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Comparative studies on Synthesis of 2-Chloro-N-(2, 5-Dihydro-1, 3, 4-Thiadiazol-2-Yl) Acetamide using Microwave irradiation and Conventional techniques and their Antioxidant activity.

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***Abstract**: The advantages of Microwave irradiation technology in the field of organic chemistry have been long known as it facilitates accelerated reaction rates, higher yields with relatively less power consumption than classical techniques. The presented study compares aspects like reaction time and percentage yield of various 2-amino-1, 3, 4-thiadiazole derivatives when synthesized by both, conventional as well by microwave irradiation along with their characterization using techniques such as FTIR and HPLC. They have a very promising role in the field of medicinal chemistry owing to their anti-cancer and anti-fungal properties The study also aims to evaluate biological activity of the compounds a simple and inexpensive technique making use of 2,2-Diphenyl-1-picrylhydrazyl(DPPH) to establish their worth as potential anti-oxidants . Thus this combination of faster synthesis and cost effectiveness can significantly reduce the cost of production of various pharmaceutical products containing thiadiazole and/or its derivatives which are known to be clinically potent.*

***Keywords :** 2-amino-1, 3, 4-thiadiazole, Microwave irradiation, anti-oxidant activity, DPPH*

I. INTRODUCTION

Ever since the first application of microwave ovens has been registered in the field of chemistry, their use over conventional methods has gained a momentum. In the field of organic synthesis, where rate of a reaction and yield of intermediates and products are of critical importance, the extension of the use of microwave radiation has been found to be of great importance and value. As a result, in 1986, the use of microwave irradiation in the field of organic synthesis sought its first publication. [1]

On one hand where conventional techniques allowed slow and inefficient method of transferring energy into the reaction system, microwaves accelerate this process by coupling directly with the molecules of the reaction mixture. [1]

The function of a dipole moment in a molecule to couple with the microwave radiation, only polar molecules interact with microwave energy [2]. Also microwave irradiated reactions require milder reaction conditions and proceed more cleanly thereby abiding to the principles of Green Chemistry [1] [3] Dimethyl Formamide (DMF) is used as a solvent in many of the chemical reactions making use of microwave irradiation as a source of energy, mainly due to reasons attributed to its polar (hydrophilic) aprotic nature, its ability to be miscible with majority of organic solvents, high boiling point of 152ºC and low evaporation rate [4]

2-amino 1, 3, 4-thiadiazole derivatives have been long known for their various anti-cancer, microbial and fungal properties owing to which it has received an indisputable position in the field of medicinal chemistry. Studies on 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT) showed inhibited proliferation of tumor cells derived from cancers of nervous system (medulloblastoma/rhabdosarcoma, neuroblastoma, and glioma) and peripheral cancers including colon adenocarcinoma and lung carcinoma[5]. Studies related to 2-(4-Bromophenylamino)-5-(2, 4-dihydroxyphenyl)-1, 3, 4-thiadiazole evaluated its neuroprotective activity [6]. Compounds of 5-substituted 2-(2, 4-dihydroxyphenyl)-1, 3, 4-thiadiazole series were reported to show anti-fungal activity [7], the effects of 2-amino 1, 3, 4-thiadiazole as an anti-leukemic agent were reported in some studies [8]

Reactive Oxygen Species (ROS) are molecules or ions that are an important component of oxidative stress in the cells. These molecules have the capability to damage the backbone of DNA in the cell and cause a series of changes that can lead to cancer. Antioxidants are molecules that can inhibit the activity of ROS and prevent the development of cancer. [9] An *in vitro* assay that estimates the ability of a compound to act as an antioxidant utilizes the stable radical forming property of DPPH. Such assays can be of great importance in determining biochemical properties of various chemical compounds that may further aid in the characterization of the compounds. [10]

II. Materials And Methods

All raw materials used in the synthesis have been obtained from M/S Fluka AG (Bachs, Switzerland) and M/S Sigma-Aldrich chemicals and Co. Inc. (Milwaukee, WI, USA). Melting points were recorded on a Thermonik Melting point Apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. IR spectra were recorded on IR-Affinity, Shimadzu using DRS system. HPLC chromatograms have been recorded on Thermo Scientific UHPLC plus Ultimate 3000 HPLC system (Thermo Scientific, USA). Elemental analysis has been carried out on a C, H, and N Elemental Analyzer (Thermo-Finnigan Flash EA 1112, Italy). Microwave synthesis was carried out in Anton Par Monowave-300.

2.1. EXPERIMENTAL

2.1.1. SYNTHESIS OF 2-CHLORO-N-(2,5-DIHYDRO-1, 3, 4-THIADIAZOL-2-YL) ACETAMIDE (1) (By Conventional method)

Dissolve 0.0495 moles (5g) of 2, 5-Dihydro-1, 3, 4-thiadiazol-2-amine in minimum quantity of Ethanol. Add equimolar concentration of Chloroacetyl chloride (3.9ml) dissolved to it. Subject this mixture to reflux on a heating mantle for 22-24hrs. Monitor the progress of the reaction using Thin Layer Chromatography after regular intervals. Solvent System**:** Chloroform: Methanol (95: 5 v/v). After confirmation using Thin Layer Chromatography, decant the liquid and transfer the solid crystals on a filter paper in a Petri dish and allow it to dry completely. Wash the crystals with Ether to ensure complete dryness.

SYNTHESIS OF 2-CHLORO-N-(2, 5-DIHYDRO-1, 3, 4-THIADIAZOL-2-YL) ACETAMIDE (1) (By Microwave irradiation)

Dissolve 0.0495 moles (5g) of 2, 5-Dihydro-1, 3, 4-thiadiazol-2-amine in minimum quantity of DMF. Add equimolar concentration of Chloroacetyl chloride (3.9ml) dissolved in DMF to it. Subject this mixture to microwave irradiation for 30-35mins. Monitor the progress of the reaction using Thin Layer Chromatography after regular intervals. Solvent System**:** Chloroform: Methanol (95: 5 v/v). After confirmation using Thin Layer Chromatography, decant the liquid and transfer the solid crystals on a filter paper in a Petri dish and allow it to dry completely. Wash the crystals with Ether to ensure complete dryness.

2.1.2. SYNTHESIS OF N-(2, 5-DIHYDRO-1, 3, 4-THIADIAZOL-2-YL)(PHENYLAMINO) ACETAMIDE 2(a-e) (By Conventional method)

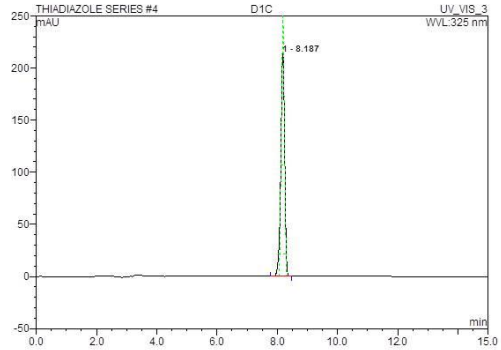
Dissolve 0.0028 moles (0.5g) of Compound 1 in minimum quantity of Ethanol. Add equimolar concentration of substituted aniline to it. Subject this mixture to reflux on an heating mantle for 6-8hrs. Monitor the progress of the reaction using Thin Layer Chromatography after regular intervals. Solvent System: Chloroform: Methanol (95: 5 v/v). After confirmation using TLC, decant the liquid and transfer the solid crystals on a filter paper in a Petri dish and allow it to dry completely. Wash the crystals with Ether to ensure complete dryness

SYNTHESIS OF N-(2, 5-DIHYDRO-1, 3, 4-THIADIAZOL-2-YL) (PHENYLAMINO) ACETAMIDE 2(a-e) (By Microwave irradiation)

Dissolve 0.0028moles (0.5g) of Compound 1 in minimum quantity of DMF. Add equimolar concentration of substituted aniline dissolved in DMF to it. Subject this mixture to microwave irradiation for 8-10mins. Monitor the progress of the reaction using Thin Layer Chromatography after regular intervals. Solvent System**:** Chloroform: Methanol (95: 5 v/v). After confirmation using TLC, decant the liquid and transfer the solid crystals on a filter paper in a Petri dish and allow it to dry completely. Wash the crystals with Ether to ensure complete dryness.

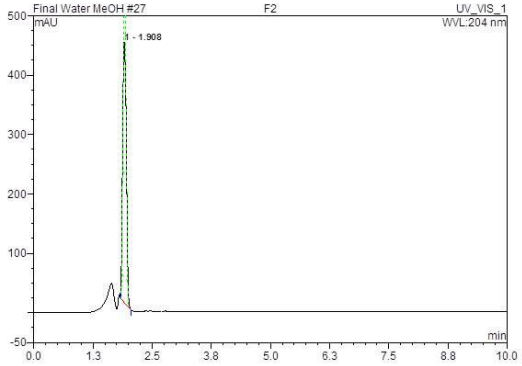
(2a): Yellow needle like crystals; M.P: 240ºC; Found: C: 35.90; H: 1.87; N: 29.42.

IR (KBr) cm^{-1}: 1629 (5 member hetero-cyclic ring), 1716.65 (C=O), 3334 (N-H), 1290.38 (N-O symmetric), 850.61 (1, 3, 5 tri-substituted 6 member ring), 1367 (C-H)



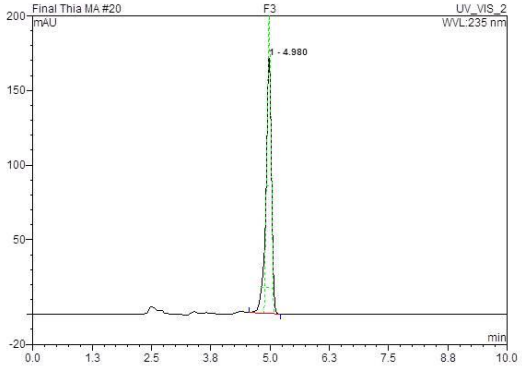
HPLC: Experimental Parameters: Column: Acclaim™ C-18, 5µ, 4.6 × 250mm, Wavelength: 325nm, Mobile phase: Water: Acetonitrile (60:40 v/v), Flow rate: 1ml/min, Column temperature: 28ºC, Injection volume: 20µL, Run time: 15mins, Retention time: 8.1mins

(2b): White needle like crystals; M.P: 255ºC; Found: C: 47.20; H: 3.00; N: 16.35.
IR (KBr) cm^{-1}: 1641 (5 member hetero-cyclic ring), 1747 (C=O), 1072.42 (N-H), 761.88 (C-Cl), 744.52 (1, 3, 5 tri-substituted 6 member ring), 2879.72 (C-H)



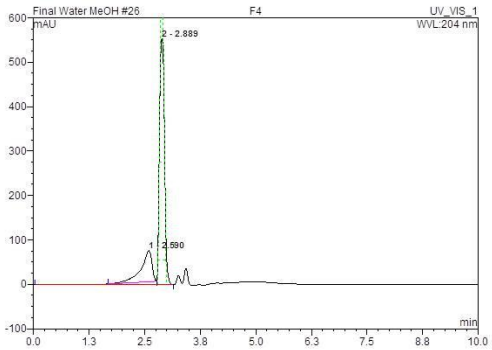
HPLC: Experimental Parameters: Column: Acclaim™ C-18, 5µ, 4.6 × 250mm, Wavelength: 204nm, Mobile phase: 100% Acetonitrile, Flow rate: 1.5ml/min, Column temperature: 28ºC, Injection volume: 20µL, Run time: 10mins, Retention time: 1.9min

(2c): White needle like crystals; M.P: 260ºC; Found: C: 38.72; H: 2.49.17; N: 22.59.
IR (KBr) cm^{-1}:1627.92 (5 member hetero-cyclic ring), 1712.79 (C=O), 1074.35 (N-H), 850.61 (C-Cl), 1533.41 (N-O symmetric), 850.61 (Para di-substituted 6 member ring), 1444.68 (C-H)



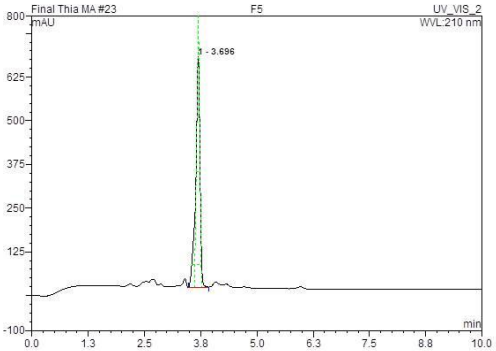
HPLC: Experimental Parameters: Column: Acclaim™ C-18, 5µ, 4.6 × 250mm, Wavelength: 235nm, Mobile phase: Methanol: Water (80:20 v/v), Flow rate: 1ml/min, Column temperature: 28ºC, Injection volume: 20µL, Run time: 10mins, Retention time: 4.9min

(2d): White needle like crystals; M.P: 266ºC; Found: C: 39.98; H: 2.24; N: 18.84.
IR (KBr) cm^{-1}: (5 member hetero-cyclic ring), 1712.79 (C=O), 3350.35 (N-H), 852.54 (C-Cl), 806.25 (1, 3, 4 tri-substituted 6 member ring), 2879.72 (C-H)



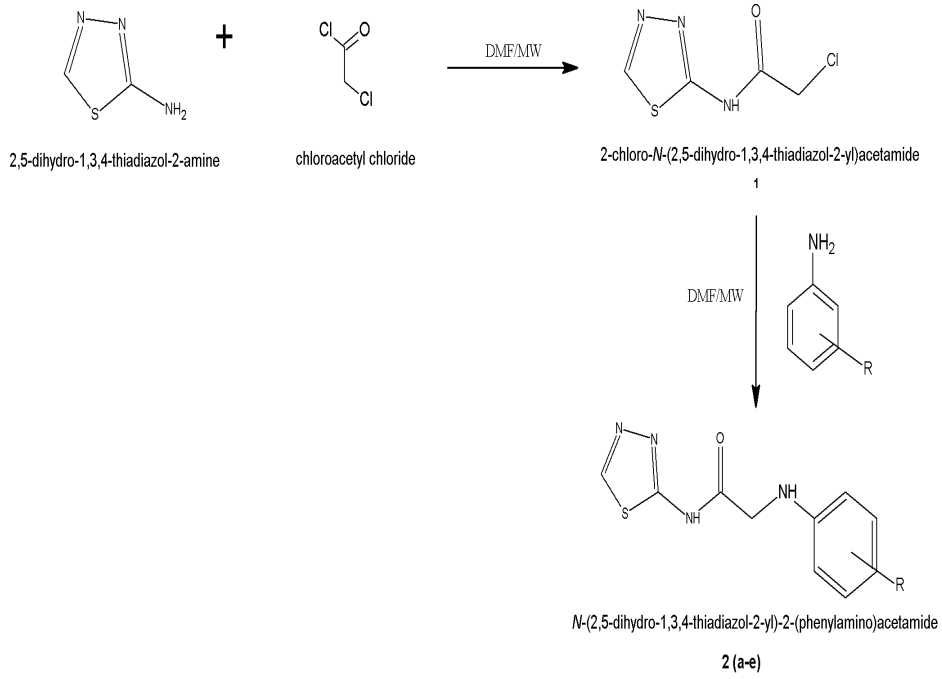
HPLC: Experimental Parameters: Column s: Acclaim™ C-18, 5µ, 4.6 × 250mm, Wavelength: 204nm, Mobile phase: 100% Acetonitrile, Flow rate: 1ml/min, Column temperature: 28ºC, Injection volume: 20µL, Run time: 10mins, Retention time: 2.8mins

(2e): brown needle like crystals; M.P: 269ºC; Found: C: 45.51; H: 2.42; N: 21.04.
IR (KBr) cm^{-1}: 1616.35 (5 member hetero-cyclic ring), 1697.36 (C=O), 3325.28 (N-H), 1290.38 (N-O symmetric), 810 (1, 3, 5 tri-substituted 6 member ring), 1365.60 (C-H)



HPLC: Experimental Parameters: Column: Acclaim™ C-18, 5µ, 4.6 × 250mm, Wavelength: 210nm, Mobile phase: Methanol: Water (85:15 v/v), Flow rate: 1ml/min, Column temperature: 28ºC, Injection volume: 20µL, Run time: 10mins, Retention time: 3.6mins

2.1.3 Table 1: Schematic Representation of Titled Compounds



2.1.4. Antioxidant assay [10]:

Antioxidant activity of the test compounds was determined by Diphenylpicrylhydrazyl (DPPH) radical scavenging method. All the synthesized test compounds were screened for their antioxidant activity by DPPH radical scavenging assay. Butyrated Hydroxy Touelne (BHT) was taken as standard. The concentrations per sample prepared were as follows: 0.5ppm, 0.75ppm and 1.0ppm using Methanol as diluent. All the observations were recorded at 517nm

Percentage inhibition= [(Absorbance of Control- Absorbance of Sample) / Absorbance of Control] * 100

III. OBSERVATIONS

3.1 Table 2: Comparison between Conventional Method & Microwave Irradiation Method.

Comp.	Substituent R	M.P (ºC)	Time		Yield		% Composition		
			Conventional (mins)	MW (mins)	Conventional (%)	MW (%)	%C	%H	%N
2a	2,4-DiCl	240	360	8	62.12	86.29	C: 35.71	C: 1.79	C: 29.15
							F: 35.90	F: 1.87	F: 29.42
2b	4-Cl	255	480	10	56.34	79.26	C: 47.34	C: 3.17	F: 16.56
							F: 47.20	F: 3.00	F: 16.35
2c	2-NO_2-5-Cl	260	420	9	50.03	79.97	C: 38.40	C: 2.25	C: 22.39
							F: 38.72	F: 2.49	F: 22.59
2d	2,4-DiCl	266	420	9	52.56	77.43	C: 39.88	C: 2.00	C: 18.60
							F: 39.98	F: 2.24	F: 18.84
2e	4-NO_2	269	360	8	63.68	80.69	C: 45.62	C: 2.68	C: 21.28
							F: 45.51	F: 2.42	F: 21.04

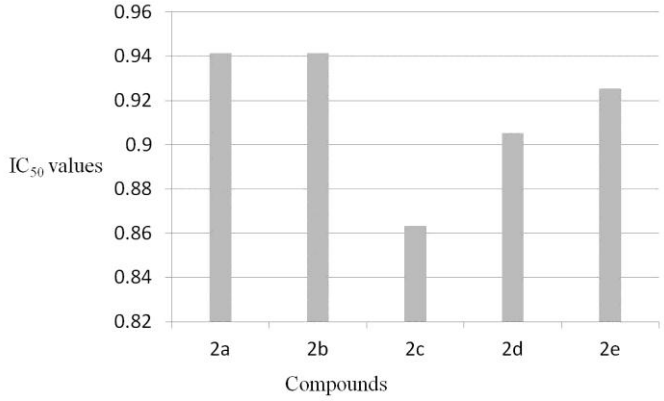
Note:- C: Calculated, F: Found.

3.1 Table 3: Estimation of antioxidant activity of the compounds using DPPH

Compound	2a	2b	2c	2d	2e
IC_{50} values	0.9414	0.9413	0.8633	0.9052	0.9254

Positive Control: 0.1575 at 517nm

3.1 Table 4: Graph of IC_{50} values of compounds



IV. Result And Discussion

The reaction time for the synthesis of all compounds by conventional methods ranged from 2 to 8 hrs in comparison with the microwave heating one (8-10 min); an obvious many-fold time reduction. Overall, a steep decrease of >95% in reaction times and a 25-60% increase in the yields were obtained.

The Melting Point of Compound 1(250ºC) was found to be significantly different from that of each of the compounds derived from it. This variation establishes a difference between the physical nature of Compound 1 and its derivatives. Difference in the retention times on TLC plate between spots of synthesized compounds and standard also was observed which further ascertains the difference in their properties.

Further, differences were also observed in the IR patterns of compounds. Each of the patterns of individual compound showed presence of a particular functional group specific to that compound thereby proving the differences in their structures. This was further confirmed using HPLC owing to the differences in the retention times.

The test compounds 2a. 2b. 2c, 2d and 2e showed IC_{50} at 0.9414, 0.9413, 0.8633, 0.9052 and 0.9254

ppm respectively.

V. Conclusion

Present research focuses on the synthesis of various substituted thiadiazole derivatives containing 2-Chloro-N-(2, 5-Dihydro-1, 3, 4-Thiadiazol-2-yl) Acetamide according to the scheme and experimental parameters presented in Table 2. Owing to the results obtained and their comparison, it is evident that microwave irradiated processes consume significantly lesser time with better yield than conventional method. These figures establish very strong points in favor of the use of microwave irradiation thereby making it an ideal technique for the synthesis of aforementioned compounds using given experimental conditions.

Also, the bioassay results establish that the derivatives express a certain degree of antioxidant activity even at concentrations as low as 0.5ppm (0.5µg/ml) indicating their strength and clinical potency.

Although, the study enlightens the antioxidant activity of the synthesized compounds at such low concentration, it does not necessarily suggest the same pattern at concentrations other than the experimental values. Thus it provides a scope for further research on the subject with different range of concentration and on more complex systems like human cell lines.

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REFERENCES

[1]. M. Surati, S. Jauhari, K. R. Desai, A brief review: Microwave assisted organic reaction, Archives of Applied Science Research, , 4 (1), 2012, 645-661,

[2]. CO Kappe, Angew Chem Int Edn: 43, 2004,6256.

[3]. C. R. Strauss, D. W. Rooney, Accounting for clean, fast and high yielding reactions under microwave conditions, Green Chem. 12, 2010, 1340-1344

[4]. C. Redlich, W. S. Beckett, J. Sparer; K. W .Barwick, C. A. Riely, H. Miller, S. L. Sigal, S. L. Shalat, Liver disease associated with occupational exposure to the solvent dimethylformamide, Annals of Internal Medicine ,108 (5), 1988, 680–686

[5]. W. Rzeskia, , J. Matysiakb, M. Kandefer-Szerszeńa, Anticancer, neuroprotective activities and computational studies of 2-amino-1,3,4-thiadiazole based compound, Bioorganic & Medicinal Chemistry, Volume 15, Issue 9, 2007, 3201–3207

[6]. M. Juszczak, K. Walczak, E. Langner, M. Karpińska, J. Matysiak, W. Rzeski, Neuroprotective activity of 2-amino-1,3,4-thiadiazole derivative 4BrABT--an in vitro study, Ann Agric Environ Med., 20(3), 2013, 575-9.

[7]. J. Matysiak, Z. Malinski, 2-(2,4-Dihydroxyphenyl)-1,3,4-thiadiazole analogues: antifungal activity in vitro against Candida species, Bioorg Khim 33(6),2007, 640-7

[8]. J. Arly Nelson, L. M. Rose, L. Lee Bennett Jr., Effects of 2-Amino-1,3,4-thiadiazole on Ribonucleotide Pools of Leukemia L1210 Cells, Cancer Res, 36 , 1976, 1375-1378.

[9]. M. Valko, M. Izakovic, M. Mazur, C. J. Rhodes, J. Telser, Role of oxygen radicals in DNA damage and cancer incidence, Molecular and Cellular Biochemistry, Volume 266, Issue 1-2, 2004, 37-56

[10]. B .S. Jayashree, A. Alam, D. Vijay Kumar, Y. Nayak, Antioxidant and Antibacterial activity of new 3-Methylflavones, Indian Journal Of Heterocyclic Chemistry, 19(3),2010,237-240