Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2025, 17(2): 676-682
(http://www.derpharmachemica.com/archive.html)

Synthesis, Characterization and Antihelmintic Activity of New N-Substituted 1,2,4-Triazole Derivatives

Pavitra Anant Hedge*, Akkamma Hanumappa Godihal, Vijay Kumar Miralagatti Mathada Jagatageri and Basavaraja Harti Shivappa

P. G. Department of Pharmaceutical Chemistry, S. J. M. College of Pharmacy, NH4-Bypass, SJMIT Campus, Chitradurga, Karnataka, India

***Corresponding author:** Pavitra Anant Hegde, P. G. Department of Pharmaceutical Chemistry, S. J. M. College of Pharmacy, NH4-Bypass, SJMIT Campus, Chitradurga, Karnataka, India; E-mail: pavitrahegde31051995@gmail.com*

Received: 16-April-2025, Manuscript no: DPC-25-167910, **Editor assigned:** 19-April-2024, Pre QC No: DPC-25-167910 (PQ), **Reviewed:** 03-May-2025, QC No: DPC-25-167910, **Revised:** 01-July-2025, Manuscript No: DPC-25-167910 (R), **Published:** 28-July-2025, DOI: 10.4172/0975-413X.17.2.676-682

ABSTRACT

The compound 2-amino-3,5-dibromobenzohydrazide was synthesized by dissolving equimolar proportions of methyl-2-amino-3,5-dibenzoate and hydrazine hydrate in water and refluxed for 6 hours. The above compound and carbon disulphide was dissolved in alcoholic potassium hydroxide and refluxed for 4 hours, then poured in to ice-cold water and obtained potassium 2-(2-amino-3,5-dibromobenzoyl) hydrazine-1-carbodithionate. The obtained product was further treated with different functional groups such as isoniazide, 2-amino-4,6-dibromo benozoic acid hydrazide, phenyl hydrazine and hydrazine hydrate in 5ml of water and refluxed for 5 to 6 hours until the evolution of H_2S ceases. The reaction mixtures were poured into ice-cold water, acidified with dil HCl. The obtained product was filtered, dried and recrystallized by using hot water. The lead compounds were characterized by melting point, TLC, calculated elemental analysis, IR, ^{1}HNMR, mass spectral studies. The compounds were tested for anthelmintic studies and showed significant activity at low and high concentration compared to standard drugs; still further studies are requested.

Keywords: 1,2,4-triazoles; Methyl-2-amino-3,5-dibenzoate; Hydrazine hydrate; 2-amino-3,5-dibromobenzohydrazide; Anthelmintic activity

INTRODUCTION

Infectious diseases such as bacterial and fungal infections have been reported to increase dramatically worldwide in recent times and one of the major causes is suppressed immunity. Suppression of immunity due to various reasons such as malignancy, immunosuppressive therapies, HIV-infection, surgeries, old age etc. is a plausible cause that places patient at high risk for catching microbial infections. The situation is further worsened by increasing incidence of microbial resistance to the majority of antibiotics available today.

Parasitic infections of the gastrointestinal tract caused by nematodes are widespread in both humans and animals. Intestinal helminths are a considerable medical and economic challenge. People become infected with intestinal nematodes by ingesting infected eggs or, in some species, by larvae actively penetrating the human host skin. The most common parasitic diseases caused by intestinal nematodes include ascariasis, ancylostomiasis/necatoriasis, trichuriasis and enterobiasis. Literature data indicate that there are approximately 800-1000 million cases of roundworm, 600-900 million cases of hookworm, 500 million cases of whipworm and 200 million cases of pinworm infection worldwide. These nematodes can cause gastrointestinal symptoms, dehydration and inhibition of psychophysical development in children. A variety of agents are used in the treatment of intestinal nematodes, e.g., Thiabendazole, Mebendazole and Albendazole (benzimidazole derivatives), Pyrantel and ivermectin. In recent years, as a result of the abuse and inadequate use of drugs in the treatment of parasitic diseases in both humans and animals, an increase in the drug resistance of parasites to available drugs has been observed. Multidrug resistance means that the pool of effective substances used in the treatment of parasitic diseases is shrinking, which generates the need to search for new compounds with antiparasitic and anthelmintic properties.

Bladin was the first scientist to name the compound triazole in 1885. Triazoles have the chemical formula $C_2H_3N_3$, meaning they are five-membered heterocyclic rings with two carbon and three nitrogen atoms. The two isomeric forms of triazoles-1,2,3-triazole and 1,2,4-triazole-depend on the location of the nitrogen atoms (Figure 1) [1].

Figure 1: Structural comparison of 1,2,3-triazole and 1,2,4-triazole isomers showing the different positions of nitrogen atoms in the five-membered ring.

1,2,4-triazole: Organic and pharmaceutical chemists have recently become interested in 1,2,4-triazoles in particular after a number of novel hybrids based on the molecular hybridization technique have been produced with a wider spectrum. Triazoles are the hardest to cleave and the most stable of the azoles. 1,2,4-triazoles, which have the chemical formula $C_2H_3N_3$, function as carboxylic, ester and amide isosteres. By replacing a carbon atom at position-4 with a nitrogen atom, it can be officially generated from pyrazole. There are two tautomeric forms of 1,2,4-triazole, A and B, with 1H 1,2,4-triazole (A) being more stable than 4H-1,2,4-triazole (B) as shown below (Figure 2) [2].

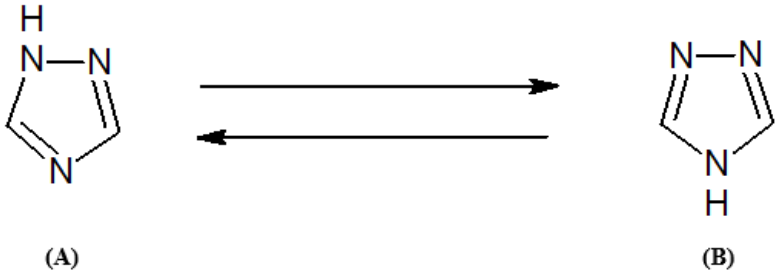


Figure 2: Tautomeric equilibrium between the two forms of 1,2,3-triazole. **Note:** (A) 1H-1,2,3-triazole and (B) 2H-1,2,3-triazole-showing proton migration between nitrogen atoms

Because of its rigidity, solubility, hydrogen bonding ability and dipole nature, 1,2,4-triazoles bind with biological receptors with high affinity and function as significant pharmacophores. Numerous medications used in clinical therapy contain this motif, such as antifungal (fluconazole, itraconazole, posaconazole, voriconazole, ravuconazole), anxiolytic, anticonvulsant and hypnotic (estazolam, alprazolam), skeletal muscle relaxant, antimigraine (rizatriptan), antiplatelet (trapidil), antidepressant (trazodone), anticancer (anastrozole), aromatase inhibitor (letrozole), antiviral (ribavirin) and anticonvulsant (loreclezole). The triazole moiety is present in several commercial plant protection fungicides, including cyproconazole, prothioconazole, triadimefon, metconazole, propiconazole, tebu conazole, epoxiconazole and triadimenol.
1,2,4-triazoles have a wide range of therapeutic effects, including anti-inflammatory, anti-cancer, antibacterial, antifungal, antitubercular, antiviral, anti-convulsant, anthelmintic, antinociceptive, analgesic, anti-corrosive, antioxidant, hypoglycaemic, urease and lipase inhibitors, anti-proliferative diuretic, sedative, anti-migraine, anti-HIV and muscle relaxants
In this work, we synthesized new compounds and focused our studies on testing anthelmintic activity by using in vitro methods. The specific aim of this study was to analyze the anthelmintic activity of the newly synthesized potassium 5-(2-mino-3-5-dibromophenyl)-4-methyl-4H-1,2,4-triaole-3-thiolate derivatives for their potential use in the treatment and control of bacteria's in humans and animals [3].

MATERIALS AND METHODS

Chemicals and reagents

All chemicals, commercial solvents and reagents were purchased from sigma-aldrich by PG department of SJM college of pharmacy, Chitradurga. All these chemicals for the reactions were procured by PG department of SJMCP. Methyl-2-amino-3,5-dibromobenzoate, hydrazine hydrate, carbon disulphide, potassium hydroxide, isoniazide, 2-amino-4,6-dibromo benzoic acid hydrazide, hydrazine hydrate, conc. hydrochloric acid, DMSO, peptone, sodium chloride.

Experimental section

Step 01: Synthesis of 2-amino-3,5 dibromobenzohydrazide: Equimolar proportions of (0.01 mol) of methyl-2-amino 3,5 dibromo benzoate and hydrazine hydrate was dissolved in water and refluxed for 6 hours, TLC was monitored for the completion of the reaction. Then the reaction mixture was poured into ice cold water. The precipitated product was filtered.
Step 02: Synthesis of 2-amino-3,5-dibromo potassium dithiocarbazinate:
Equimolar proportion of 2-amino-3,5-dibromobenzohydrazide and carbon disulphide was dissolved in alcoholic potassium hydroxide and refluxed for 4hours, the reaction was monitored by TLC and the reaction mixture was poured into ice cold water, filtered and dried. Recrystallized from hot water [4].

Step 03: Synthesis of derivatives T1-T4

Synthesis of derivative T1: Equimolar proportions of 2-amino-3,5-dibromo potassium dithiocarbazinate (Intermediate 01) and isoniazide was dissolved in water and refluxed for 5-6 hours until the evolution of H2S ceases. Then poured into ice cold water, acidified with dil HCl until the precipitate forms, filtered and dried, recrystallized from hot water. The melting point was determined. (MP-207°C).
Synthesis of derivative T2: Equimolar proportions of 2-amino-3,5-dibromo potassium dithiocarbazinate (Intermediate 01) and 2-amino-4,6-dibromo benzoic acid hydrazide was dissolved in 5 ml of water and refluxed for 5-6 hours until the evolution of H2S ceases. Then poured into ice cold water, acidified with dil HCl until the precipitate forms, filtered and dried, recrystallized from hot water. The melting point was determined. (MP-205°C).
Synthesis of derivative T3: Equimolar proportions of 2-amino-3,5-dibromo potassium dithiocarbazinate (Intermediate 01) and dinitro phenyl hydrazine was dissolved in 5ml of water and refluxed for 5-6 hours until the evolution of H_2S ceases. Then poured into ice cold water, acidified with dil HCl until the precipitate forms, filtered and dried, recrystallized from hot water. The melting point was determined. (MP-200°C).
Synthesis of derivative T4: Equimolar proportions of 2-amino -3,5-dibromo potassium dithiocarbazinate (Intermediate 01) and hydrazine hydrate was dissolved in 5 ml of water and refluxed for 5-6 hrs until the evolution of H_2S ceases. Then poured into ice cold water, acidified with dil HCl until the precipitate forms, filtered and dried, recrystallized from hot water. The melting point was determined (MP-208°C).

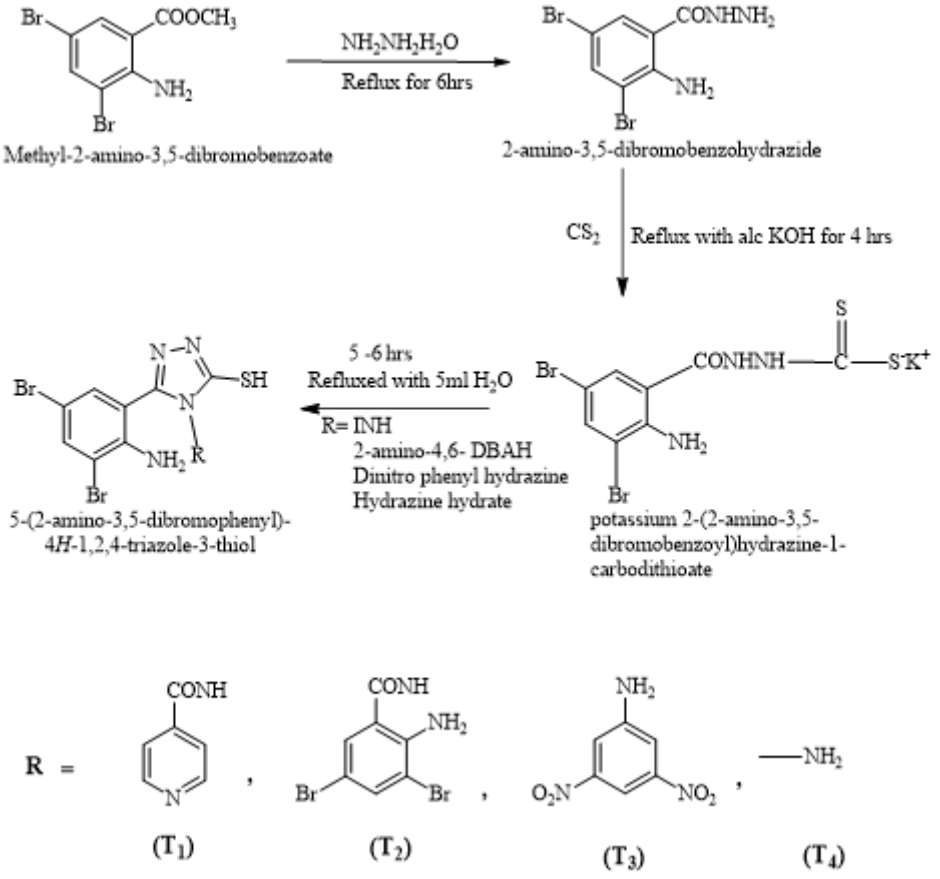


Figure 3: Synthetic pathway for the formation of 5-(2-amino-3,5-dibromophenyl)-4H-1,2,4-triazole-3-thiol derivatives (T_1–T_4) starting from methyl 2-amino-3,5-dibromobenzoate.

General procedures: Melting points were determined in open capillaries and are uncorrected (Table 1). IR spectra (KBr pellet technique) were recorded using OPUS-2000 Bruker spectrophotometer. ^{1}H NMR spectra were recorded on Agilent AM 400 instrument (at 400 MHz) using Tetramethylsilane (TMS) as an internal standard and DMSO-d6 as a solvent. Chemical shifts are given in parts per million (ppm). Splitting patterns are designated as follows: s-singlet, d-doublet, t-triplet, q-quartet and m-multiplet. Mass Spectra (MS) were recorded on Schimadzu GC-MS operating at 70eV. All the synthesized compounds were purified by recrystallization. The reactions were followed up and the purity of compounds was monitored on pre-coated TLC plates and visualizing the spots in iodine chamber and ultraviolet light (Tables 2-4).

Table 1: Analytical data.

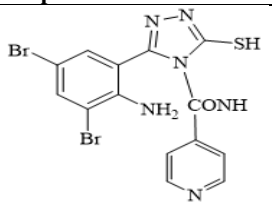
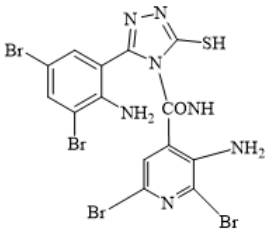
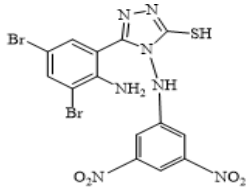
S. No	Compound code	MP 0C	% Yield	Molecular formula	Molecular weight	Calculated %			
						C	H	N	O
1	T1	207	78%	$C_{14}H_{10}Br_2N_6OS$	470.14	33.09	1.79	16.54	31.15
2	T2	205	82%	$C_{15}H_{10}Br_4N_6OS$	642.95	24.69	1.18	14.4	2.35
3	T3	200	74%	$C_{14}H_9Br_2N_7O_4S$	531.14	29.54	1.42	17.22	11.24

4	T4	210	65%	$C_8H_7Br_2N_5S$	365.05	23.24	1.5	17.37	--

Table 2: Characteristics of IR absorption bands.

S No	Compound code	Ar-NH_2 (in cm^{-1})	Ar-CH stretching (in cm^{-1})	C-N stretching (in cm^{-1})	C=C stretching (in cm^{-1})	C=N stretching (in cm^{-1})	C=O of amide	SH stretching (in cm^{-1})	C-Br stretching (in cm^{-1})	Ar-No_2
1	T1	3350	3071.59	1448.79	1680	1606.92, 1568.96, 1525.81	1661.92	2580	586	--
2	T2	3356	3065.52	1450.1	1660	1610.81, 1570.64 ,1525.80	1650.41	2565	575	--
3	T3	3370	3090	1440.82	1670	1606.92, 1560.2	--	2570	560	1516
4	T4	3354.71	3384	1448.24	1685	1590.24, 1640.32	--	2604.99	582	--

Table 3: Characteristics of ^1H NMR spectra.

S. No	Compound structure	Compound code	Hydrogen	δ(ppm)	Multiplicity	Solvent
1		T_1	-Ar-4H	7.43, 8.63	Doublet	(DMSO-d6): 400 MHz
			-Ar-2H	7.24,7.93	Singlet	
			-NH2-2H	5.32	Singlet	
			-SH-1H	13.05	Singlet	
			-NH-1H	1.5	Singlet	
2		T_2	-Ar-1H	8.14	Singlet	(DMSO-d6): 400 MHz
			-Ar-2H	7.24, 7.93	Singlet	
			-NH_2-4H	5.32, 5.82	Singlet	
			-SH-1H	13.20	Singlet	
			-NH-1H	1.5	Singlet	
2		T_3	-Ar-3H	8.13, 8.22	Doublet	(DMSO-d6): 400 MHz
			-Ar-2H	7.24, 7.93	Singlet	
			-NH_2-2H	5.42	Singlet	

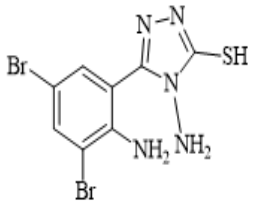
			-SH-1H	13.79	Singlet	
			-NH-1H	4.9	Singlet	
4		T_4	-Ar-2H	7.24, 7.93	Singlet	
			$-NH_2$-4H	5.32, 5.74	Singlet	
			-SH-1H	13.89	Singlet	(DMSO-d6): 400 MHz

Table 4: Characteristics of mass spectra.

S. No	Compound code	Calc. Mol. weight	Mol. formula	Fragmentation	ESI-MS m/z
1	T3	470.14	$C_{14}H_{10}Br_2N_6OS$	M^{+1} (NO_2,NO_2)	440.91
				M^{+2} (Br, Br, NH_2)	268.08
				M^{+3} (SH)	236.28
2	T4	642.95	$C_{15}H_{10}Br_4N_6OS$	M^{+1} (Br, Br, NH_2)	297.34
				M^{+2} (SH)	265.28
				M^{+3} (C_5H_5N)	188.19

***In-vitro* anthelmintic activity**

The synthesized compounds are screened for anthelmintic activity by using earthworms. Five earthworms of nearly equal size were placed in standard drug solution and test compounds solutions at room temperature. Normal saline used as control. The standard drug and test compounds were dissolved in minimum quantity of Dimethyl Formamide (DMF) and adjusted the volume up to 15 ml with normal saline solution to get the concentration of 0.1% w/v, 0.2% w/v and 0.5% w/v. Albendazole was used as a standard drug. The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive. The mean lethal time and paralysis time of the earthworms for different test compounds and standard drug are tabulated (Table 5) [5].

Table 5: Anthelmintic activity.

S. No	Name	Time in minutes					
		For paralysis		For death			
		% of concentration		% of concentration			
		0.1	0.2	0.5	0.1	0.2	0.5
1	Control (0.9% concentration)	--	--	--	--	--	--
2	Albendazole	9	9	4	10	10	8
3	T1	2	2	1	6	4	3
4	T2	7	2	1	8	7	5
5	T3	9	9	7	13	15	16
6	T4	3	3	2	10	8	4

RESULTS AND DISCUSSION

Synthesis of 2- amino- 3,5 dibromobenzohydrazide

Equimolar proportions of (0.01 mol) of methyl-2-amino 3,5 dibromo benzoate and hydrazine hydrate was dissolved in water and refluxed for 6hours, TLC was monitored for the completion of the reaction. Then the reaction mixture was poured into ice cold water. The precipitated product was filtered.
Yield: 75%. FT-IR (υ max, cm^{-1}): 3357 (Ar-NH_2), 3386 (Ar-CH stretching), 1651.92 (C=O of amide),1597 (C=N), 586 (C-Br stretching).

Synthesis of 2-amino -3,5-dibromo potassium dithiocarbazinate

Equimolar proportion of 2-amino-3,5-dibromobenzohydrazide and carbon disulphide was dissolved in alcoholic potassium hydroxide and refluxed for 4 hours, the reaction was monitored by TLC and the reaction mixture was poured into ice cold water, filtered and dried. Recrystallized from hot water.
Yield: 70%. FT-IR (υ max, cm^{-1}): 3354 (Ar-NH_2), 3388 (Ar-CH stretching), 1655 (C=O of amide),1597 (C=N), 586 (C-Br stretching), 1200 (C=S) [6].

Synthesis of derivative T_1

Equimolar proportions of 2-amino -3,5-dibromo potassium dithiocarbazinate (Intermediate 01) and 2-amino-4,6-dibromo benzoic acid hydrazide was dissolved in 5ml of water and refluxed for 5-6 hours until the evolution of H_2S ceases. Then poured into ice cold water, acidified with dil HCl until the precipitate forms, filtered and dried, recrystallized from hot water. The melting point was determined.
Yield:78%. FT-IR (υ max, cm^{-1}): 3350 (Ar-NH_2), 3071.59 (Ar-CH stretching), 1661.92 (C=O of amide),1448.79 (C-N), 586 (C-Br stretching), 1680 (C=C stretching), 1606.92, 1568.96, 1525.81 (C=N stretching), 2580 (SH stretching). 1H NMR (DMSO-d6: 400 MHz δppm): 7.43, 8.63 (d, Ar-4H), 7.24,7.93 (s, Ar-2H), 5.32 (s, NH_2-2H), 13.05 (s, SH-1H), 1.5 (s, NH-1H).

Synthesis of derivative T_2

Equimolar proportions of 2-amino -3,5-dibromo potassium dithiocarbazinate (Intermediate 01) and 2-amino-4,6- dibromo benzoic acid hydrazide was dissolved in 5 ml of water and refluxed for 5-6 hours until the evolution of H_2S ceases. Then poured into ice cold water, acidified with dil HCl until the precipitate forms, filtered and dried, recrystallized from hot water. The melting point was determined
Yield:82%. FT-IR (υ max, cm^{-1}): 3356 (Ar-NH_2), 3065.52 (Ar-CH stretching), 1650.41 (C=O of amide),1450.10 (C-N), 575 (C-Br stretching), 1660 (C=C Stretching), 1610.81, 1570.64, 1525.80 (C=N stretching), 2565 (SH stretching). ^{1}H NMR (DMSO-d6: 400 MHz δppm): 8.14 (s, Ar-1H), 7.24,7.93 (s, Ar-2H), 5.32, 5.82 (s, NH_2-4H), 13.20 (s, SH-1H), 1.5 (s, NH-1H) [7].

Synthesis of derivative T_3

Equimolar proportions of 2-amino -3,5-dibromo potassium dithiocarbazinate (Intermediate 01) and dinitro phenyl hydrazine was dissolved in 5 ml of water and refluxed for 5-6 hours until the evolution of H_2S ceases. Then poured into ice cold water, acidified with dil HCl until the precipitate forms, filtered and dried, recrystallized from hot water. The melting point was determined.
Yield: 74%. FT-IR (υ max, cm^{-1}): 3370 (Ar-NH_2), 3090 (Ar-CH stretching), 1440-0.82 (C-N), 560 (C-Br Stretching), 1670 (C=C stretching), 1606.92, 1560.20 (C=N stretching), 2570 (SH stretching), 1516 (Ar-No_2). ^{1}H NMR (DMSO-d6: 400 MHz δppm): 8.13, 8.22 (d, Ar-3H), 7.24,7.93 (s, Ar-2H), 5.42 (s, NH_2-2H), 13.79 (s, SH-1H), 4.9 (s, NH-1H). ESI-MS m/z: 440.91 (M^{+1} -NO_2, NO_2), 268.08 (M^{+2} -Br, Br, NH_2), 236.28 (M^{+3}-SH) Mol wt: 470.14 [8].

Synthesis of derivative T_4

Equimolar proportions of 2-amino-3,5-dibromo potassium dithiocarbazinate (Intermediate 01) and Hydrazine hydrate was dissolved in 5ml of water and refluxed for 5-6 hrs until the evolution of H_2S ceases. Then poured into ice cold water, acidified with dil HCl until the precipitate forms, filtered and dried, recrystallized from hot water. The melting point was determined.
Yield: 65%. FT-IR (υ max, cm^{-1}): 3354.71 (Ar-NH_2), 3384 (Ar-CH stretching), 1448-.24 (C-N), 582 (C-Br stretching), 1685 (C=C stretching), 1590.24, 1640.32 (C=N stretching), 2604.99 (SH stretching). ^{1}H NMR (DMSO-d6: 400 MHz δppm): 7.24, 7.93 (s, Ar-2H), 5.32, 5.74 (s, NH_2-4H), 13.89 (s, SH-1H). ESI-MS m/z: 297.34 (M^{+1} -Br, Br, NH_2), 265.28 (M^{+2} -SH), 188.19 (M^{+3} -C_5H_5N) Mol wt: 642.95.
Anthelmintic activity: Synthesized compounds of 5-(2-amino-3,5-dibromophenyl)- 4H-1,2,4-triazole-3-thiol derivatives were tested for anthelmintic activity by using earth worms compared to standard Albendazole. T1 and T4 showed significant anthelmintic activity against standard Albendazole [9,10].

CONCLUSION

Results of present study demonstrated that, a new class of different Isoniazide, 2-amino-4,6-dibromo benozoic acid hydrazide, phenyl hydrazine and Hydrazine hydrate encompassing 1,2,4- triazole derivatives were synthesized and evaluated as anthelmintic drugs by using earthworms compared to standard albendazole. The newly synthesized heterocyclics exhibited promising anthelmintic activity. It can be concluded that this class of compounds certainly holds great promise towards good active leads in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress.

ACKNOWLEDGEMENT

I am thankful to principal, PG department of pharma chemistry, SJM college of pharmacy, Chitradurga for providing necessary facilities to carry out this work.

REFERENCES

[1] Kołodziej P, Wujec M, Doligalska M, et al. J Adv Res. **2024**; 60: p. 57-73.
[2] Strzelecka M, Swiątek P. Pharmaceuticals. **2021**; 14(3): p. 224.
[3] Aggarwal R, Sumran G. European J Med Chem. **2020**; 205: p. 112652.
[4] Omer R, Rashid RF, Othman K. J Phy Chem Func Mat. **2023**; 6(1): p. 43-56.
[5] Matin MM, Matin P, Rahman MR, et al. Front Mol Biosci. **2022**; 9: p. 864286.
[6] Ameen DS, Hamdi MD, Khan AK. Al Mustansiriyah J Pharmaceu Sci. **2022**; 22(3): p. 65-81.
[7] Emami L, Sadeghian S, Mojaddami A, et al. BMC Chem. **2022**; 16(1): p. 91.
[8] Kumari M, Tahlan S, Narasimhan B, et al. BMC Chem. **2021**; 15(1): p. 5.
[9] Abdelli A, Azzouni S, Plais R, et al. Tetrahedron Lett. **2021**; 86: p. 153518.
[10] Amin NH, El-Saadi MT, Ibrahim AA, et al. Bioorg Chem. **2021**; 111: p. 104841.