SYNTHESIS OF TRIAZOLE AND OXADIAZOLE DERIVATIVES OF 6-METHYLBENZOTHIAZOL-2-YL – HYDRAZINE AND EVALUATION OF THEIR ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES

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Abstract:

Several triazole derivatives of 6-methylbenzothiazol-2-yl – hydrazine was synthesized by the cyclization of the acid hydrazides with CS2 in KOH followed by treating it with hydrazine hydrate and benzaldehyde. The chemical structures were valid with the help of ability of infrared spectroscopic analysis proton nuclear resonance spectroscopic analysis carbon -13 nuclear magnetic resonance spectroscopic analysis and mass bauer spectroscopic analysis. The resulted compounds are assessed for analgesic, anti-inflammatory drug, opposing microorganism and opposing fungous activity. Triazole subsidiaries was found to possess good analgesic and antiinflammatory properties. The oxadiazole subsidiaries showed against bacterial and hostile to fungous exercises. Likewise, the hindrance impact can increment with the enhance in concentration of those mixtures.

Keywords: 6-Methylbenzothiazol-2-Yl–Hydrazine; Triazoles; Oxadiazoles; Analgesic; Anti Inflammatory; Antimicrobial Activities.

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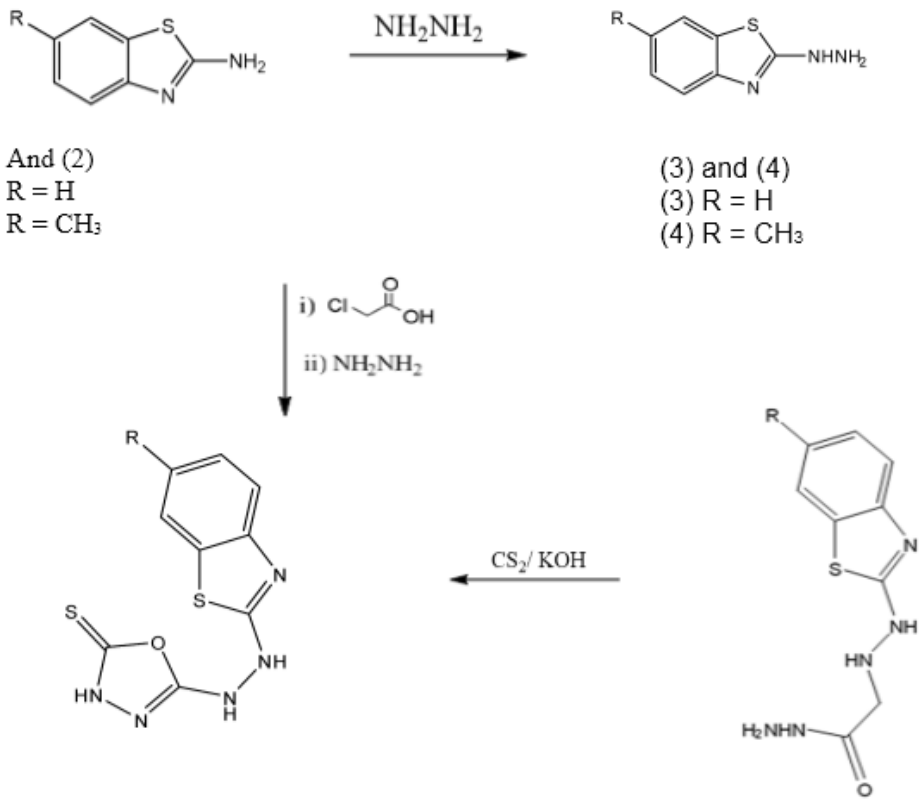
1. Introduction

Triazoles are regarded to possess a range of organic activities viz, antibacterial [1]. anti-fungicidal, Anti tubercular, hypoglycemic [2] and diuretic [3] properties. Further more, the intermediate compounds of Schiff's bases possess anti cancer activity in animal screening [4]. Taking this into consideration and our persevered notice within the preparation of biologically active heterocyclic compounds it used to be a topic of interest to synthesize some new triazole derivatives. The heterocyclic derivatives primarily Hexa cyclic and Penta cyclic species have inhabited a distinguished point among a range of organic derivatives for their multitudinous medicinal properties [5]. Amidst of broad form of triazoles and oxadiazoles are compete necessary |a vital| position in medicative chemistry that investigates for improving pharmacologically predominant derivatives. A number of them have entered good attention as implicit anti-fungal and antibacterial agents. Furthermore, triazole ring obtained a novel location within the heterocyclic field because of the very fact it's a usually experienced structural motive in several pharmaceutically pertinent organic compounds.

Triazole and oxadiazole have sparked a large amount of synthetic effort and biological activity [6]. The essential intermediary for subsequent synthesis was the derivatives of methanoic acid hydrazide. According to scheme-I, Benzothiazole was used to prepare the methanoic acid hydrazide derivatives, which were then reacted with carbon disulfide and potassium hydroxide to produce oxadiazole derivatives. Oxadiazole derivatives were used to syntheses triazole derivatives, which were then used to syntheses a triazole that was put into a Benzothiazole moiety. A review of their anti microbial, narcotic and anti-inflammatory action was deemed relevant in light of these considerations and our ongoing heed in the preparation of triazoles and oxadiazoles. Based on their elemental analyses and various spectral data, the synthesised compounds' structures were determined.

Synthesis of compounds

Figure 1 depicts the reaction plan for the synthesis of Compounds 3 - 12.



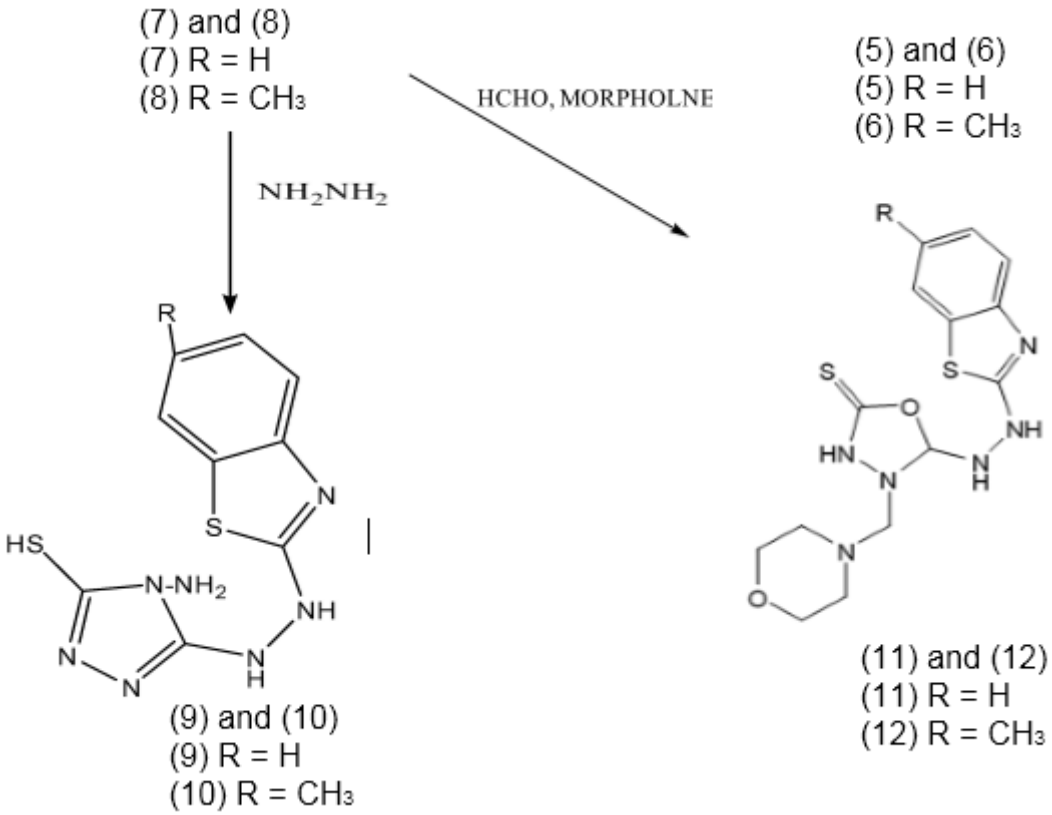


Figure 1: The reaction scheme for the synthesis of compounds

6-methylbenzothiazol-2yl-hydrazine (3,4).
Conc. HCl (6 mL) was gradually added while stirring to 6 mL of hydrazine hydrate between 5 and 10^{O}C. Then, during the next three hours, ethylene glycol (24 mL) and 6-methylbenzothiazolamine (I) (0.03 mol) were refluxed together. When cooled, it gives 6-methylbenzothiazol-2-ylhydrazine(II), which was drenched, water washed, and recrystallized from ethanol. This derivative was also dried. m.p.220.225 ^{0}C: IR (KBR) (Cun^{-1}): 3320, 3221, 1650, 1460 (C-N), 1192, 1157 and 820.

2-hydrazineyl-N'-(6-methylbenzothiazol-2-yl)acetohydrazide (**5,6**).
Chloroacetic acid (1 mole) and hydrazine hydrate(2 mole) were added to 6-methylbenzothiazol-2-yl-hydrazine [3,4] (1 mole) in 100 ml of ether . The mixture was continuously stirred as it was refluxed for 4 hours. The mixture was poured over crushed ice. The precipitate was collected and recrystallized from absolute alcohol after being filtered and dried.

5-(2-(6-methylbenzothiazol-2-yl) hydrazineyl)-1, 3, 4 - oxadiazole - 2 (3H) - thione (**7,8**).
A solution of KOH (1.5 moles in 20ml ethanol) and CS_2 were added to 2-hydrazineyl-N'-(6-methylbenzothiazol-2-yl) acetohydrazide (5.6) (1 mole) in ethanol (250 ml). This mixture was re-fluxed for 8 hours, concentrated, and acidified with diluted hydrochloric acid. The solid that resulted from these steps was then gathered, cleaned with water, and recrystallized from AcOH/H2O. m.p.177-179 ^{0}C. m/z: 279.02 (100.0%), 280.03 (11.0%), 281.02 (9.1%), 280.02 (3.4%), 282.02 (1.2%), 281.03 (1.1%). Elemental Analysis: C, 43.00; H, 3.25; N, 25.07; O, 5.73; S, 22.95.

4 - amino - 5 - (2 -(6 -methylbenzothiazol-2-yl) hydrazineyl)-4H-1, 2, 4-triazol - 3 - thiol (9,10)
A mixture of the oxadiazole derivative (7,8) (1.5 mol) and hydrazine hydrate (4.5 mol) in alcohol (60 mL) was heated for 3 hours to prepare Compound (5) which was ether washed and then recrystallized from AcOH/H2O . The excess hydrazine hydrate was removed under

reduced pressure. m.p.160.162 ^{0}C. m/z: 293.05 (100.0%), 294.06 (10.9%), 295.05 (9.6%), 294.05 (4.2%), 296.05 (1.1%). Elemental Analysis: C, 40.94; H, 3.78; N, 33.42; S, 21.86

5-(2-(6-methylbenzothiazol-2-yl)hydrazineyl)-4-(morpholinomethyl)-1,3,4-oxadiazolidine-2-thione (11,12)

Paraformaldehyde (0.5 mol) and morpholine (7.5 mol), were added to triazole derivatives (7,8) (1 mole) in absolute ethanol. For three hours, this derivative was heated over an ice bath. After cooling, the admixture attained was dried off. From AcOH/H2O, the product was recrystallized. m.p.120.122 ^{0}C. m/z: 380.11 (100.0%), 381.11 (20.1%), 382.10 (9.1%), 383.11 (1.5%), 382.12 (1.3%), 382.11 (1.1%). Elemental Analysis: C, 47.35; H, 5.30; N, 22.09; O, 8.41; S, 16.85

Pharmacology

Analgesic studies by tail clip method

The tail clip method [7] was used to conduct analgesic tests on albino rats weighing 80–100gms. Prior to the administration of the drug, food and water were withheld for 24 hours. Applying a bulldog clamp with its arms wrapped in a rubber tube to the base of the tail allowed researchers to examine all of the rats. Animals were disqualified if they didn't start making consistent attempts to remove the clip within 15 seconds. Six groups of six rats each were created from the rats that responded favourably. The animal's tail clip was placed at the base of its tail, and observations were performed up to 120 minutes after the test compound was administered. From the moment the clip was inserted until the animal attempted to remove it, the reaction time was measured in seconds. Prolongation of the reaction time in the compounds treated animals when compared to the controls gave the analgesic effect.

Table 1 displays the substances under study's analgesic effects

TABLE 1. Analgesic activities of the compounds

Compound	Dose level in mg/Kg	Reaction time in minutes observed					
		0	15	30	60	90	120
Control	-	3.08 ±0.204	3.17 ±0.258	3.17 ±0.258	3.33 ±0.258	3.17 ±0.258	3.08 ±0.204
Morphine	15	3.16 ±0.258	6.33 ±0.258	14.40 ±0.490	10.90 ±0.418	9.75 ±0.273	8.16 ±0.258
Compound 7	50	3.08 ±0.491	3.33 ±0.258	3.50 ±0.316	3.75 ±0.418	3.75 ±0.273	3.33 ±0.258
	100	3.25 ±0.273	3.92 ±0.376	4.41 ±0.491	5.30 ±0.605	5.25 ±0.273	4.83 ±0.408
Compound 8	50	3.08 ±0.204	3.75 ±0.27	3.83 ±0.25	4.00 ±0.31	3.92 ±0.49	3.58 +0.376
	100	3.33 ±0.25	4.08 ±0.37	4.75 ±0.27	5.416 ±0.373	5.35 ±0.48	4.75 ±0.68
Compound 9	50	3.50	3.91	4.25	4.25	3.82	3.58

		±0.447	±0.376	±0.418	±0.689	±0.258	±0.376
	100	3.83 ±0.258	4.41 ±0.376	5.50 ±0.447	5.75 ±0.273	5.33 ±0.258	4.42 ±0.441
Compound 10	50	3.16 ±0.251	3.50 ±0.44	3.58 ±0.37	3.67 ±0.408	3.66 ±0.40	3.43 ±0.40
	100	3.40 ±0.123	4.02 ±0.30	3.58 ±0.37	5.23 ±0.32	5.21 ±0.44	5.17 ±0.40
Compound 11	50	3.08 ±0.61	4.60 ±0.43	7.60 ±0.43	7.60 ±0.22	6.02 ±0.22	3.58 +0.22
	100	3.10 ±0.45	6.30 ±0.43	7.60 ±0.43	7.60 ±0.22	6.02 ±0..07	5.8 ±0.48
Compound 12	50	3.05 ±0.74	3.19 ±0.766	4.52 ±0.81	4.52 ±0. 89	3.28 ±0. 58	3.85 ±0. 76
	100	3.83 ±0.58	7.14 ±0.76	7.05 ±0.74	7.57 ±0.73	6.33 ±0.58	5.24 ±0.41

Mice's hind paw oedema was investigated by carrageenin's anti-inflammatory studies.

The compounds' anti-inflammatory properties were investigated using the studies by the system of Carrageenin instigated hind paw oedema in mice. The animals were split up into various groups of six apiece. After giving the compounds to the mice at varying dosages, 0.03 mL of carrageenin diluted in normal saline was injected into the right hind paw after 30 minutes. Four hours following the delivery of carrageenin, the animals were sacrificed. At the level of the ankle joint, the hind paw was severed, then weighed. Calculating the percentage of inhibition of hind paw oedema using control animals and comparing it to those receiving regular doses of phenylbutazone. Due to the volume of research utilising it as the standard, phenylbutazone was utilised as a standard for anti-inflammatory studies . The compounds were dosed to the animals at 50 and 100 mg per kilo gram of body weight. These substances were dissolved in 0.0002 L of DMSO0. It was discovered that DMSO had no anti-inflammatory effects of its own at this dosing level.

The Anti-inflammatory effect of the compounds under study is presented in Table 2.

TABLE 2. Anti-inflammatory activities of the compounds

Compound	Dose level mg/Kg body weight	Difference in weight of hind paw in mg mean ± SE	Percentage of inhibition
Control	-	36.25 ± 0.6892	-
Phenylbutazone	100	23.08 ± 0.3760	36.33
Compound 7	50	33.00 ± 0.5477	08.97

	100	29.17 ± 0.2582	19.54
Compound 8	50	31.75 ± 0.2582	12.41
	100	26.17 ± 0.6831	27.78
Compound 9	50	29.17 ± 0.6050	19.54
	100	31.50 ± 0.2582	13.10
Compound 10	50	31.41 ± 0.6128	13.34
	100	25.84 ± 0.1634	28.73
Compound 11	50	31.73 ± 0.5568	12.48
	100	27.95 ± 0.2143	22.91
Compound 12	50	30.46 ± 0.4757	15.97
	100	27.53 ± 0.3142	24.06

Each value represents the mean value ± SE of 6 observations

Well diffusion method for testing Antibacterial activity

The sterile Petri dishes received 20 millilitres of the nutritious agar medium. A cork borer with a 10 mm diameter was used to drill wells into the solidified plates. The nutrient broth medium was inoculated with the bacteria that had been subcultured for one day. Following inoculation, the compounds were individually dissolved in DMSO solvent and then pipetted into the wells at doses of 125 g, 250 g, and 500 g per 100 liters. The plates were kept at 37ºC for one day. For comparative analysis, the antibiotic streptomycin served as a benchmark.

The Antibacterial effect of the compounds under study is presented in Table 3.

TABLE 3. Anti-bacterial activities of the compounds

Human pathogens	Zone of inhibition of the compounds in 100 µL of DMSO against the Human pathogens						
	7 8 9 10					11 12	
	0.0 µg	250µg	250µg	2500µg	250µg	250µg	250µg
Staphylococcus aureus	-	15.0	19.0	22.5	14.0	16.0	18.6
Bacillus subtilis	-	12.0	14.0	15.0	14.6	15.4	16.0
Pseudomonas aeruginosa	-	18.4	22.0	25.8	18.0	21.0	24.8
Salmonella typhi	-	16.0	18.0	19.0	14.5	19.0	22.0
Salmonella paratyphi ‘A’	-	21.4	25.0	28.7	18.4	22.0	26.0

Salmonella paratyphi 'H'	-	18.0	20.5	22.0	16.7	18.0	24.5
Klebsiella pneumoniae	-	12	14.0	16.5	13.5	15.8	18.0
Proteus mirabilis	-	23.0	25.0	27.4	12.6	16.0	20.0
Proteus vulgaris	-	13.0	14.0	14.7	17.0	19.4	22.7
Escherichia coli	-	13.0	17.5	21.0	15.7	18.0	21.4

Antifungal activity by poison plate method.
To test each compound's antifungal activity, each was individually dissolved in the DMSO solvent. Using a micropipette, the derivatives of 125, 250, and 500 mg per 100 ml were added to the Petri plates. Then each Petri dish received 20 mL of the sterilised Sabourads agar media. The 8 mm-diameter fungus mycelia were inserted into the centre of the newly prepared culture plate after the medium had solidified. The solvent DMSO was utilised as a standard for comparison research in place of the chemical. As a control, the plate containing only the solvent and the mycelia was preserved. After 21 days of room temperature incubation, the growth of the mycelia on the plates was assessed.

Table 4 lists the compounds under investigation's antifungal effects.

TABLE 4. Anti-fungal activities of the compounds

Dermatophytes	Zone of inhibition of the compounds in 100 µL of DMSO against the Dermatophyte (diameter in mm)									
	7		8	9	10	11		12		
	0.0 µg	250µg	250µg	250µg	250µg	250µg	250µg			
Microsporum gypseum	-	9.0	12.0	16.4	10.0	13.0	16.5			
Microsporum nanum	-	12.0	14.5	17.4	11.0	14.2	17.0			
Trichophyton mentagrophytes	-	18.0	16.0	20.0	12.0	13.0	18.0			
Trichophyton rubrum	-	11.0	13.0	29.0	12.0	14.0	28.0			

2. Results and Discussion

The distinctive band of compounds 7 and 8 can be seen in their FT-IR spectra. Both =N-NH2 group and triazole moiety N-H stretching produce bands with peaks at 3420 cm^{-1} and 3252 cm^{-1}, respectively. The aromatic ring's stretching from =C-H is what causes the peak at 3024 cm^{-1}. At 2908 cm^{-1} and 2847 cm^{-1}, respectively, the methylene group's symmetric and asymmetric C-H stretching is seen. The S-H stretching is represented by an absorption band at 2620 cm^{-1}. At 1621 cm^{-1}, N-H bending vibration happens. At 1578 cm^{-1}, the oxadiazole moiety's C=N stretching may be seen. At 1600 cm^{-1}, 1558 cm^{-1}, and 1462 cm^{-1} areas, skeleton vibrations involving aromatic C=C stretching within the ring are absorbed. The prominent band seen at 1273 cm^{-1} is attributed to the C-N stretching. Peaks at 1120 cm^{-1} are visible in the

oxadiazole moiety's C-O stretching. Due to the thiol-thione tautomers SH and C=S, the C=S stretching is represented as a medium absorption band at 1016 cm^{-1}. It is possible to attribute the peaks at 752 cm^{-1} and 699 cm^{-1} to aromatic C-H out of plane bending vibrations. The N-H wagging vibrations that are out of plane may be responsible for the peak at 502 cm^{-1}.

Compounds 9 and 10's FT-IR spectra display the following distinguishing band. A sharp band at 3426 cm^{-1} results from the triazole moiety's N-H stretching (arising out of thiol - thione tautomers). The N-H stretching of the =N-NH and >N-NH2 groups is responsible for the peaks at 3310 cm^{-1} and 3272 cm^{-1}. The stretching of the aromatic ring by =-C-H is what causes the peak at 3023 cm^{-1}. At 2950 cm^{-1} and 2872 cm^{-1}, respectively, the methylene group's symmetric and asymmetric C-H stretching is seen. The stretching of the S-H atoms leads to an absorption band at 2623 cm^{-1}. At 1625 cm^{-1}, N-H bending vibration happens. At 1581 cm^{-1}, the triazole moiety's C=N stretching is seen. At 1598 cm^{-1}, 1523 cm^{-1}, and 1408 cm^{-1} areas, skeleton vibrations involving aromatic C=C stretching within the ring are absorbed. The C-N stretching is thought to be responsible for the strong band seen at 1252 cm^{-1}. Due to the thiol-thione tautomers SH and C=S, the C=S stretching is represented as a medium absorption band at 1028 cm^{-1}. It is possible to attribute the peaks at 756 cm^{-1} and 607 cm^{-1} to the aromatic C-H out of plane bending vibrations. The N-H wagging vibrations that are out of plane may be responsible for the peak at 514 cm^{-1}.

The distinctive band of compounds 11 and 12 can be seen in their FT-IR spectra. Due to the =N-NH2 group's N-H stretching, a wide band with a centre point of 3309 cm^{-1} is produced. The aromatic ring's stretching from =C-H is what causes the peak at 3025 cm^{-1}. The methylene group's symmetric and asymmetric C-H stretching is reserved at 2908 cm^{-1} and 2850 cm^{-1}, respectively. At 1621cm^{-1}, N-H bending vibration happens. At 1582 cm^{-1}, the oxadiazole moiety's C=N stretching may be seen. At 1593 cm^{-1}, 1558 cm^{-1}, and 1462 cm^{-1} areas, skeleton vibrations involving aromatic C=C stretching within the ring are absorbed. The C-N stretching is responsible for the strong bands seen at 1378 cm^{-1} and 1349 cm^{-1}. The oxadiazole and morpholine moieties exhibit a peak in C-O stretching at 1286 cm^{-1}, 1223 cm^{-1}, and 1035 cm^{-1}. For the C=S stretching, a medium absorption band at 1013 $cm^{-1'}$ is appropriate. The aromatic C-H out of plane bending vibrations may be responsible for the high values at 753 cm^{-1} and 697cm^{-1}. The N-H wagging vibrations that are out of plane may be responsible for the peak at 547 cm^{-1}.

The following signals can be seen in the compound 7 and compound 8's H-NMR spectrum. A singlet is visible at 12.86 ppm for the thiol proton, SH proton, and NH proton resulting from thiol-thione tautomerism. Due to the NH2 protons, the molecule exhibits singlet resonance signal at 7.90 ppm. The resonance signals of aromatic protons are represented by the multiplet signals between 7.27 and 7.01 ppm. The triplet signals in the methine protons are focused at 3.85 ppm. The nitrogen atom of the moiety's connected methylene group exhibits a singlet at 3.61 ppm. At 1.69 ppm, the methylene protons in the third and fifth positions of the ring exhibit doublet signals.

The following signals can be seen in the chemical 9 and 10's H-NMR spectra. A singlet peak at 12.88 ppm may be seen in the thiol proton, SH proton, and NH proton that result from the thiol-thione tautomerism. The hydrazine -(N-NH2) protons are responsible for a singlet at 7.96 ppm. At 7.70 ppm, the singlet resonance signal caused by -(N=NH2) protons is detected. The resonance signals of aromatic protons are represented by the multiplet signals between 7.29 and 7.02 ppm. The aromatic moiety of methine protons exhibits doublet signals in the range of 3.86 to 3.90 ppm. The resonance singlet peak for the methylene group, which is linked to the nitrogen ring, is located about 3.60 ppm. The ring-presented methylene protons exhibit a doublet at 1.69 ppm.

The following signals can be seen in the compound 11 and compound 12's H-NMR spectrum. The hydrazoyl - (N=NH2) protons are responsible for the resonance signals at 7.70 ppm. The resonance signals of aromatic protons are represented by multiplet signals between 7.28 and 7.03 ppm. At 3.67 ppm, a doublet is

visible for the methylene groups joined to the oxygen atom of the morpholine moiety. Signals between 2.89 and 3.61 ppm are displayed by the other methylene group of the morpholine moiety and the methylene group joined to the nitrogen atom of the aromatic moiety. A singlet peak is present at 3.73 ppm for the methylene group that joins the morpholine moiety to the oxadiazole ring. The methine protons are thought to be responsible for the triplet proton signals centred at 3.88 ppm. At 1.68 ppm, a doublet is seen for the methylene protons bound to the third and fifth carbon atoms of the aromatic ring.

Compounds 7 and 8's 13C-NMR spectra display the following distinctive signature. At 156.65 ppm, the carbon atom that is joined to the sulphur atom resonates. The carbons connected to the hydrazono group are what cause the peak to be seen at 154.84 ppm. The other carbon atom in the oxadiazole ring that is linked to the oxygen is what causes the resonance signals at 153.35 ppm. At 139.21, 129.92, 128.12 and 127.80 ppm, the aromatic carbon resonance signals are detected. At 54.27 ppm, the methylene carbon signal that is sandwiched between the aromatic and oxadiazole rings may be seen. At 53.79 ppm, signals resulting from carbons linked to the phenyl group in a hydrazono ring may be seen. The hydrazono aromartic ring's other carbons have a 38.18 ppm resonance.

Compounds 9 and 10's 13C-NMR spectra display the following distinctive signature. The other tertiary carbon atom in the oxadiazole ring is what is responsible for the signal at 159.83 ppm. At 147.56 ppm, the carbon molecule to which the thiol group is bonded resonates. The carbon atom that is linked to the hydrazono group produces a signal at 155.10 ppm. The resonance signals of the aromatic carbons are responsible for the peaks seen at 138.29, 129.92, 128.46, and 126.91 ppm. At 53.12 ppm, the signals resulting from the phenyl group connected to methine carbons are visible. At 37.53 ppm, the methylene carbon signal is detected between the aromatic and triazole rings.At 39.18 ppm, the other carbons in the hydrazono aromatic ring resonant.

Compounds 11 and 12's 13C-NMR spectra display the following distinctive signal. At 157.87 ppm, the carbon atom that is joined to the sulphur atom resonates. The carbon with the formula -C=N-NH2 has a peak at 154.96 ppm. The other tertiary carbon atom in the oxadiazole ring is what is responsible for the signal at 153.68 pom. The aromatic carbon atoms' resonance signals can be seen at 136.59, 129.31, 128.54 and 127.02 ppm. The carbon atom bound to the oxygen in the morpholinyl ring is the cause of the resonance signal at 71.36 ppm. At 70.29 ppm, the methylene carbon bordered by the morpholine and oxadiazole rings is responsible for the signal. At 54.51 ppm, the methylene carbon signal that is bordered by the aromatic and oxadiazole rings may be seen.The carbon atom in the morpholinyl ring next to the nitrogen atom is what causes the resonance signal at 54.02 ppm. At 53.73 ppm, the methine carbons linked to the phenyl group are visible. At 38.83 ppm, the hydrazono-aromatic ring's methylene carbon signal may be seen.

The synthesized compounds have the benzothiazole moiety and possess analgesic activity. Though these compounds are toxic these could still be used with other adjuvant or synergistic agent to bring down the toxicity. In addition, this novel series would provide better SAR for benzothiazole analogues in general and possible some new promising analgesics. The treatment of synthesized test compounds shows significant anti-inflammatory activity but still the synthesized test compounds are less potent than the phenylbutazone. All pathogens, including Pseudomonas aeruginosa, Salmonella Typhi, Salmonella Paratyphi 'A', and Salmonella Paratyphi 'H', are effectively combatted by the prepared compounds. In contrast to other pathogens like Staphylococcus Aureus and Klebsiella pneumoniae, which had modest action, compounds were ineffective against infections like Bacillus Subtilis. Additionally, it is evident that when compound concentration rises, the inhibition zone widens. When compared to dermatophytes such Microsporum Gypseum, the compounds are more active, while Microsporium Nanum, Trichophyton Mentagrophytes, and Trichophyton Rubrum are less active. Thus, the presence of Morpholinyl and triazole moiety int

the compound enhances the antifungal activity. Moreover, the antifungal activity depends primarily on the voluminosity of the substituent in the compounds.

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