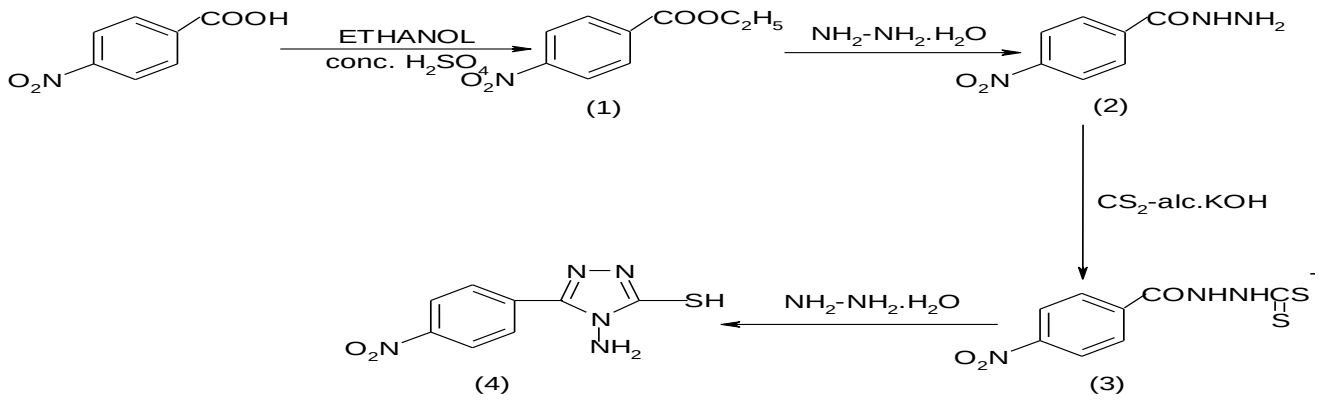
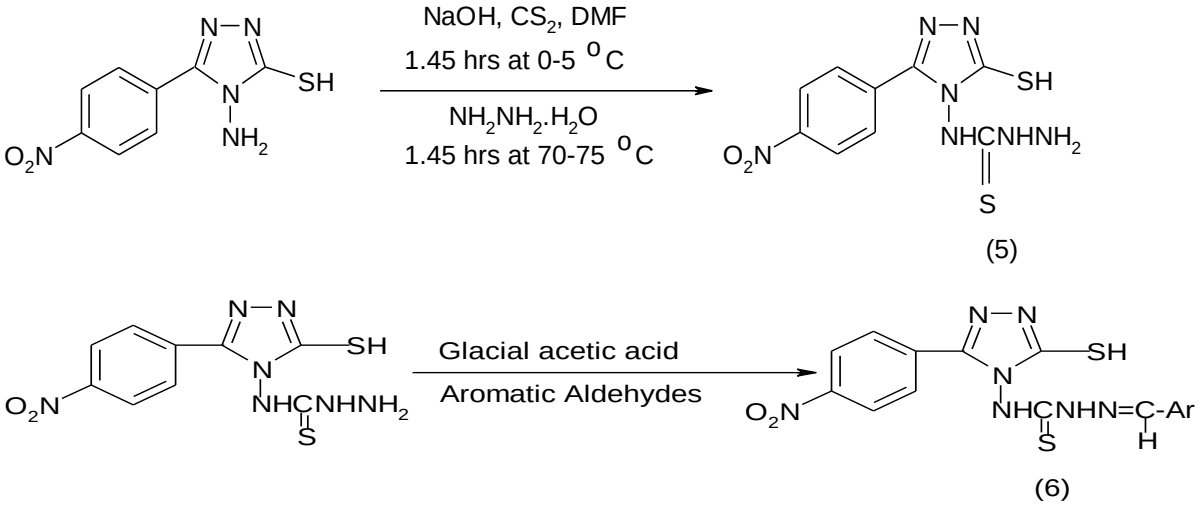


Fig 2. Scheme of Synthesis



IR (KBr, cm^{-1}): 3276.83(NH_2),1504.37(C-NO_2) 1618.17(C=N),1309.58(C=S),1188.07(C-N C),1269.07(C=S),3093,2920.03 (-CH),1477.37(C-C), 1677.95(-CSNH)

1H-NMR: 13.617 (S,1H,SH); 9.31 (S,1H,N-NH); 5.63-5.77(T,1H, NH-NH_2), 6.59-6.61 (D, 2H, NH-NH_2) , 7.6-7.9(M,Ar-H)

Synthesis of Schiff's bases of 5-Mercapto-3-(p-nitrophenyl)-4H-1,2,4-triazole-4-yl-thiosemicarbazone (General Microwave Procedure) (6)

5-Mercapto-3-(p-nitrophenyl)-4H-1,2,4- triazole-4-yl-thiosemicarbazide 0.22gm (0,0007mole) and sufficient glacial acetic acid was taken in a 150ml microwave flask. pH was adjested in 3-5 range. To this mixture various aromatic aldehydes (0.001 mole) were added and reaction mixture was refluxed in microwave oven at power 7 (455 watt, 65%) for 2.5 hrs. Reaction was monitored by TLC. Then the reaction mixture was poured into crushed ice, solid schiff base was precipitated out. It was filtered on simple funnel by using whatman paper and solid was washed with water, dried and recrystallized from ethanol-water (4:1).

2-chloro benzaldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1, 2, 4-triazol-4-yl)-thiosemicarbazone (6a)(Table 1)

TLC: Ethyl acetate: Hexane (3.2)

IR (KBr, cm^{-1}): 1591.16 (-N=CH) ;1271 (N C=S)1355.86(C=S);835.12(Ar.-CH); 2933.53 (ArC-H str.assy.) 1423.37 (ArC-C multiple bond str),3105.18(NH str),1508.23(C NO_2).1H-NMR: 14.23 (S,1H,SH);

10.2(S,1H,NH);10.41(S,1H,NH), 7.51- 8.0 (M,8H,2-phenyl); 8.9(S,1H,N=CH).

2-nitro benzaldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1, 2, 4-triazol-4-yl)-thiosemicarbazone (6b)

TLC: Ethyl acetate: Hexane (3:2)

IR (KBr, cm^{-1}): 1602.74 (-N=CH) , 1263.29 (N-C=S),838.98(Ar.-CH),2939.31(Ar-CHstr.assy.),1421.44(ArC-Cmultiple bond str),3105.18 (NHstr),1340.43(C=S), 1525.59(C-NO_2)

1H-NMR: 14.23(S,1H,SH),10.46 (S,1H,NH);10.20(S,1H,NH), 6.60-8.17 (M,8H,2-phenyl); 6.6(S,1H,N=CH)

3-nitro benzaldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1, 2, 4-triazol-4-yl)-thiosemicarbazone (6c)

TLC: Ethyl acetate: Hexane (3:2)

IR (KBr, cm^{-1}): 1602.74 (-N=CH), 1265.22 (N-C=S),837.05 (Ar.-CH),2945.1(ArC-H str.assy.),1421.44(ArC-Cmultiple bond str),3103.25 (NHstr),1350.O8(C=S),1529.45(C-NO_2)

1H-NMR: 14.2(S,1H,SH),10.01 (S,1H,NH),10.21(S,1H,NH), 7.53-8.90 (M,8H,2-phenyl), 6.62(S,1H,N=CH)

2-hydroxy benzaldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1, 2, 4- triazol-4-yl)-thiosemicarbazone(6d)

TLC Ethyl acetate: Hexane (3:2);

IR (KBr, cm^{-1}): 1606.59 (-N=CH),1271 (N-C=S),842.83 (Ar.-CH bend),2929.67 (ArC-H str. assy.), 1423.37(Ar C-C multiple bond str),1359.72(C=S),3105.18(NHstr), 1512.09(C-NO_2),3400(OH)

1H-NMR14.1(S,1H,SH),10.20(S,1H,NH),10.46(S,1H,NH),6.96-7.79(M,8H,2-phenyl), 6.59 (S,1H,N=CH),9.89(S,1H,OH).

4-hydroxy benzaldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1, 2, 4- triazol-4-yl)-thiosemicarbazone(6e)

TLC Ethyl acetate: Hexane (3:2)

IR (KBr, cm^{-1}): 1598.88 (-N=CH),1259.43 (N-C=S), 833.19 (Ar.-CH bend),2923.88 (ArC-H str.assy.), 1425.30(Ar C-C multiple bond str), 1365.51(C=S),3174.61(NH str),1512.09 (C-NO_2),3400 (OH).

p-Dimethyl amino benzaldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1,2,4-triazol-4-yl)-thiosemicarbazone(6f)

TLC: Ethyl acetate: Hexane (3:2)

IR (KBr, cm^{-1}): 1587.31 (-N=CH);1259.43 (N-C=S)813.9 (Ar.-CH bend);2920.03 (ArC-H str.assy.); 1431.08(Ar C-C multiple bond str)1367.44(C=S),3107.11(NH str)1529.45 (C-NO_2),1170.71 (C-N-C).

4-methoxy benzaldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1, 2, 4- triazol-4-yl)-thiosemicarbazone (6g)

TLC Ethyl acetate: Hexane (3:2)

IR (KBr, cm^{-1}): 1602.74 (-N=CH);1251.72 (N-C=S)833.19 (Ar.-CH bend);2925.81 (ArC-H str.assy.); 1423.37(Ar C-C multiple bond str)1301.86(C=S),3120.61(NH str)1508.23 (C-NO_2),1166.85(C-N-C).

Cinnamaldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1, 2, 4-triazol- 4-yl)-thiosemicarbazone (6h)

TLC Ethyl acetate: Hexane (3:2),

IR (KBr, cm^{-1}): 1602.74(-N=CH);840.91 (Ar.-CH bend);2925.81 (Ar-CHstr.assy.); 1421.44(Ar C-C multiple bond str)1311.5(C=S),3056.96(NH str)1498.59 (C-NO_2),1182.28(C-N-C)

Furfuraldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1, 2, 4-triazol- 4-yl)-thiosemicarbazone(6i)

TLC Ethyl acetate: Hexane (3:2),

IR (KBr, cm^{-1}): 1606.59 (-N=CH);1274.86 (N-C=S)838.98 (Ar.-CH bend);2920.03 (Ar-CH str.assy.); 1340.43(C=S),1508.23 (C-NO_2),1166.85(C-N-C).

Benzaldehyde N-(5-Mercapto-3-(p-nitrophenyl)-4H-1,2,4-triazol-4-yl)-thiosemicarbazone (6j)

TLC Ethyl acetate: Hexane (3:2),

IR (KBr, cm^{-1}): 1604.59 (-N=CH);1271.86 (N-C=S)837.98(Ar.-CH bend);2920.03(Ar-CH str.assy.);1340.43(C=S),1508.23(C-NO_2), 1164.84(C-N-C).

Table 1: Physical Data of 5 and 6a-6j

Comp.	R	M.F.	m.p. (°C)	Yield (%)
5		$C_9H_9N_7O_2S_2$	235	8.39
6a	2-Chloro phenyl	$C_{16}H_{12}N_7O_2S_2Cl$	160	50.00
6b	2-Nitro phenyl	$C_{16}H_{12}N_8O_4S_2$	200	64.51
6c	3-Nitro phenyl	$C_{16}H_{12}N_8O_4S_2$	210	64.51
6d	2-hydroxy phenyl	$C_{16}H_{13}N_7O_3S_2$	200	51.72.
6e	4-hydroxy phenyl	$C_{16}H_{13}N_7O_3S_2$	205	51.00
6f	4-dimethylamino phenyl	$C_{18}H_{19}N_8O_2S_2$	220	32.25
6g	4-methoxy phenyl	$C_{17}H_{15}N_7O_3S_2$	223	50.00
6h	cinamaldehyde	$C_{18}H_{15}N_7O_2S_2$	147	49.00
6i	formaldehyde	$C_{14}H_{11}N_7O_3S_2$	215	37.03
6j	benzaldehyde	$C_{16}H_{13}N_7O_2S_2$	223	35.71

Antibacterial activity

The compounds 5 and 6a-6j were screened in vitro for their antibacterial activity against pathogenic organisms E. Coli and S. Aureus using disc diffusion method (nutrient agar) at a concentration of 100 µg/ml and 150 µg/ml with DMF as the solvent[11-14]. After 24 hrs of incubation at 37 °C the zones of inhibition formed were measured in mm with standard drug Streptomycin (Table 2).

Table 2: Inhibition Zones (mm)

Sr No	Concentrations (µg/ml)	E. coli	S. aureus	C. albicans
5	150	18	15	28
	100	14	13	25
6a	150	15	28	**40**
	100	07	21	**35**
6b	150	**26**	**35**	**35**
	100	18	12	28
6c	150	**34**	**27**	**36**
	100	**29**	21	**31**
6d	150	**35**	**31**	**41**
	100	**30**	**26**	**34**
6e	150	22	17	18
	100	18	15	16
6f	150	21	18	16
	100	18	16	15
6g	150	13	15	17
	100	12	14	16
6h	150	16	17	18
	100	14	15	14
6i	150	14	16	22
	100	12	13	18
6j	150	17	21	29
	100	13	19	24
Std Antibacterial (Streptomycin)	125	25	32	-
Std Antifungal (Fluconazole)	125	-	-	29

Antifungal activity

The compounds 5 and 6i-6j were screened in vitro for their antifungal activity against Candida albicans using disc diffusion method (sabhraud dextrose agar) at a concentration of 100 µg/ml and 150 µg/ml with DMF as the solvent[15-17]. After 24 hrs of incubation at 37 °C the zones of inhibition formed were measured in mm with standard drug Fluconazole (Table 2).

3. RESULTS AND DISCUSSION

The synthesized compounds were evaluated for their antibacterial and antifungal activity. The activity data revelead that the 6b,6c, 6d compounds exhibited good activity against *E. coli* and *S. aureus*. In comparision to the standard drug and 6a,6b,6c, 6d compounds exhibited good activity against *C. albicans*. These activities were better than their respective standards.

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