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Characterization & Invitro Antioxidant Activity of 1, 3, 4 Thiadiazole Derivatives of Thiazolidinone

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Abstract

In view of the considerable importance of thiadiazoles and thiazolidinones, which are the core structures in a variety of pharmaceuticals with a broad spectrum of biological activity. Synthesis of series of potential biological active 1, 3, 4 thiadiazole linked 4 thiazolidinone derivatives were obtained via a multistep synthesis sequence with a simple and convenient approach by using substituted benzoic acids, which are expected to possess enhanced antioxidant activity based on the literature survey reports. In the present study the initial compound, 5-phenyl-1, 3, 4-thiadiazol-2-amine was treated with different substituted aromatic aldehydes to produce Schiff base. The resulting Schiff base were subjected to addition reactions with thioglycolic acid to form title compounds of 2-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one. The structure of the synthesized compounds was characterised by FT-IR, H1NMR and mass spectral analysis. The synthesized compounds were tested for antioxidant activities with standard drug using DPPH method. The results of this study revealed that, among the compound tested for antioxidant activity, TZD 5 and TZD 3 exhibited promising antioxidant activity with the IC50 value 27.50µM and 28.00µM while the value of reference compound, ascorbic acid 29.2µM. The antioxidant screening results indicate that exciting DPPH radical scavenging activity was observed in compounds (TZD 3 and TZD 5) in comparison with standard ascorbic acid. These results may also provide some significance guidance for the development of new class antioxidant.

Keywords: 1, 3, 4 Thiadiazole, Thiazolidinone, Schiff base, Antioxidant activity, FT-IR, DPPH method.

INTRODUCTION

Several reactive oxygen species are important cellular components, enzymatically generated in aerobic living organisms, which show significant role in various physiological and pathological process. Under physiological conditions, the concentration of free radicals including reactive oxygen, nitrogen, sulphur and carbon species is regulated by antioxidant defence systems. An antioxidant functions by inhibiting the oxidation of other molecules that is the chemical reaction involving the loss of electrons or an increase in oxidation state [1]. Oxidation reactions can produce free radicals and which can start chain reactions in the cell leads to the damage or death to the cell. By removing free radicals intermediates they can terminate the chain reaction and inhibit other oxidation reactions. They do this by being oxidised themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid and polyphenols. The ROS produced in the cells include hydrogen peroxide, hypochlorous acid and free radicals such as hydroxyl radical and superoxide anion. The hydroxyl radical is particularly unstable and will react rapidly specifically with most biological molecules. This species is produced from hydrogen peroxide in metal-catalyzed redox reactions such as the Fenton reaction. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, while damage to proteins causes enzyme inhibition, denaturation and protein denaturation [2]. It is well known that free radicals cause autoxidation of unsaturated lipids in food. In addition, antioxidants are known to interrupt the free radicals chain of oxidation and to donate the hydrogen from phenolic hydroxyl

groups, there by forming stable free radical and prevent the further oxidation of lipids. Antioxidant activity of any compound was evaluated through four different methods namely, superoxide radical-scavenging, hydrogen peroxide scavenging, reducing power, and DPPH radical scavenging assays. The antioxidant potential of the compound was related to its (i) hydrogen or electron donating capacity, (ii) its ability to stabilize and delocalize the unpaired electron, and (iii) potential to chelate the transition metal ion. The antioxidant activity of the flavanoids due to the inhibition of the enzyme responsible for the superoxide radical production, chelation of the metal ions and scavenging of ROS. So we are tried to found the antioxidant activity of 1,3,4- thiadiazole linked 4-thiazolidinone derivatives. DPPH is a common abbreviation for an organic chemical compound 2, 2- diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free-radical molecules. DPPH has two major applications, both in laboratory research: one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay, and another is a standard of the position and intensity of electron paramagnetic resonance signals [2,3].

In view of the considerable importance of thiadiazoles and thiazolidinones, which are the core structures in a variety of pharmaceuticals with a broad spectrum of biological activity, specifically referencing their ability to prevent ROS formation, the present work is intented to synthesize new heterocyclic compounds bearing the thiadiazole moiety [5].

The prevalent existence of the heterocycles in bioactive natural products, drugs, and agrochemicals has made them as important synthetic target. Five-membered heterocyclic compounds; oxadiazoles and thiadiazoles have attracted significant interest in medicinal chemistry, pesticide chemistry, polymer sciences, material science and they are the building blocks of new molecular systems for biologically active molecules. The Nitrogen-oxygen heterocycles are also of synthetic interest as they constitute an important class of natural and non-natural products and many of them exhibit useful biological activities. 1,3,4-oxadiazoles are biologically versatile. 1,3,4-thiadiazoles also possess various biological properties such as antitumor, anticonvulsant, antihypertensive, anesthetic, antibacterial and cardiotonic activities [4-6].

MATERIALS AND METHODS

The synthetic strategy to synthesis the target compounds is depicted in schemes. 5-phenyl-1,3,4-thiadiazol-2-amine was treated with different substituted aromatic aldehydes to produce Schiff base. The resulting Schiff base were subjected to addition reactions with thioglycolic acid in the presence of zinc chloride to form title compounds of 2-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one [20]. The target compounds were prepared and the purity of the compounds were ascertained by routine TLC, Chloroform: Methanol (6:4) and the consistency in melting points were checked by open capillary tube method and were uncorrected. The molecular weight, percentage yield of all the synthesized compounds was calculated and reported. The characterization of the derivatives was also carried out by various spectroscopic methods such as FT-IR, 1 HNMR, and MASS [7].

The antioxidant activity of the synthesized compounds was performed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [24]. All the well characterized 2-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one derivatives were dissolved to prepare a stock solution of 1 mg/mL using DMSO. Fifty microliter solutions of the compounds were added to 1 mL of a 0.1 mM solution of DPPH in methanol. After 2 h, absorbance values were measured at 517 nm. Ascorbic acid was used as standard [8-10].

Step 1: General procedure for synthesis of 5-phenyl-1,3,4-thiadiazol-2-amine (BA1)

A mixture of 9.11g (0.1 mol) of thiosemicarbazide, 12.2g (0.1 mol) of benzoic acid and 5 ml of conc. sulphuric acid in 50 ml of ethanol in a 250 ml round bottom flask was refluxed for 2 hours. Reaction mixture was poured on to crushed ice. The solid separated out was filtered, washed with cold water, dried and recrystallized from ethanol. [11, 12]

Step 2: General procedure for synthesis of 5-phenyl-*N*-[(*E*)-phenylmethylidene]-1,3,4-thiadiazol-2-amine

Placed 2g (0.01 mol) of 5-phenyl-1,3,4-thiadiazol-2-amine, 1.11g (0.01 mol) of benzaldehyde and 20 ml of methanol in a 250 ml round bottom flask attached to a reflex condenser on a water bath, introduced 1 ml glacial acetic acid drop by drop while the reaction mixture was warming, and refluxed for $5^1/_2$ hrs, till the reaction mixture was almost clear and homogenious. Transfered the filterate to a 100 ml wide-mouthed container containing crushed ice and stirred well till the product was completely formed. Filtered and washed with distilled water, dried and recrystallized from chloroform. [14, 16]

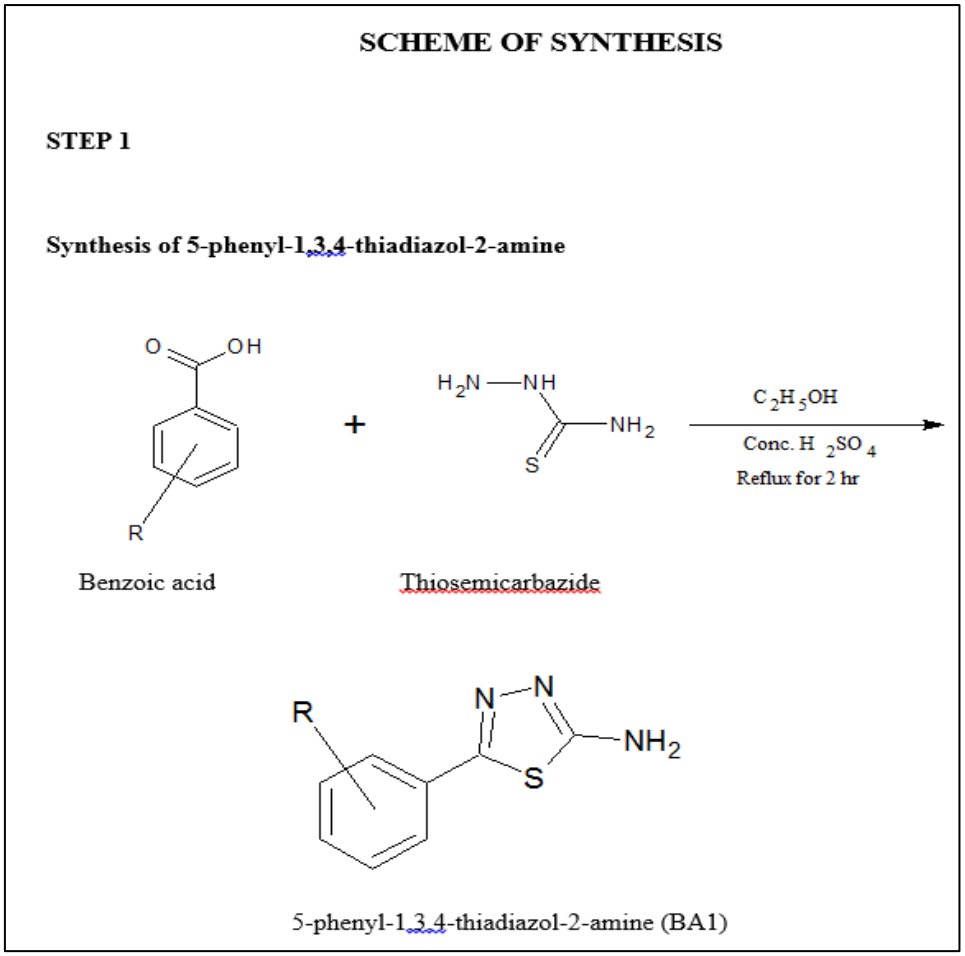


Figure 1: Synthesis of thiadiazoles

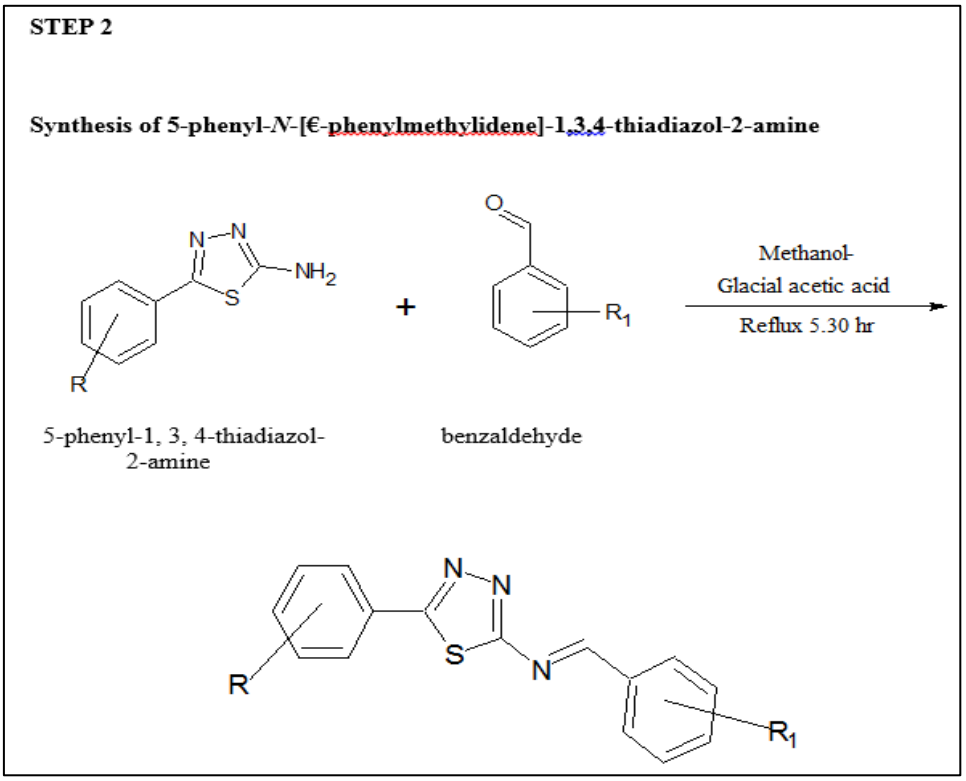


Figure 2: Synthesis of Schiff bases

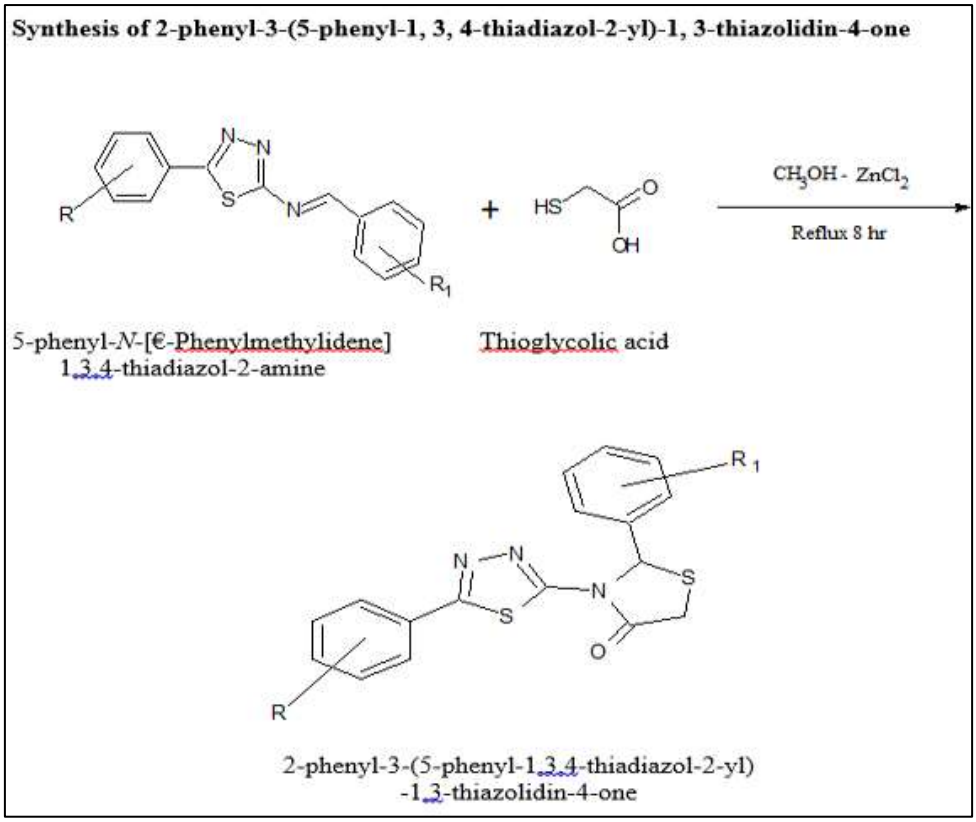
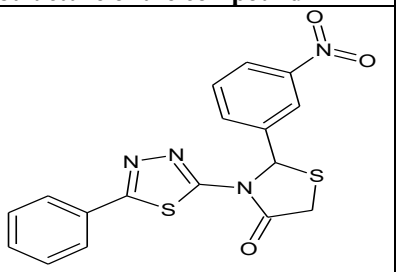
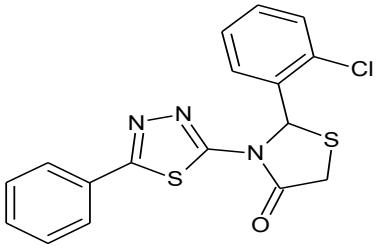
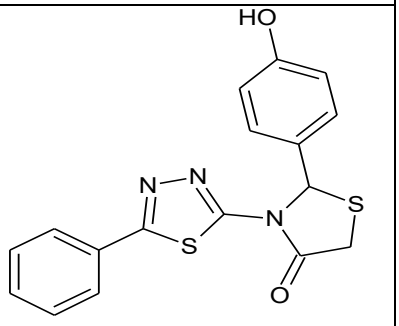


Figure 3: Synthesis of thiazolidinone derivatives

Table 1: Structures of synthesized derivatives

Compound code	Name of the Compound	Structure of the compound
TZD 3	2-(3-nitrophenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one	
TZD 5	2-(2-chlorophenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one	
TZD 10	2-(4-hydroxyphenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one	

Step 3: Synthesis of 2-(3-nitrophenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidi-4-none (TZD 3)

Placed 1g (0.003 mol) of N-[(E)-(3-nitrophenyl) methylidene]-5-phenyl-1,3,4-thiadiazol-2-amine, 0.331g (0.003 mol) of thioglycolic acid and 20ml methanol in a 250 ml round bottom flask attached to a reflux condenser and introduced 0.05g zinc chloride as catalyst. Refluxed the contents for 8 hrs, till the reaction mixture was almost clear and homogenious. Poured the hot reaction mixture to crushed ice by stirring to form the product. Filtered and washed with distilled water, dried and recrystallized from ethanol. [13, 15]

Step: 3 Synthesis of 2-(2-chlorophenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one (TZD 5)

Placed 1g (0.003 mol) of *N*-[(*E*)-(2-chlorophenyl) methylidene]-5-phenyl-1,3,4-thiadiazol-2-amine, 0.331g (0.003 mol) of thioglycolic acid and 20ml methanol in a 250 ml round bottom flask (RBF) attached to a reflux condenser and introduced 0.05g zinc chloride as catalyst. Refluxed the contents for 8 hrs, till the reaction mixture was almost clear and homogeneous. Poured the hot reaction mixture to crushed ice by stirring to form the product. Filtered and washed with distilled water, dried and recrystallized from ethanol.

Step 3: Synthesis of 2-(4-hydroxyphenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one (TZD 10)

Placed 1g (0.003 mol) of 4-{(*E*)-[(5-phenyl-1,3,4-thiadiazol-2-yl)imino]methyl}phenol, 0.331g (0.003 mol) of thioglycolic acid and 20ml methanol in a 250 ml round bottom flask attached to a reflux condenser and introduced 0.05g zinc chloride as catalyst. Refluxed the contents for 8 hrs, till the reaction mixture was almost clear and homogenious. Poured the hot reaction mixture to crushed ice by stirring to form the product. Filtered and washed with distilled water, dried and recrystallized from ethanol.

RESULTS AND DISCUSSION

Characterization of synthesized compounds

Compound TZD 3 - 2-(3-nitrophenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidi-4-none

Recrystallized with ethanol. Yield: - 55%, M.p- 190°C, Molecular weight- 384.43, Molecular Formula- $C_{17}H_{12}N_4O_3S_2$. IR peaks (cm^{-1}) - 3068(Ar-CH str); 1608(C=N); 1529(C=C str); 1067(N-N); 650(C-S-C); 1693(C=O); 1349(C-NO2).

Compound TZD 5 - 2-(2-chlorophenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one

Recrystallized with ethanol. Yield: - 58%, M.p.- 182°C, Molecular weight-373.87, Molecular Formula- $C_{17}H_{12}ClN_3OS_2$. IR peaks (cm-1) - 3025(Ar-CH str); 1575(C=N); 1450(C=C str); 1064(N-N); 657(C-S-C); 1674(C=O str); 700(C-Cl).

Compound TZD 10 - 2-(4-hydroxyphenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one

Recrystallized with ethanol. Yield: - 60%, M.p.- 183°C, Molecular Formula- $C_{17}H_{13}N_3O_2S_2$. IR peaks (cm-1) - 3047(Ar-CH str); 1574(C=N); 1450(C=C str); 1064(N-N); 657(C-S-C); 1673(C=O str); 1322(C-O str); 3647(O-H str);

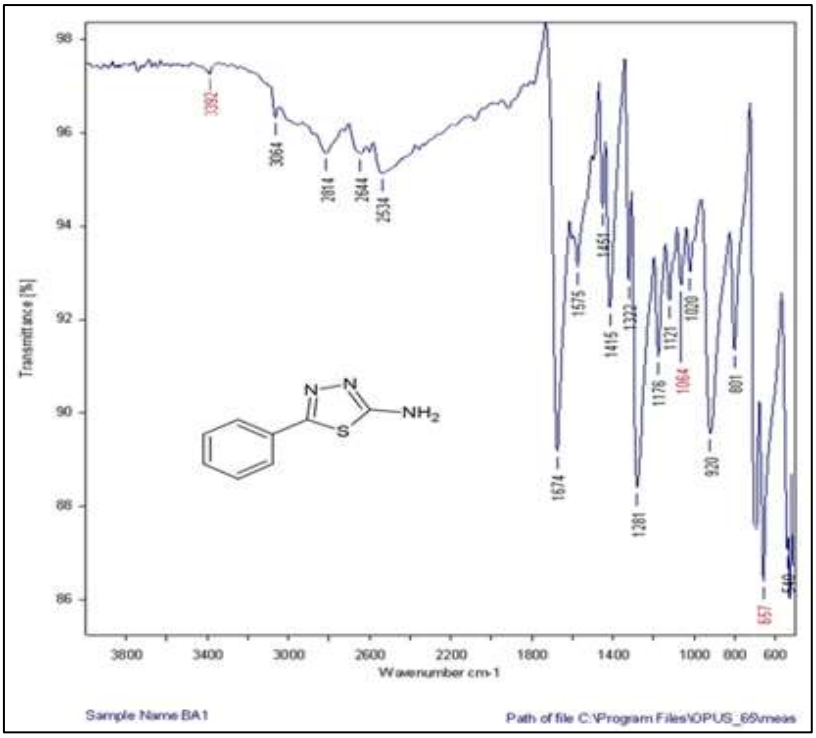


Figure 4: IR Spectrum of BA1

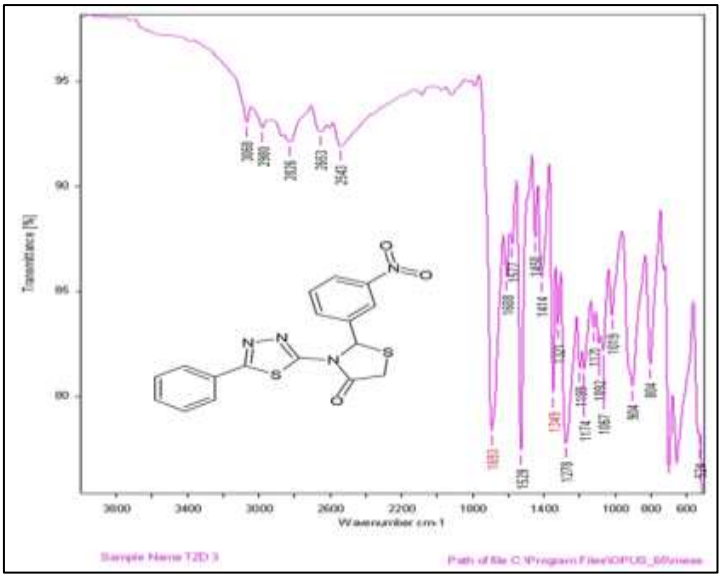


Figure 5: **IR Spectrum of TZD 3**

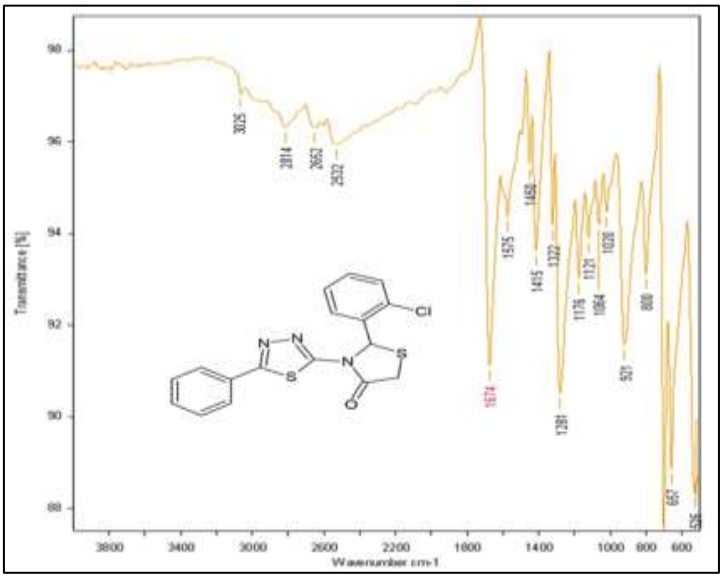


Figure 6: IR Spectrum of TZD 5

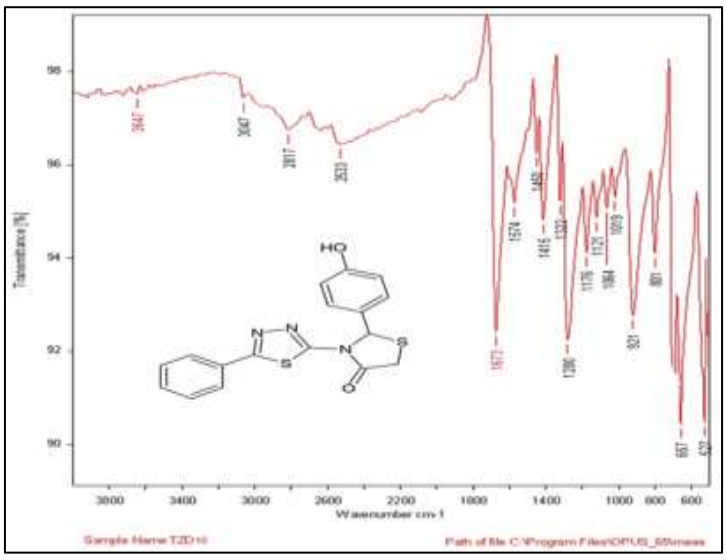


Figure 7: IR Spectrum of TZD 10

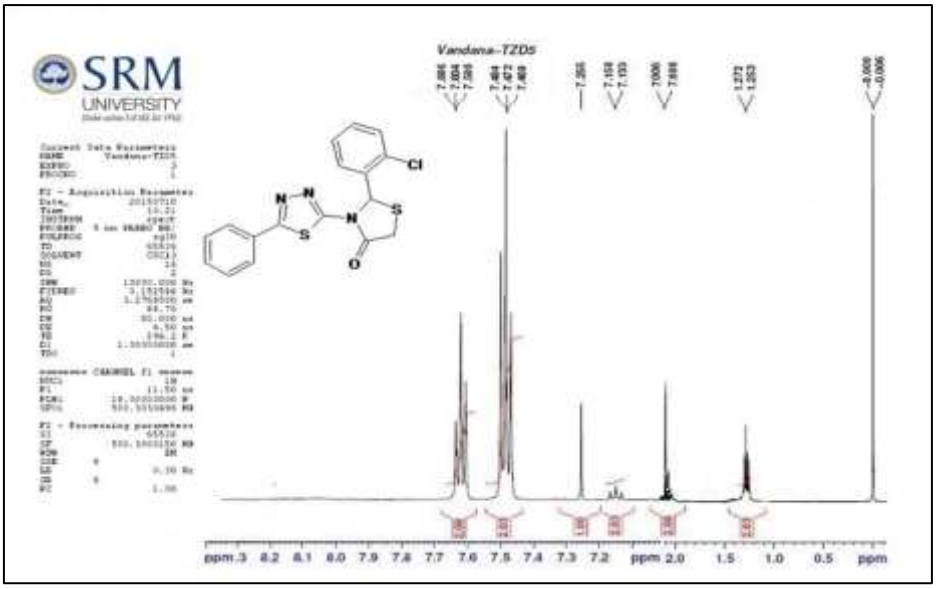


Figure 8: NMR Spectrum of TZD 5

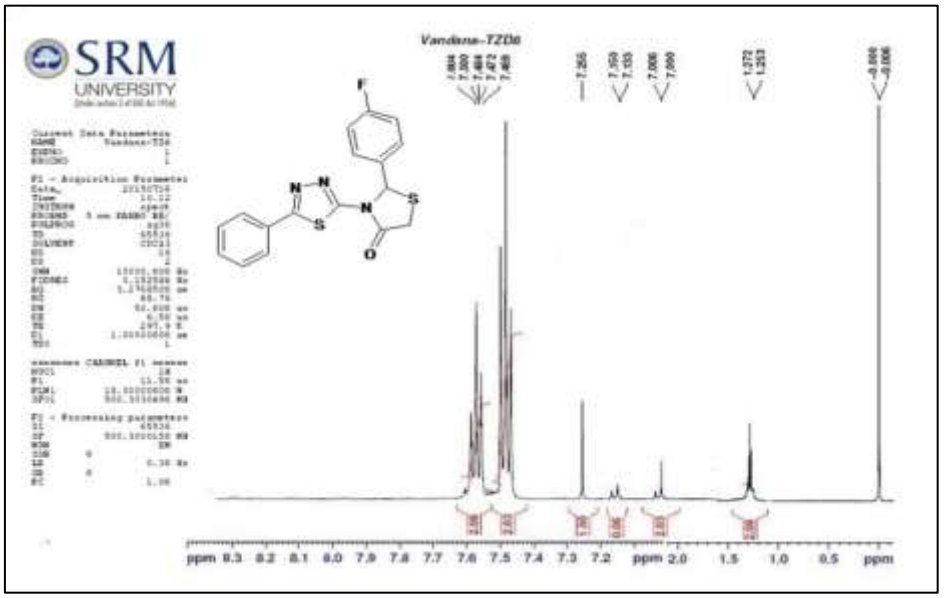


Figure 9: NMR Spectrum of TZD 10

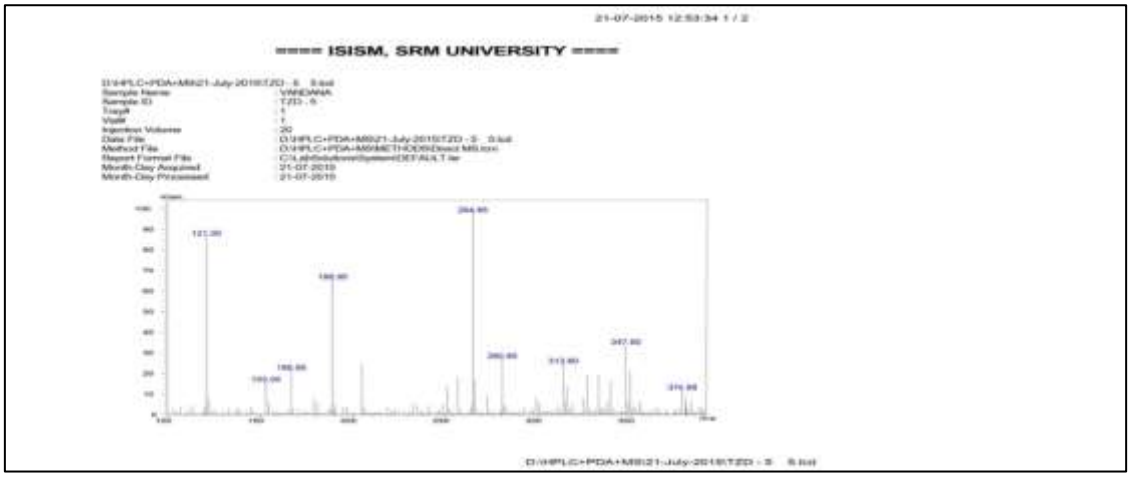


Figure 10: MASS Spectrum of TZD 5

1HNMR spectral analysis of TZD 5 shows peak at 7.000-7.006 (2H(d) of phenyl), 7.133-7.150 (2H(d) of phenyl), 7.255 (1H(s) of N-CH-S in 4-thiazolidinone), 7.469-7.606 (5H(m) of Ar), 1.253-1.272 (2H(d) of CH2 in 4-thiazolidinone.

Mass spectra of synthesized compounds were recorded with LC-MSD Trap-SL 2010 A-Shimadzu. Mass spectral data of TZD 5: molecular ion peak (M+H+) shows 374.98 from the above we confirmed exact molecular mass of the above titled compound was 373.87. Base peak of the compound TZD 5 was 264.85. Mass spectral data of TZD 10: molecular ion peak (M+H+) shows 356.67 from the above we confirmed exact molecular mass of the above titled compound is 355.43. Base peak of the compound was 262.56.

By considering the IR, 1HNMR, MASS spectra of the synthesized compounds, we can confirm that the expected structure of the derivatives.

In-vitro antioxidant activity

Antioxidant activity of organic molecules is related to their electron or hydrogen atom donating ability to DPPH radical, so that they become stable diamagnetic scaffolds. The interaction of synthesized compounds with stable DPPH free radical indicates their free radical scavenging ability. The reduction ability of DPPH radicals was determined by decline in their absorbance at 517 nm enthused by antioxidants. Majority of the tested compounds in these series showed good interaction with the DPPH radical at 1 mg/mL concentration. The scavenging effects of all the synthesized compounds on DPPH radical are presented as percentage inhibition in (Table 2). DPPH radical scavenging activity of the synthesized compounds exhibited outstanding results as compared to the standard Ascorbic acid. Antioxidant screening of synthesized compounds were also carried out by using DPPH method. Among the compound tested for antioxidant activity, TZD 5 and TZD 3 exhibited the good activity with the IC_{50} value 27.50 µM and 28.00 µM, while IC_{50} value of reference compound was ascorbic acid 29.2 µM. In Antioxidant results showed that good efficacy and derivatization of the parent compound has resulted in good antioxidant efficacy [17, 19].

The synthesized analogs were screened for antioxidant activity by DPPH assay. The percentage inhibition of samples TZD 3, TZD 5 and TZD 10 of different concentrations was tabulated.

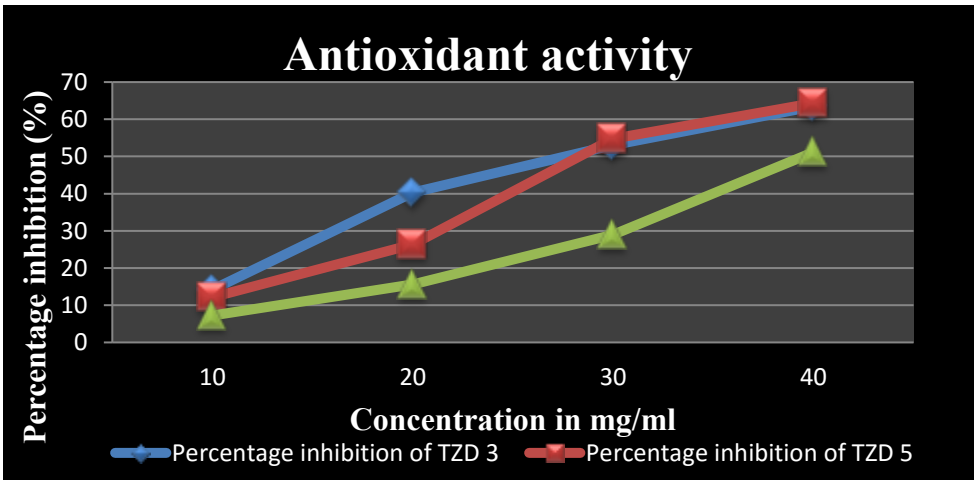


Figure 11: Antioxidant activity of TZD 3, TZD 5 & TZD 10

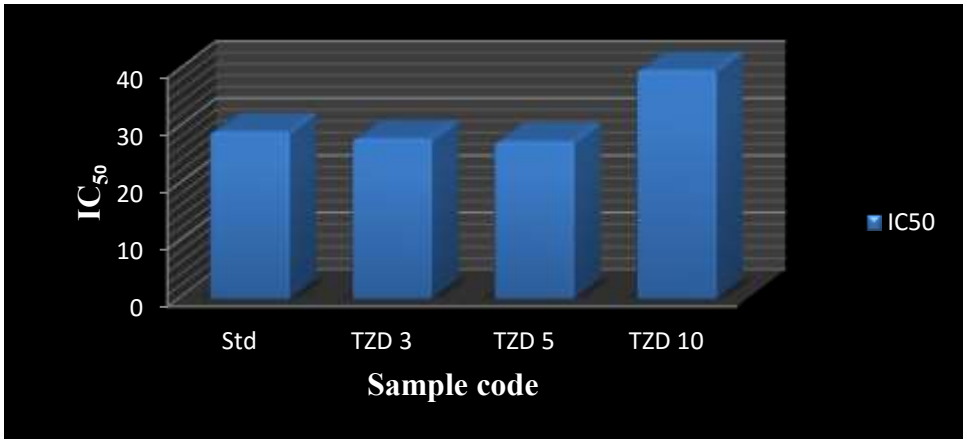


Figure 12: Histogram of antioxidant activity

Table 2: IC50 value of tested compounds for antioxidant activity

No	Sample in µg/ml	DPPH ASSAY		
		OD	Percentage inhibition (%)	IC_{50}
1	**Control 0.00**	3.45	0.00	
2	TZD 3 -**10**	2.95	14.19	
3	TZD 3 -**20**	2.06	40.28	
4	TZD 3 -**30**	1.62	53.04	28.00
5	TZD 3 -**40**	1.26	63.47	
1	TZD 5 -**10**	3.02	12.46	
2	TZD 5 -**20**	2.54	26.37	
3	TZD 5 -**30**	1.56	54.78	27.50
4	TZD 5- **40**	1.23	64.34	
1	TZD 10 -**10**	3.20	7.24	
2	TZD 10 -**20**	2.91	15.65	
3	TZD 10 -**30**	2.45	28.98	40.0
4	TZD 10 -**40**	2.03	51.15	
	Standard	Ascorbic acid		29.2

DISCUSSION

The DPPH radical scavenging activity results are shown in table 2, Figure 1 and figure 2 by comparing with the standard drug Ascorbic acid. The ability of different samples (TZD 3, TZD 5 and TZD 10) to scavenge DPPH was investigated at various concentration of the solution. From the observed results the percentage inhibition of sample TZD 3 and TZD 5 was concentration dependent with an effective concentration at fifty percent of 28.00 µg/ml & 27.50 µg/ml compared to that of standard with IC 50 of 29.2 µg/ml, the results revealed the values were also remarkably excellent for the derivative with nitro group and chloro group (TZD 3 & TZD 5), then the derivatives TZD 10 shows comparable poor result with the IC 50 value 40.00 µg/ml.

CONCLUSION

In conclusion, we have achieved a convenient protocol for the synthesis 1,3,4-thiadiazole and thiadiazolidine incorporated derivatives in good yield and evaluated their in vitro antioxidant activity by using DPPH radical scavenger assay. Our antioxidant screening results indicate that exciting DPPH radical scavenging activity was observed in compounds (TZD 3 and TZD 5) in comparison with standard ascorbic acid. The lead compounds emerging with the most potent antioxidant activity in this study will be further structurally modified towards the discovery of a compound with optimal antioxidant activity. These results may also provide some significance guidance for the development of new class antioxidant.

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