# SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITIES OF NOVEL 2,4-DITHIOBIURET

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**Abstract**
*Drugs containing heterocyclic compounds showed remarkable and noticeable drug effect: hence they create their own importance in medicinal, agricultural and drug sciences. A simple and efficient method has been developed for the synthesis of a series of novel 2,4-dithiobiuret derivatives. In this work, a new series of 2-(5-subtituted-2,4-dithiobiurete) benzothiazole derivatives have been prepared by the interaction 1:1 molar proportion of 1-(benzo[d]thiazol-2-yl)thiourea (I a) with substituted isothiocyanates (II a-g) in 60% acetone-ethanol medium and the reaction mixture refluxed on water bath, recrystallized the product by using ethanol. This method provides the rapid and easy access of the product in very good yield. The recrystallized products were characterized on the basis of elemental analysis, chemical characteristics and spectral data. Screening of antioxidant activities of novel synthesized compounds.*
***Keywords:*** *1-(benzo[d]thiazol-2-yl) thiourea, substituted isothiocyanates, 60% acetone-ethanol medium, Biological evaluation.*

## Introduction

Heterocyclic compounds exhibits number of application in numerous fields like pharmaceutical, medicinal, therapeutic, drug and agricultural. 2, 4-Dithiobiuret it is a significant biological moiety[1-3] and also a good intermediate in the synthesis of different type of vital active heterocycles. Synthesis and biological valuation of novel 2,4-dithiobiurets is thrilling field in the organic chemistry. 2,4-dithiobiuret have many application in biological[4], medicinal[5], pharmaceutical[6.] Further specifically, nitrogen and sulphur containing heterocycles and derived compounds from the benzothiazole moiety possess a variety of antimicrobial activity[7-10] and antioxidant activities[11-12]. 2,4-Dithiobiurates are used as drug and also showed biological applications and significances, hence many analogs of them are used in various medicinal, industrial, agricultural, biochemical sciences[13-14]. 1,2,4-Dithiazoles were obtained by an oxidative cyclisation of 2,4-dithiobiuret making use of bromine in chloroform[15], 1,3,5-dithiazines were synthesized from 2,4-dithiobiurets and isocyanodichlorides[16]and these 1,3,5-dithiazines gave directly 1,3,5-triazines by simple isomerization[17].

Considering all these facts, it was planned to design, synthesize and to explore reactions of 1-(benzo[d]thiazol-2-yl) thiourea (Ia) with different alkyl/arylisothiocynates (IIa-f) in 60% acetone-ethanol medium were investigated.

## Materials and Method

All the chemical used in the present research were MERCKS (India Made). Starting compounds (I) were synthesized by literature method[18]. Method employed in the present experiments for the synthesis of various substituted 2,4-dithiobiuertes based benzothiazole is conventional refluxing under electronic water bath for different hours for different experiments. Melting points of all the synthesized compounds estimated using paraffin oil and uncorrected. The purity of the compounds was check by TLC on silica gel in petroleum ether and ethyl acetate [80:20]. The carbon, hydrogen, sulphur and nitrogen analysis was carried out on Carlo-Ebra-1106 analyzer and Colman-N-analyzer-29. IR spectra were recorded on SCIMADZU FTIR spectrometer in the range 4000-400 cm-1:1 in KBr pellets. PMR spectra were recorded on BRUKER AVANCE II 400 NMR spectrometer with TMS as an internal standard using CDCl3 and DMSO-d6 as a solvent. Mass spectra were recorded on WATERS,Q-TOF micromass(ESI-MS).

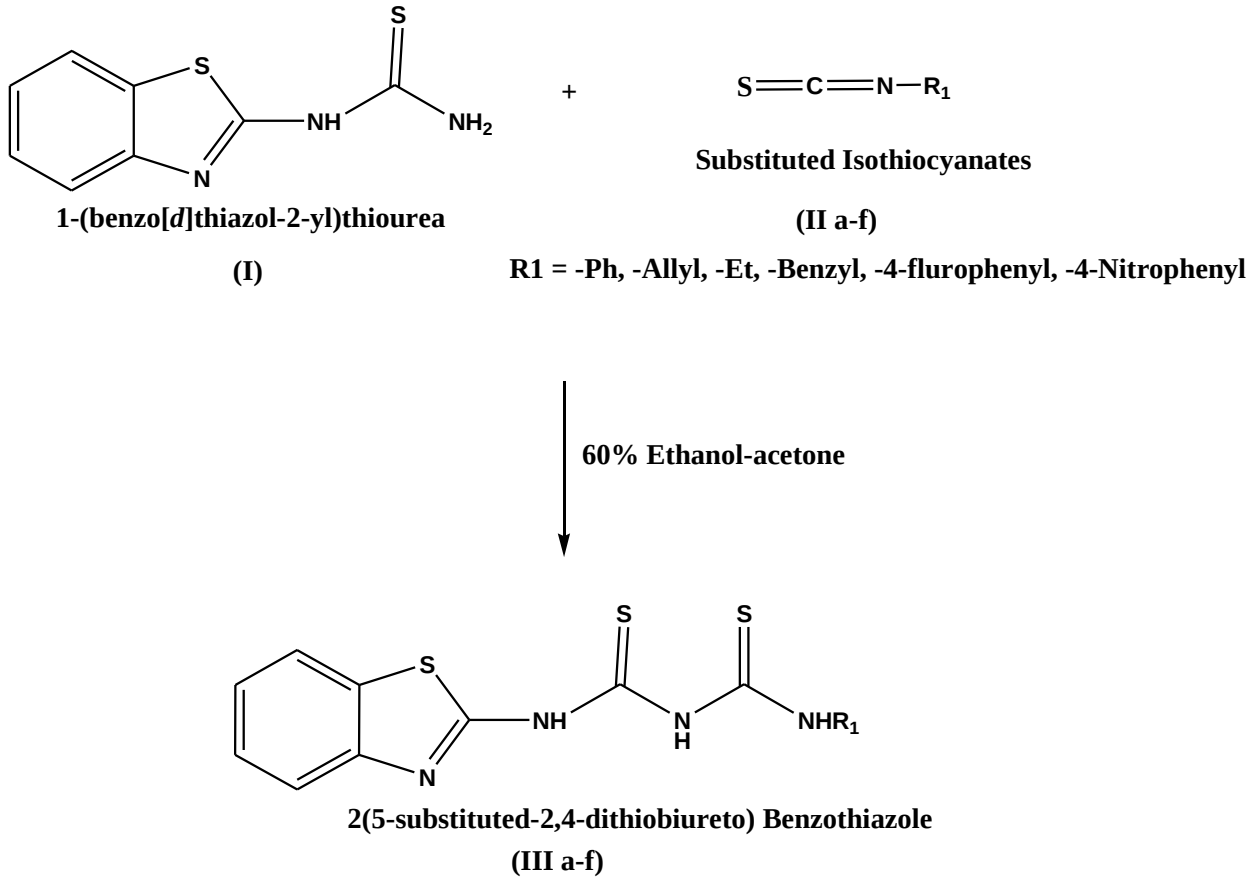
## Experimental

### General Procedure

2-(5-substituted-2,4-dithibiurete) benzothiazole (III a-f) was synthesized by the interaction of 1-(benzo[d]thiazol-2-yl) thiourea (I) with different

isothiocyanates(II a-f) in 60% ethanol - acetone medium reflux for four hours. During heating reactant dissolved into the solvent. After distillation of excess solvent crystals were obtained, which recrystallized from ethanol to obtain 2-(5-substituted-2,4-dithibiurete) benzothiazole (IIIa-f).

General reaction scheme for synthesis of various 2,4-dithiobiuret




**1-(benzo[*d*]thiazol-2-yl)thiourea**

**(I)**

**2(5-substituted-2,4-dithiobiureto) Benzothiazole**

**(III a-f)**

**Synthesis of 2-(5-Phenyl-2,4-dithibiurete) benzothiazole (III a)**

In 100 ml round bottom flask a reaction mixture of 1-(benzo[d]thiazol-2-yl) thiourea (I) and Phenylisothiocyanate (IIa) in 1:1 molar proportions was refluxed in 60% acetone-ethanol medium for 4 hours on water bath, brownish crystals were separated out, filtered and dried at room conditions. Recrystallized from ethanol, Completion of reaction was monitoring by TLC. Yield 80%, melting point-199$^{o}$C.

Similarly, 1-(benzo[d]thiazol-2-yl) thiourea (I) interacted with methylisothiocyanate (IIb), Ethlylisothiocyanate (IIc), Benzylisothiocyanate (IId), 4-flurophenylisothiocyanates (IIe), 4-Nitrophenylisothiocyanate (IIf) to isolate 2-(5-allyl-2,4-dithibiurete) benzothiazole (IIIb), 2-(5-Ethyl-2,4-dithibiurete) benzothiazole (IIIc), 2-(5-Benzyl-2,4-dithibiurete) benzothiazole (IIId), 2-[5-(4-flurophenyl)-2,4-dithibiurete]benzothiazole (IIIe), 2-[5-(4-Nitrophenyl)-2,4-dithibiurete]benzothiazole (IIIf), by above mentioned method and enlisted in **Table No.1**.

**Table No. 1**

| Sr.No. | 2-(5-substituted-2,4-dithibiurete) benzothiazole (III a-f) | Yield % | M.P. $^{o}$C |
|---|---|---|---|
| **1** | 2-(5-methyl-2,4-dithibiurete) benzothiazole (IIIb) | 81 | 198 |
| **2** | 2-(5-Ethyl-2,4-dithibiurete) benzothiazole (IIIc) | 83 | 201 |
| **3** | 2-(5-Benzyl-2,4-dithibiurete) benzothiazole (IIId) | 79 | 200 |
| **4** | 2-[5-(4-flurophenyl)-2,4-dithibiurete]benzothiazole (IIIe) | 89 | 223 |
| **5** | 2-[5-(4-Nitrophenyl)-2,4-dithibiurete]benzothiazole (IIIf) | 91 | 241 |

**Result and Discussion**

Spectral characterization results for all the synthesized compounds are given below.

**Spectral Characterization**

**Synthesis of 2-(5-Phenyl-2,4-dithibiurete) benzothiazole (III a)**

**Colour**-Brownish, **Molecular formula**-$C_{15}H_{12}N_4S_3$, **Yield** 81%, **M.P**. 198$^0$C, **% Composition found (calculated)** C-52.30 , H-3.83 , N-26.20, S-26.21 , **FTIR (Kbr) vcm**- 3323.22 N-H stretching, 3001.47 (C-H Ar Stretching), 3136.38 (N-H Amido), 1946.37 (C-H Ar Bending,), 1184.99 (C=S Stretching), 680.04(=C-H bending); **1H NMR (400MHz CDCL$_3$ δ ppm)**, δ 2.02 ppm (1H, Singlet, -NH), Singlet of 2H of –NH at δ 3.8 ppm, doublet of 2CH aromatic benzothiazole at δ 8.20ppm and δ 8.10 ppm, multiplate 5H of Ph at δ 6.46 ppm - δ 7.04 ppm. **Mass** m/z 344.12.

**Synthesis of 2-(5-Methyl-2,4-dithibiurete) benzothiazole (III b)**

**Colour**-Green Solid, **Molecular formula**-$C_{10}H_{10}N_4S_3$, **Yield** 80%, **M.P**. 199$^0$C, **% Composition found (calculated)** C-40.30 , H-2.90 , N-18.10, S-33.12 , **FTIR (Kbr) vcm**-1 3414 N-H stretching., 2972 C-H stretching.,1734 N=C-N stretching., 1616 C=C stretching., 1541 N-C=S stretching., 1149 C-N stretching **1H NMR (400MHz CDCL$_3$ δ ppm)**, doublet of 3H of –CH3 at δ 2.38 ppm, singlet of 1H of aromatic –NH at δ 3.80 ppm, singlet of 1H at δ 1.90 ppm, doublet of 2CH of aromatic benzothiazole at 8.20ppm and 8.10 ppm. **Mass** m/z 280.12.

**Synthesis of 2-(5-ethyl-2,4-dithibiurete) benzothiazole (III c)**

**Colour**-Brownish Solid, **Molecular formula**-$C_{11}H_{12}N_4S_3$, **Yield** 83%, **M.P**. 201$^0$C, **% Composition found (calculated)** C-42.57 , H-3.08 , N-17.20, S-33.12 , **FTIR (Kbr) vcm**-1 3004.80 Ar-H streching, 3380.98, 3371.34 N-H stretching, 1589.23 N-H Bending, 1145.64, 1089.71 C=S Streching, 1149 C-N stretching. **1H NMR (400MHz CDCL$_3$ δ ppm)**, Ar-H proton at δ 7.55 ppm – δ 8.23 ppm, , -NH protons at δ 3.90 ppm, CH2 protons at ™ 3.30 ppm,CH3 protons at ™1.16 ppm. **Mass** m/z 295.43.

**2-(5-Benzyl-2,4-dithibiurete) benzothiazole (IIId)**

**Colour**-Brownish Solid, **Molecular formula**-$C_{16}H_{14}N_4S_3$, **Yield** 79%, **M.P**. 200$^0$C, **% Composition found (calculated)** C-52.60 , H-2.94 , N-15.20, S-26.24 , **FTIR (Kbr) vcm**-1 3295.87 N-H stretching, 3098.44 C-H Ar Stretching, 650.8, 752.25 C=C bending, 1168.73 C=S Stretching, 1370, 1554 C-H bending. **1H NMR (400MHz CDCL$_3$ δ ppm)**, Multiplate of 5H of Ph at ™ 7.06 ppm – ™ 7.14 ppm, singlet of 2H of –CH3 at ™ 4.70 ppm, Singlet of 2H of –NH at δ 2.20 ppm, singlet of Ar-NH protons at δ 3.90 ppm, doublet of 2CH aromatic benzothiazole at δ 8.20ppm and δ 8.10 ppm . **Mass** m/z 357.50.

**2-[5-(4-flurophenyl)-2,4-dithibiurete]benzothiazole (IIIe)**

**Colour**- Greenish Solid, **Molecular formula**-$C_{15}H_{11}FN_4S_3$, **Yield** 89%, **M.P**. 223$^0$C, **% Composition found (calculated)** C-48.70 , H-3.06 , F-5.10, N-14.52, S-26.24 , **FTIR (Kbr) vcm**-1 3295.87 N-H stretching, 3098.44 C-H Ar Stretching, 752.25 C=C bending, 1168.73 C=S Stretching, 1370, 1554 C-H bending. **1H NMR (400MHz CDCL$_3$ δ ppm)**, Singlet of 2H of –NH at δ 3.20 ppm, singlet of -NH protons at δ 2.30 ppm, doublet of 2CH aromatic benzothiazole at δ 8.20ppm and δ 8.10 ppm . **Mass** m/z 361.00.

**2-[5-(4-Nitrophenyl)-2,4-dithibiurete]benzothiazole (IIIf)**

**Colour**- Yellow Solid, **Molecular formula**-$C_{15}H_{11}N_5O_2S_3$, **Yield** 91%, **M.P**. 241$^0$C, **% Composition found (calculated)** C-46.70 , H-2.60, N-17.52, O-8.22, S-24.26 , **FTIR (Kbr) vcm**-1 1523 N-O stretching, 3295.87 N-H stretching, 3098.44 C-H Ar Stretching, 752.25 C=C bending, 1168.73 C=S Stretching, 1370, 1554 C-H bending. **1H NMR (400MHz CDCL$_3$ δ ppm)**, doublet of 2H of Ph –CH at δ 7.60 ppm and δ 6.50 ppm Singlet of 2H of –NH at δ 3.20 ppm, singlet of -NH protons at δ 2.30 ppm, doublet of 2CH aromatic benzothiazole at δ 8.20ppm and δ 8.10 ppm . **Mass** m/z 390.16.

**Pharmacological Studies**

**Antioxidant Activity**

All the synthesized compounds (IIIa) to (IIIf) were screened for their in Vitro antioxidant activity. Recent global interest has surged in the identification of pharmacologically potent antioxidant compounds devoid of adverse effects. These compounds play a crucial role in health protection by neutralizing free radicals, which are unstable molecules implicated in the pathogenesis of various degenerative diseases and conditions, including hepatic disease, immune dysfunction, cataracts, and macular degeneration. Scientific evidence suggests that antioxidants mitigate the risk of chronic diseases and conditions. Antioxidant compounds, such as phenolic acids, polyphenols, and flavonoids, scavenge free radicals like peroxide, hydroperoxide, and lipid peroxyl radicals, thereby reducing the risk of diseases associated with oxidative stress.

The anti-oxidant activity of compounds can be determine by using the colorimetric DPPH assay, as described by Shimada et al., (1992) to determine the radical scavenging activity of the plant extracts.

In-vitro Antioxidant screening of synthesized compounds was done by using only DPPH method. This method chosen because it is fast, simple and accurate method. Moreover, the method can be applied to the sample in small quantities. DPPH is a purple organic compound and reacts with the antioxidant compound, DPPH would be reduced and its color would be turned yellow. The hydrogen donating capacity of test samples is quantified in terms of their ability to scavenge the relatively stable, organic free radical DPPH and by consequent reduction. The absorption of the deep violet DPPH solution is measured at 517 nm, after which absorption decreases due to decolonization to a yellow-white color, in the event of reduction. This decrease in absorption is stoichiometric according to the degree of reduction (Arulpriya et al., 2010).

The free-radical scavenging activity was estimated by DPPH assay. The reaction mixture contained 10 µl of test sample and 190 µl of methanolic solution of 0.3 mM DPPH radical. The mixture was then shaken vigorously and incubated at 37° C for 5 min. The absorbance was measured at 517 nm on ELISA plate reader indicated higher free radical scavenging activity (Awaley et al. 2020), which was calculated using the following equation:

$$(\%)Free\ radical\ scavenging\ effect = \frac{[Absorbance\ of\ control\ (Ac) - Absorbance\ of\ sample(As)]}{Absorbance\ of\ control\ (Ac)} \times 100$$

| Sr. No. | Sample Code | Antioxidant Potential (Mean ±SD) |
|---|---|---|
| 1 | III a | 26.442±2.058 |
| 2 | III b | 45.851±2.631 |
| 3 | III c | 51.828±2.452 |
| 4 | III d | ----- |
| 5 | III e | 67.511±3.002 |
| 6 | III f | ----- |
| 7 | +Ve Control (Ascorbic acid) | 89.781±3.232 |

**Table: - DPPH radical scavenging activity of titled compounds**

## Conclusion

In the present research of synthesis of compounds (IIIa-IIIf), percentage of yield of compound (IIIf) is highest i.e. 91%. Variation in the yield of each compound is due to substitution at Nitrogen in the alky/aryl isothiocynate (IIa-f). It is also Observed that, change in the substituent at nitrogen leads not only the yield of product but also it affects the melting point and antioxidant activities. Among synthesized series of compounds of 2,4-dithiobiurets i.e. (IIIa-IIIf), it can conclude that compound (IIIc) and (IIIe) displayed the excellent anti-oxidant results in compare with the a standard drug. After studying the toxicities of the (IIIc) and (IIIe), these compounds may be act as good drugs for the living beings.

## References


1. M. S. Lunge, R. S. Shaikh, D. T. Tayade., synthesis of 1-(4-pyridine) imino-5-substituted dithiobiurets, *International Journal of Advanced Research*, 2016, 4(1), 908- 912.
2. D. T. Tayade, P. R. Kale., synthesis of 2-[substituted-2,4- dithiabiureto]-11-(piperazin-1-yl) dibenzo [b,f][1,4] oxazepines, *International Journal of Applied Sciences*, 2016, 03(03), 370-374.
3. D.T.Tayade P.R.Kale., Synthesis of 8-(Substituted-2,4-Dithiabiureto)-1-Methyl-6-Phenyl-4H-[1,2,4] Triazolo[4,3-A] [1,4] Benzodiazepines, *Journal of Scientific Research and Management International*, 2016, 4 (1), 10-13.
4. K.S.Panpaliya, Rahimullah Sharaftullah, D.T.Tayade., Synthesis of 2-(N'-Substituted)Thiocarbamido-5-(N''-Substituted)-2,4-Dithiobiurato)-1,3 Benzothiazoles and Antimalarial Activity *International Journal of ChemTech Research*, 2018, 11(05), 495-499.
5. D. T. Tayade and S. A. Waghmare., Design, synthesis, characterization and anti-microbial screening of some novel 2,4-dithiobiueret based chalcones, *Der Pharma Chemica*, 2016, 8(8), 78-81.
6. Farhanullah A. Khan, Rahim S. Sheikh and Shailesh G. Atram., Synthesis and Antimicrobial Screening of 2,4-Dithiobiuret Derivatives *G P Globalize Research Journal of Chemistry*, 2020, 3, 60-65.

7. ]. Murthi Y, Pathak D. Synthesis and Antimicrobial screening of Substituted 2-Mercaptobenzothiazoles. J Pharm Res, 2008; 7(3); 153-155
8. Mahran MA, El-Nassry SMF, Allam SR, ElZawawy LA, Synthesis of some new benzothiazole derivatives as potential antimicrobial and antiparasitic agents, Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2003; 58(8): 527-530.
9. Trivedi B and Shah, VH, Synthesis of 2 (substituted benzal hydrazine carbomyl methyl thio) benzothiazoles for antimicrobial activity, In Chem Abstr, 1992; 116: 151637.
10. S.M. Shantakumar et al. Synthesis and Antimicrobial Activity of Some New 2-Substituted Benzothiazole Derivatives.,EJournal of Chemistry,Research Article,775,(2009).
11. Cressier, D. Prouillac, C. Hernandez, P. Amourette, C. Diserbo, M. Lion, C. Rima. Synthesis, antioxidant properties and radioprotective effects of new benzothiazoles and thiadiazoles. Bioorg. Med. Chem. 2009, 17, 5275–5284.
12. Xiao, J. Zhang, Q. Gao, Y., Q.Tang, J.J. Zhang, A.L. Gao, J.-M. Secondary metabolites from the endophytic Botryosphaeria dothidea of Melia azedarach and their antifungal, antibacterial, antioxidant, and cytotoxic activities. J. Agric. Food Chem. 2014, 62, 3584–3590.
13. TayadeDT, KalePR.(Synthesis of 2-[substituted-2,4-dithiabiureto]-11-(piperazin-1-yl)dibenzo [b,f][1,4] oxazepines),Int. J. of Applied Sciences, 2016, 3(3); 370-374.
14. Tayade DT, Ingole SP.(Synthesis of 1-phenylthiocarbamido-5-formamidinosubstitu-ted thiocarbamido2-imino-4-thiobiurates by microwave technique),Int. J. of research & Research Methodology,2016, 4(2); 49-53.
15. Tayade D.T., Waghmare S.A., (Design, synthesis and characterization of novel series of 1,2,4- dithiazole based chalcones). J. Chemical and Pharmaceutical Res,2016, 8(5); 934-937.
16. Tayade D.T., Lunge M.S., (Synthesis of a novel series of 2-substitutedimino-4-substi-tutedimino-6-[4- pyridineimino]amino-1,3,5-dithiazines).International Journal of Current Research, 2016, 7(8); 18956- 18959.
17. Tayade D.T., Bhagwatkar A.K., (Novel synthesis of 2-chloro-4,6-distituted thiocarbamido-1,3,5- triazines). Int. J of Chemical and Physical Sci., 2013, 2(3); 23-27.
18. Harinath Yapatia, Subba Rao Devinenib, Suresh Chirumamillaa And Seshaiah Kallurua, Synthesis, characterization and studies on antioxidant and molecular docking of metal complexes of 1-(benzo[d]thiazol-2-yl)thiourea. J. Chem. Sci. Vol. 128, No. 1, January 2016, pp. 43–51. DOI 10.1007/s12039-015-0999-3.