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Synthesis and evaluation of 1,2,4-triazole derivatives as antimicrobial, antifungal and anthelmintic agents

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Current research work is focused on incorporation of thiosemicarbazide, triazole in one framework and observed for antifungal, antibacterial and anthelmintic activities. Derivatives of 1,2,4-triazole-3-thiol are synthesized with thiosemicarbazide and semicarbazide as a starting material. Thiosemicarbazide are subjected to intermolecular cyclization in alkaline medium followed by acidification to give 1,2,4-triazole-3-thiol. Equimolar mixture of thiosemicarbazide and different consecutive acids are heated, fusion occurred to give different triazoles derivatives. The minimum inhibitory concentrations for synthesized compounds are in the range of 3.12-25 μg/mL.

Keywords: Thiosemicarbazide, 1,2,4-triazole, antimicrobial activity, antifungal activity, anthelmintic activity

A large variety of 1,2,4-triazole derivatives possess diverse biological activities like antibacterial, antifungal, antiviral, anticonvulsant, antidepressant, antitubercular, antitumor, antihypertensive, anti-inflammatory activities[1]. Triazoles fused with other ring systems such as thiadiazine, quinazolone, imidazole, naphtharidine and pyrimidine have shown broad spectrum of activities. The main aim of synthesis of triazolopyrimidine is that they are well known to show higher antifungal activity, in addition, it also shows other important activities such as anticancer, bronchodilator, antiviral, and antibacterial[2]. Fungal infections remain a significant cause of morbidity & mortality in world over leading disease Acquired Immune Deficiency Syndrome (AIDS)[3]. Superficial fungal infections are classified into dermatomycoses like infection of skin, hair, nails and candidiasis *i.e.* most common systemic fungal infection which affects mucus membranes[4].

For potent antifungal activity, firstly 1,2,4 -Triazole should possess two or three aromatic rings at least one of which is halogen substituted and this halogen substituted ring should be separated from the azole moiety by two carbon atoms and secondly only 2 and/or 2,4 substitution yields effective azoles compounds.

Results and Discussion

All Compounds were evaluated for antimicrobial activities against some bacterial strains *pseudomonas aerugenosa, Bacillus subtilis* and *Staphylococcus aureus* by broth dilution method. It is quantitative method for determining the Minimum Inhibitory Concentration (MIC) of the antibiotic against bacteria to be tested[5-7]. The stock solution of (100 μg/mL) of compounds was prepared in DMSO. To each tube containing sterilized Nutrient broth medium (5 mL), 5 mL of drug solution was added. The serial dilutions were made to obtain concentrations (in μg/mL) such as 100, 50, 25, 12.5, 6.125, and 3.125. Each tube was inoculated with the microorganisms *P. aerugenosa, B. subtilis* and *S. aureus*. All the tubes were incubated at 35-37 °C for 18 h. Positive control tubes (organism + broth + DMSO) and negative control tubes (broth + drug) were also prepared. Streptomycin used as standard drug which was adjusted to about concentration of 100 μg/mL[8-10].

Antifungal activity carried out against two fungal strains of *Aspergillus niger* and *Candida albicans* by turbidimetric method. The stock solution of (100 μg/mL) of compounds was prepared in DMSO. To each tube containing sterilized Sabouraud's liquid medium (5 mL), 5 mL of drug solution was added. The serial dilutions were made to obtain concentrations (in μg/mL) such as 100, 50, 25, 12.5, 6.125, 3.12, and 1.56. Each tube was inoculated with the microorganism and was incubated to 30 °C for

14 days. Positive control tubes (organism + broth + DMSO) and negative control tubes (broth + drug) were also prepared. The readings were taken and expressed as (-), if inhibition of growth is seen and (+), if inhibition of growth is not seen. Fluconazole was used as standard drug for this activity.

The result of measuring the MIC (Table 1) indicated good activities of synthesized compounds compared to standard drugs. The newly synthesized compounds were tested for anthelmintic activity. *Pheretima posthuma* from of nearly equal size (6±1 cm) were selected randomly for present study. The earthworms were divided into four groups of six earthworms in each. Albendazole diluted to with normal saline solution to obtained 0.1% w/v, 0.2% w/v, 0.5% w/v and 1% w/v served as standard and poured into Petri dishes. The synthesized compounds were prepared in minimal quantity of DMSO and diluted to prepare four concentrations *i.e*. 0.1% w/v, 0.2% w/v, 0.5% w/v and 1% w/v for each compound. Normal saline serves as control. Six earthworms nearly equal size (6±1 cm) are taken for each concentration and placed in Petri dishes at RT. The time taken for complete paralysis and death are recorded. The mean paralysis time and mean lethal time for each sample was calculated. The time taken for worms to become motionless was noted as paralysis time and to ascertain death, each worm was frequently applied with external stimuli which stimulates and induce movement in the earthworms if alive[11-14]. The result of anthelmintic (Table 2) indicated good activities of synthesized compounds compared to standard drugs.

Experimental Details

ll the chemicals and reagents were used as purchased (from Sigma–Aldrich, India) and used as received. Melting points of all compounds were

Table 1 — Minimum Inhibitory Concentration (MIC) in µg/mL of compounds for antibacterial and antifungal activity

Compound	MIC (µg/mL)				
	Bacterial strain			Fungal strain	
	Bacillus subtilis	*Staphylococcus aureus*	*pseudomonas aerugenosa*	*Candida albicans*	*Aspergillus niger*
3a	25	25	25	25	25
3b	12.5	12.5	25	12.5	25
3c	25	25	25	25	25
3d	25	25	25	12.5	25
3e	25	25	12.5	12.5	25
4	12.5	12.5	25	12.5	25
Streptomycin	3.12	3.12	3.12	-	-
Fluconazole	-	-	-	3.12	3.12

Table 2 — Anthelmintic activity of compounds

Compound	Time in min (mean ± SD) for paralysis			Time in min (mean ± SD) for death		
	Concentration (%w/v)			Concentration (%w/v)		
	0.1	0.2	0.5	0.1	0.2	0.5
3a	2.12 ±0.034	2.52 ±0.015	2.18 ±0.015	4.39 ±0.025	3.88 ±0.03	3.04 ±0.035
3b	5.12 ±0.041	4.14 ±0.011	3.81 ±0.021	5.60 ±0.025	4.64 ±0.040	4.32 ±0.030
3c	5.31 ±0.021	4.22 ±0.025	3.32 ±0.015	6.91 ±0.021	6.57 ±0.030	4.78 ±0.03
3d	3.73 ±0.023	2.41 ±0.021	1.71 ±0.025	3.43 ±0.022	2.70 ±0.031	2.41 ±0.025
3e	2.92 ±0.021	2.02 ±0.032	1.52 ±0.02	3.59 ±0.021	2.51 ±0.025	1.86 ±0.030
4	3.50 ±0.025	3.15 ±0.030	2.20 ±0.021	4.30 ±0.030	3.22 ±0.041	2.38 ±0.025
Albendazole	3.12 ±0.015	2.71 ±0.021	2.21 ±0.022	4.41 ±0.025	3.17 ±0.025	3.45 ±0.035

Where n = 6

determined by open cup capillary method and are uncorrected. Thin Layer Chromatography (TLC) analysis was performed on glass plates using silica gel G_{60} and spots were visualized by iodine vapour. All Infra-Red (IR) spectra were recorded using Jasco-4100 spectrometer using potassium bromide pellet to confirm presence of functional group.^{1}H NMR spectra were recorded by Bruker Avance-2 400 NMR spectrometer using deuterated chloroform ($CDCl_3$) and dimethyl sulphoxide (DMSO) depending on solubility of synthesized compounds for structural confirmation.

Ammonium Thiocynate (0.3 mol) was added in a mixture of hydrazine hydrate (0.47 mol) and 12.5 mL of water followed by evaporation to evolve ammonia immediately. The mixture was heated to boiling until temperature reached up to 130 °C then the mixture cooled to 15 °C, add 5 mL of water and thiosemicarbazide was separated from mother liquor. Yield: 14.04 g (50%), mp 180-182°C[15].

General procedure for Synthesis of various 1,2,4 triazole derivatives

An equimolar mixture of an acid (0.1 mol) and thiosemicarbazide (0.1 mol) was heated in an oil bath till the contents melted and maintained at same temperature for 7 h, then cooled and treated with dilute sodium bicarbonate solution to remove any unreacted acid left. The solid filtered was washed with water, dried and recrystallized to obtained triazole[16].

5-[(*E*)-2-phenylethenyl]-4*H*-1, 2, 4-triazole-3-thiol (3a): Yield 51.92%, mp 177-179 °C. IR, ν, cm^{-1}, 3201 s (2º NH), 1667 s (C=N), 1494 s (C=C), 1078 s (C=S), 1340 s (C-N). ^{1}H NMR spectrum, δ, ppm: 3.9 s (1H, S-H), 6.6 d (1H, C-H), 6.8 d (1H, C-H), 7-7.5 m (5H, aromatic), 8 s (1H, N-H).

5-(pyridin-3-yl)-4*H*-1, 2, 4-triazole-3-thiol (3b): Yield 56.42%, mp 120-122 °C. IR, ν, cm^{-1}, 3338 s (2º NH), 1669 s (C=N), 1416 s (C=C), 1237 s (C=S), 1299 s (C-N). ^{1}H NMR spectrum, δ, ppm: 3.4 s (1H, SH), 9.1 s (1H, C-H_a), 9.3 d (1H, C-H_b), 8.6 d (1H, C-H_c), 8.2 d (1H, C-H_d), 13.2 s (1H, N_4-H).

5-benzyl-4*H*-1, 2, 4-triazole-3-thiol(3c): Yield 42.62%, mp 116-118 °C. IR, ν, cm^{-1}, 3265 s (2º NH), 1649 s (C=N), 1476 s (C=C), 1206 s (C=S). ^{1}H NMR spectrum, δ, ppm: 3.52 s (1H, S-H), 3.6 s (2H, C-H), 7.2 -7.3 m (5H, aromatic), 12.5 s (1H, N-H).

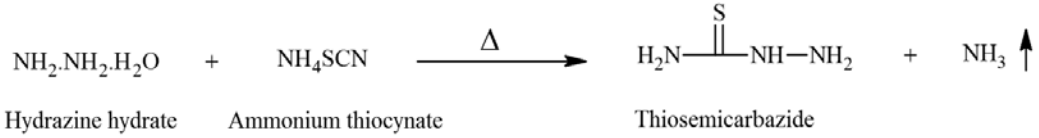
5-(chloromethyl)-4*H*-1, 2, 4-triazole-3-thiol (3d): Yield 73.97%, mp 155-157 °C. IR, ν, cm^{-1}, 3138 s (2º NH), 1594 s (C=N), 725 s (C-Cl), 1018 s (C=S), 1335 s (C-N).

5-(trichloromethyl)-4*H*-1, 2, 4-triazole-3-thiol (3e).Yield 77.41%, mp 252-254 °C. IR, ν, cm^{-1}, 3289 s (2º NH), 1618 s (C=N), 734 s (C-Cl), 1126 s (C=S), 1325 s (C-N).

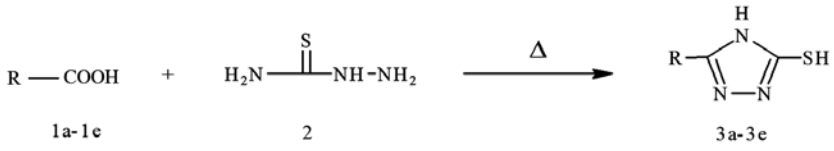
Semicarbazide (0.06 mol) was added in 100 mL sodium hydroxide (0.125 mol) solution and then benzoyl chloride (0.071 mol) was added slowly with continuous stirring. The reaction mixture was maintained at lower temperature. An intermediate, 2-benzoylhydrazine-carboxamide (3) was separated, collected and dried. Yield 2.9 g (74.93%), mp 156-158°C[17-18].

2-benzoyl hydrazine carboxamide (0.014 mol), was added in sodium hydroxide (0.0225 mol) solution and then it was mixed in 4.46 mL of water and heated on a steam bath for 1 h. The reaction mixture was cooled for 30 min in ice bath and treated with concentrated hydrochloric acid (0.061 mol). Further the reaction mixture was cooled on ice bath for 2 h. 5-Phenyl-1,2-dihydro-3*H*-1,2,4-triazol-3-one was separated, collected and dried. The whole synthesis schemes are presented in Scheme 1-3.

5-phenyl-1,2-dihydro-3*H*-1,2,4-triazol-3-one (4): Yield 4.35 g (66.45%), mp 120-122°C. IR, ν, cm^{-1}, 3442 s (2º NH), 1651 s (C=N), 1686 s (C=O), 1326 s (C-N), 1454 s (C=C). ^{1}H NMR spectrum, δ, ppm: 11.9 s (1H, N-H), 7.4-7.8 m (5H, aromatic), 10.05 s [1H, N-H(enol form of compound)], 10.38 s (1H, N-H), 11.4 s [1H, O-H(enol form of compound)][19-23].

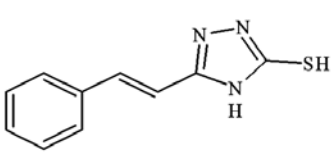


Scheme 1 — Synthesis of Thiosemicarbazide



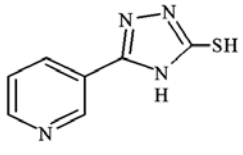
1a-1e 2 3a-3e

1 = Cinnamic acid (a), Nicotinic acid (b), Phenyl acetic acid (c), Chloroacetic acid (d), Trichloroacetic acid (e)



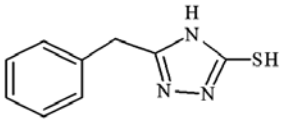
5-[(E)-2-phenylethenyl]-4H-1,2,4-triazole-3-thiol

3a



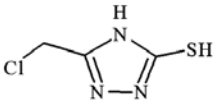
5-(pyridin-3-yl)-4H-1,2,4-triazole-3-thiol

3b



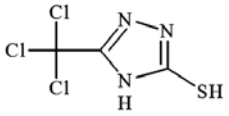
5-benzyl-4H-1,2,4-triazole-3-thiol

3c



5-(chloromethyl)-4H-1,2,4-triazole-3-thiol

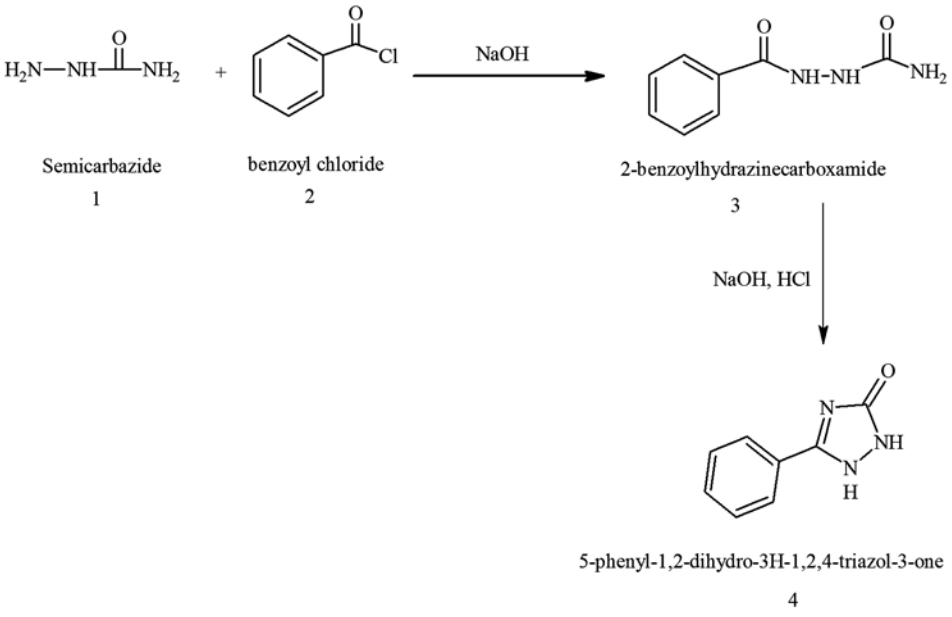
3d



5-(trichloromethyl)-4H-1,2,4-triazole-3-thiol

3e

Scheme 2 — Synthesis of various 1,2,4 triazole derivatives



Semicarbazide 1

benzoyl chloride 2

2-benzoylhydrazinecarboxamide 3

5-phenyl-1,2-dihydro-3H-1,2,4-triazol-3-one 4

Scheme 3 — Synthesis of phenyl-1,2-dihydro-3*H*-1,2,4-triazol-3-one (benzoylaton of semicarbazide was done to prepare benzoyl semicarbazide)

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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