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# BIOLOGICAL EVALUATION OF SOME SYNTHESIZED N- SUBSTITUTED 1, 3, 4 THIADIAZOLE DERIVATIVES BY USING INVITRO MODEL

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## ABSTRACT

1,3,4-Thiadiazole and its derivatives continue to be of great interest to a large number of researchers.1,3,4-thiadiazole is one of the most potent heterocyclic containing carbonic anhydrase and antibacterial inhibitor from the natural and synthetic origin. At present many 1,3,4-thiadiazole nucleus containing drugs are available in the market such as acetazolamide, methazolamide, megazol. In the present investigation we have synthesized 1,3,4 thiadiazoles and tested for brine shrimp lethality assay, possibly due to the presence of =N–C–S moiety. All the synthesized compounds were tested for toxicity by using brine shrimp model. The compound 3d, 3k, 3i, 3n having highest cytotoxic effect according standard drug 5-Fluorouracil.

**Keywords*:*** - 1,3,4-Thiadiazole, toxicity study, Brian shrimp, 5-Fluorouracil

## INTRODUCTION

1,3,4-Thiadiazole and its derivatives continue to be of great interest to a large number of researchers.[(1)]1,3,4-thiadiazole is one of the most potent heterocyclic containing carbonic anhydrase and antibacterial inhibitor from the natural and synthetic origin. Till date many 1,3,4-thiadiazole nucleus containing drugs are available in the market such as acetazolamide, methazolamide, megazol.[(2)] 1,3,4 thiadiazoles exhibit divers biological activities possibly due to the presence of =N–C–S moiety. Moreover, some bi-heterocyclic compounds incorporating 1,3,4thiadiazole or 1,2,4triazole ring have been produced as antimicrobial agents.The cyclization of the compounds having thiosemicarbazide structure has shown to be an excellent strategy for the synthesis of 1,2,4 triazole, 1,3,4 thiadiazole, 1,3,4 oxadiazole and or 1,3,4 triazine derivatives.[(3)] Heterocycles bearing a symmetrical triazole or 1,3,4-thiadiazole moieties, represent an interesting class of compounds possessing a wide spectrum of biological activities such as anti-inflammatory, antiviral and antimicrobial properties. It has also been reported that derivatives of 1,2,4-triazole and 1,3,4-thiadiazole condensed

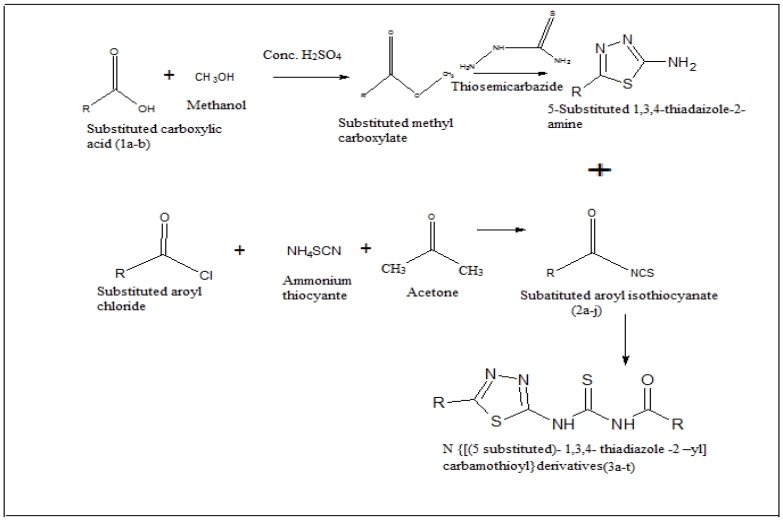
nucleus systems exert diverse pharmacological activities such as anti-inflammatory, antitumor, antifungal and antibacterial.(4) A large no of thiadiazoles have been patented in the medicine field for the treatment of a wide variety of diseases and some of them have become commercial products compound in medicinal chemistry because of its therapeutic value .it is also known to have unique antibacterial and anti-inflammatory activities.(5) The thiazole moiety belongs to an important class of N and S- containing heterocycles and when attached to a thiourea functional group forms the building block for pharmaceutical agents. They exhibit a wide spectrum of pharmaceutical activity.(6) Thiadiazole is a very multifaceted moiety that shows a wide range of pharmacological activity. Thiadiazoles are bioisosteres of heterocycles such as oxadiazole, oxazole and benzene. Substitution of thiadiazoles with their bioisosteres increases activity.(7) The potency andselectivity of NSAIDs appear to be directly related totheir gastric, renal and hepatotoxicity.A number of 1-acylthiosemicarbazides, 1,3,4-oxadiazoles,1,3,4-thiadiazoles and 1,2,4-triazole-3-thioneswere identified as potent anti-inflammatory compounds.(8)

A compound's toxicity is measured using two indicators: the median effective concentration ($EC_{50}$) and the median lethal concentration ($LC_{50}$). The concentration-response curve is generated using 24 h of data to obtain the $EC_{50}$ (teratogenic effects) and LC50 (embryotoxic effects). The ratio of $LC_{50}$ to $EC_{50}$ is the corresponding therapeutic index (TI)[9,10]. In pharmaceutical administration, a TI value < 1 is optimal. In the present study thiourea is important sulfur and nitrogen containing compound and they are useful substances in drug research because it has wide range of Pharmacological activity[11]. The azole when attached to Thiourea functional group forms the building block for pharmacological agents. They exhibit a wide spectrum of pharmaceutical activity. In the design of new Thiourea and 1, 3, 4-thiadiazole derivatives, this novel dual inhibitory activity of enzyme pathways holds promise as anti-inflammatory agent with improved efficacy and safety profile.

**Methodology:**[12,13,14,15]

Step 1: Microwave synthesis Method:

a. Synthesis of 5 (phenyl)-1, 3, 4-thiadiazole -2-amine.
b. Synthesis of substituted benzoyl isothiocyanate (1a-j).
c. Synthesis of substituted N-{[5-(phenyl)-1, 3, 4-thiadiazole -2-yl] carbamothioyl} derivatives




**A. Synthesis of 1,3,4-Thiadiazole derivatives by microwave method:**

**Step 1st: - Synthesis of 5-(substituted)-1,3,4-thiadiazole - 2-amine:**

Methyl substituted carboxylate was prepared by using 0.01 mole of substituted carboxylic acid in 35ml methanol. Carboxylic acid is esterified with methanol. The heat the reaction in microwave under reflux for 20 to 25 min at 340 watts.by adding few drops of $H_2SO_4$ as catalyst. The mixture of above compound (0.01mol) and 2 ml thiosemicarbazide was refluxed for 15 to 20 min at 34 watts. TLC tested reaction after completion and solid precipitate was dried and recrystallize from methanol the solid precipitate was washed, dried and recrystallize from ethanol.

**Step 2nd: - Synthesis of substituted benzoyl isothiocyanate:**

A chloride substituted for benzoyl (10mmol) solution in C3H6O (50ml) Was introduced in absolute terms acetone (30ml) to ammonium thiocyanate (10mmol). At 340 watts. The blend of reactions was Shot for 15 to 20 min under reflux. TLC tested after the reaction is complete. refrigerated to room temperature, and filtered off the formed precipitate (NH4Cl). To ready for the fresh. To the freshly formulated solution of derivative aroyl isothiocyanate.

**Step 3rd: - Synthesis of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl] carbamothioyl} derivatives:**

A solution of 5-(substituted)-1,3,4-Thiadiazole -2-amine (10mmol) in acetone (10ml) was added and the resulting mixture was stirred with refluxed for 15 to 20 min at 340 watts. TLC tested after the reaction is complete. The solid prod was washed with water and purified by washing with ethanol absolute. The physical data of 5-(substituted)-1,3,4-Thiadiazole -2-amine are presented in table and the physical data of N-{[5-(substituted)-1,3,4-thiadiazole-2-

yl] carbamothioyl} are presented in table 6.1.2.2 all the compounds are stable and can be stored at room temperature.

**Table no. 1 Physicochemical data of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl] carbamothioyl} by conventional and microwave method (3a-t).**

| Sr. no | Compounds | Molecular Formula | Molecular Weight | MP $^0C$ | Percentage Yield% | | Rf value |
|---|---|---|---|---|---|---|---|
| | | | | | Conventional | Micro-wave | |
| 1 | 3a | $C_{17}H_{14}N_4O_2S_2$ | 370.44 | 134- 136 | 50% | 56% | 0.84 |
| 2 | 3b | $C_{17}H_{13}ClN_4O_2S_2$ | 404.89 | 142- 144 | 61% | 64% | 0.91 |
| 3 | 3c | $C_{17}H_{13}ClN_4O_2S_2$ | 404.89 | 138- 140 | 66% | 70% | 0.84 |
| 4 | 3d | $C_{17}H_{12}Cl_2N_4O_2S_2$ | 439.33 | 166-168 | 47% | 54% | 0.86 |
| 5 | 3e | $C_{18}H_{18}N_4O_3S_2$ | 400.47 | 144- 146 | 70% | 76% | 0.94 |
| 6 | 3f | $C_{17}H_{13}FN_4O_2S_2$ | 388.43 | 136- 138 | 61% | 67% | 0.75 |
| 7 | 3g | $C_{17}H_{13}N_5O_4S_2$ | 415.44 | 112- 114 | 73% | 79% | 0.82 |
| 8 | 3h | $C_{12}H_{11}ClN_4O_2S_2$ | 342.82 | 126- 128 | 60% | 75% | 0.84 |
| 9 | 3i | $C_{15}H_{18}N_4O_2S_2$ | 350.45 | 124- 126 | 72% | 79% | 0.84 |
| 10 | 3j | $C_{11}H_9Cl_2N_4O_2S_3$ | 396.31 | 136- 138 | 76% | 82% | 0.89 |
| 11 | 3k | $C_{16}H_{10}N_6O_5S_2$ | 430.41 | 102- 104 | 61% | 64% | 0.94 |
| 12 | 3l | $C_{16}H_9ClN_6O_5S_2$ | 464.86 | 144-146 | 65% | 69% | 0.84 |
| 13 | 3m | $C_{16}H_9ClN_6O_5S_2$ | 464.86 | 152- 154 | 76% | 82% | 0.76 |
| 14 | 3n | $C_{16}H_8Cl_2N_6O_5S_2$ | 499.30 | 124-126 | 79% | 85% | 0.74 |
| 15 | 3o | $C_{17}H_{12}N_6O_6S_2$ | 460.44 | 128- 130 | 69% | 76% | 0.78 |
| 16 | 3p | $C_{16}H_9FN_6O_5S_2$ | 448.40 | 118- 120 | 65% | 79% | 0.83 |
| 17 | 3q | $C_{16}H_9N_7O_7S_2$ | 475.41 | 128-130 | 72% | 78% | 0.92 |
| 18 | 3r | $C_{11}H_7ClN_6O_5S_2$ | 402.79 | 196-198 | 80% | 87% | 0.82 |
| 19 | 3s | $C_{14}H_{14}N_6O_5S_2$ | 410.42 | 144-146 | 78% | 87% | 0.84 |
| 20 | 3t | $C_{10}H_5Cl_2N_6O_5S_3$ | 456.28 | 132-134 | 82% | 88% | 0.78 |

**Cytotoxicity Test** [16,17]

Recently, brine shrimp (*Artemia salina*) lethality assay is commonly used to check the cytotoxic effect of bioactive chemicals. Bioassay of brine shrimp deaths is simple, rapid, inexpensive, easily mastered, and requires small amounts of test material (10-20 mg or less). For those kinds of human solid tumors the bioassay has good correlation with cytotoxic activity.

**Serial dilution of synthesized compound**

All test tubes were clean taken and labeled properly. An analytical balance was weighed on the synthesized 10 mg compound. Stock solution was then prepared with 10 mg soluble of synthesized compound. Serially Prepared dilution from stock solution. Four it has been the test tubes labeled as 1-4.

**Hatching brine shrimp**

Measure 3 liters of water using measuring cylinder and pour into the rectangular jar. Weigh about 27 g of table salt by a balance and supplemented with 4.2 mg/l dried yeast add it into the jar containing water. Mix salt in water with help of spatula. Place the tip of an airline air pump in the bottom of the pot by continually maintaining the aeration. At the top water level of the jar, add about 10 g of brine shrimp eggs and mix with water. Switch on a light, Placed within a few inches of the pot. After 20-24 hours the nauplii will hatch. Observe the nauplii. Select the nauplii after 20-24 hours from next. Hatched nauplii from empty egg should be separated and through the shutting the air off and the light off. Pour 10 nauplii it into test tube by using Pasteur pipette.

**Toxicity testing**

Expose the nauplii to various concentrations of the synthesized compound. Count the no. of live and calculate the percentage of death after 24 hours

Number of % dead nauplii calculated by using following formula,

$$\% \text{ Death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{Number of live nauplii}} \times 100$$

**Observation:**

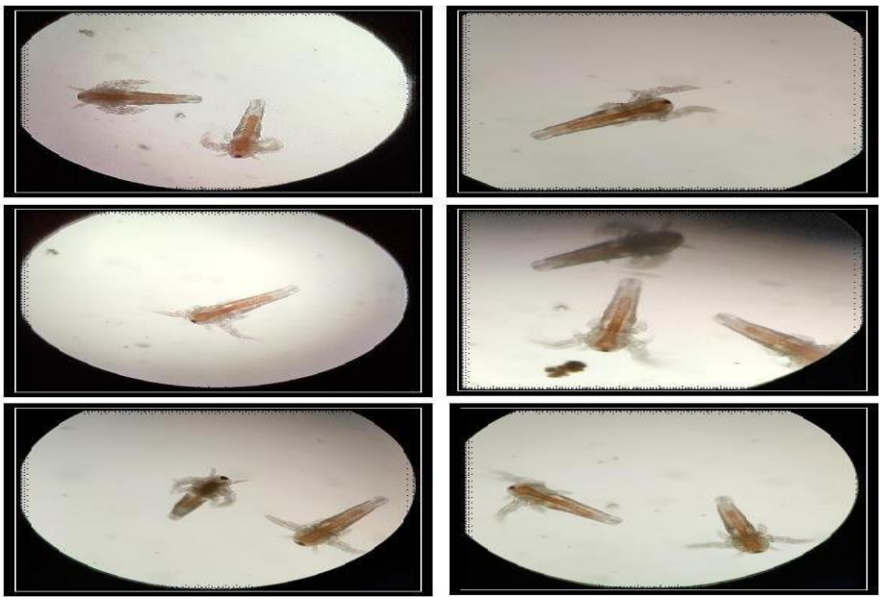
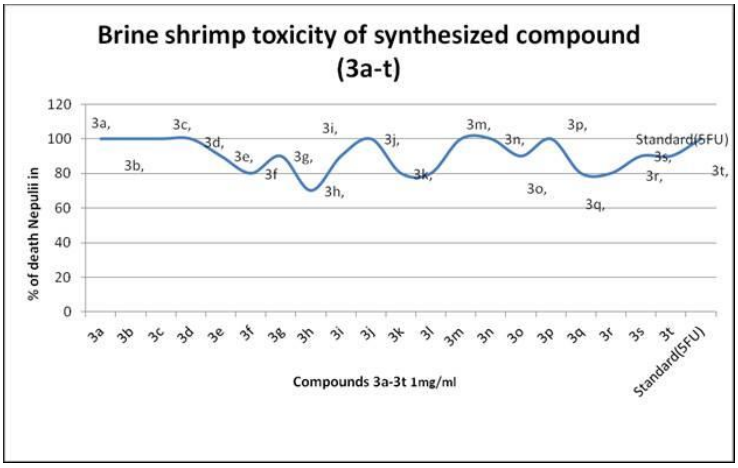


**Fig.no.1: Brine shrimp nauplii dead images**

**Table no.2: Brine shrimp toxicity of synthesized compound (3a-t):**

| Sr. no | Compound | % dead nauplii | | | |
|---|---|---|---|---|---|
| | | 1mg | 100ug/ml | 10ug/ml | 1ug/ml |
| 1 | 3a | 100 | 80 | 50 | 10 |
| 2 | 3b | 100 | 80 | 60 | 50 |
| 3 | 3c | 100 | 70 | 40 | 20 |
| 4 | **3d** | **100** | **80** | **60** | **10** |
| 5 | 3e | 90 | 70 | 70 | 20 |
| 6 | 3f | 80 | 60 | 60 | 20 |
| 7 | 3g | 90 | 80 | 50 | 10 |
| 8 | 3h | 70 | 70 | 70 | 30 |
| 9 | **3i** | **90** | **70** | **70** | **30** |
| 10 | 3j | 100 | 80 | 50 | 30 |
| 11 | **3k** | **80** | **70** | **70** | **10** |
| 12 | 3l | 80 | 70 | 50 | 20 |
| 13 | 3m | 100 | 70 | 50 | 40 |
| **14** | **3n** | **100** | **80** | **50** | **30** |
| 15 | 3o | 90 | 50 | 40 | 10 |
| 16 | 3p | 100 | 80 | 70 | 30 |
| 17 | 3q | 80 | 70 | 70 | 40 |
| **18** | 3r | 80 | 70 | 70 | 40 |
| 19 | 3s | 90 | 70 | 70 | 30 |
| 20 | 3t | 90 | 50 | 40 | 10 |
| **21** | **Standard(5FU)** | **100** | **70** | **70** | **20** |




**Graph No :1: Brine shrimp toxicity of synthesized compound (3a-t)**

## RESULT AND DISCUSSION:

Check sample lethality test in a zoological organism, such as the saline Artemia (shrimp). Brian Shrimp Cytotoxicity Test sampling a large-scale compound for their various bioactivities is very useful. The synthesized compound 3a-t used for the cytotoxicity test by using brine shrimp. The compound 3d, 3k, 3i, 3n having highest cytotoxic effect according standard drug 5-Fluorouracil.

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