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Research Paper

Eco-friendly solvent free synthesis and antioxidant activities of N-substituted iminothiazodin-4-one derivatives

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ABSTRACT: *Heterocyclic compound are a highly significant family of chemicals in organic chemistry. It has a wide range of medical, agronomic and chemical characteristics. Consequently, many people participate in the synthesis of these chemicals. A comprehensive description is provided in the first section, including the heterocyclic compounds. Heterocycles are widely utilized in the medical, chemical and agricultural industries. Also commercial and university research organizations are being utilized to develop methods which may be used for molecular assembly consisting of heterocyclic structures. Microwave irradiated solvent free method were used for the synthesis of N-substituted iminothiazodin-4-one* ***5a-g*** *derivatives by the treatment of substituted anilines with thiourea in presence of chloroacetic acid in the first step and then natural catalyst citric acid was used in the second step for few minutes. In an anti-oxidant result point of view, all the prepared compounds,* ***5a-g*** *showed very excellent scavenging activity compared to standard.*

KEYWORDS: *Microwave synthesis, iminothiazodin-4-one derivative, antioxidant activities*

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I. INTRODUCTION

The creation and discovery of new physiologically and pharmacologically effective substances includes heterocyclic compounds including nitrogen and sulphur. Therapeutic value molecules are designed, synthesized and produced for medicinal chemistry. The development of various privileged systems of proven utility in medicinal chemistry has been the result of progress in areas such as combinatory chemistry and heterocyclic chemistry in the last several decades [1]. Most of the ring systems derived by replacement of a methane group with a nitrogen atom are formally derived from pyrrole, furan and thiophene. It is the shift in nitrogen location contributing to the structural heterogeneity of the heterocycle community. The heteroatom's, such as oxygen in isoxazoles, oxazoles, oxadiazoles, or isothiazoles, thiadiazoles, sulphide can be contained in aromatic nitrogen-containing heterocycles with five members. Heterocyclic compounds are very common in nature. Throughout a majority of the identified organic compounds tend to be heterocyclic. Five-member aromatic ring heterocycles are widely distributed in nature and are often important in different biochemical processes. The bio-isosteres of several substituent are also hetero-aromatic rings including phenyl rings and carboxyl and their ester analogues, thus rendering the resulting substances more medicinal. As a result, medicinal chemists are usually included in new chemical entities [2,3].

Thiazolidinone, a fourth carbon saturated carbonyl thiazole band, is said to have mystical properties with a wide range of biological functions. In a five-part loop, it is part of a large group of sulphur and nitrogen-containing heterocyclic compounds [4]. This physiologically active scaffold sparked interest in creating a slew of novel compounds by modifying the 4-thiazolidinone moieties at the 2, 3, and 5 levels using a variety of replacements. The structure and characteristics of substitutes in the 2-, 3-, and 5-positions vary, but the group linked to the carbon atom in the 2-position imposes the largest variation [5]. Thiazolidine is a Thaiazoletetrahydro derivative, while thiazolidinone is a thiazolidinone. Many halogen-substituted monocyclic-lactams have potent antibacterial, antimicrobial, anti-inflammatory, anticonvulsant, and antitubercular properties. These have an effect on the central nervous system and function as enzyme inhibitors. 4-thiazolidinone and 2-azetidinone derivatives are important in medicinal chemistry because they have diverse microbiological properties. 2-aryl-4-thiazolidinone has recently been developed and shown to have potent

selective anti-platelet triggering 538 mechanisms in vitro and in vivo, as well as anti-inflammatory [8, antibacterial [9], anticancer [10], and anti-HIV-1 action [11]. Carbon-nitrogen-double-bond synthesis plays a vital role in chemistry research progress. In the field of coordination chemistry, ship base ligands are important, particularly in the production of Schiff bases complexes because these compounds may theoretically shape stable complexes with metal ions [12]. These ligands reflect widely utilized groups of new compounds in coordination chemistry [13]. Schiff bases are organic compounds of considerable use in different fields [14] such as pharmacy, food, cosmetics, etc. Recently, Schiff base complexes drew attention in biochemistry and biomedicine due to their unique properties [15-16].

The use of microwave energy sources to induce synthetic chemical changes has been widely recognized in recent years. Thermal heating facilitates the weak medium of chemical reactions, opening up new opportunities for synthetic chemistry in the context of revolutionary reactions that cannot be achieved with traditional reflux [17]. Microwave-assisted technique unlocks new opportunities for synthetic chemistry in the context of revolutionary reactions that cannot be accomplished with traditional reflux. Green chemistry's environmentally friendly approach is now required in the synthesis of organic or heterocyclic components for the production of safer and more convenient end products [18]. Microwave radiation is a powerful tool for accelerating chemical processes. Chemical reactions happen even faster in the microwave than they do in conventional heating. Increased material yield and clean manufacturing in minutes will result from the microwave frequency at which the reactions occur [19]. Microwave-assisted effective organic synthesis solvent and pollution-free methods have a significant advantage. Microwave reactions are a green chemistry technique that is quicker, stronger, and healthier than traditional and other practical reactions [20]. Because the microwave improves reaction speed, optimizes reaction frequency, and prompts corresponding synthesis, microwave-assisted organic synthesis has a number of benefits over conventional reflux systems [21]. As a result, compared to traditional techniques, microwave-assisted organic synthesis has been demonstrated to significantly decrease reaction time, enhance product yield, and improve material quality by reducing undesired side reactions [22]. Therefore the researcher has been synthesized the different substituted iminothiazodin-4-one and iminooxazolidin-4-one derivatives followed by their Schiff bases by using microwave assisted clean and efficient reactions as a contemporary way, to get better yield and eco-friendly reaction [23]. Given these beneficial biological activities, the pharmacological characteristics of iminothiazodin-4-one and iminooxazolidin-4-one derivatives, and the significance of microwave aided method. For their synthesis, several techniques have been devised. Similarly, certain microwave-assisted techniques are discussed in this research paper.

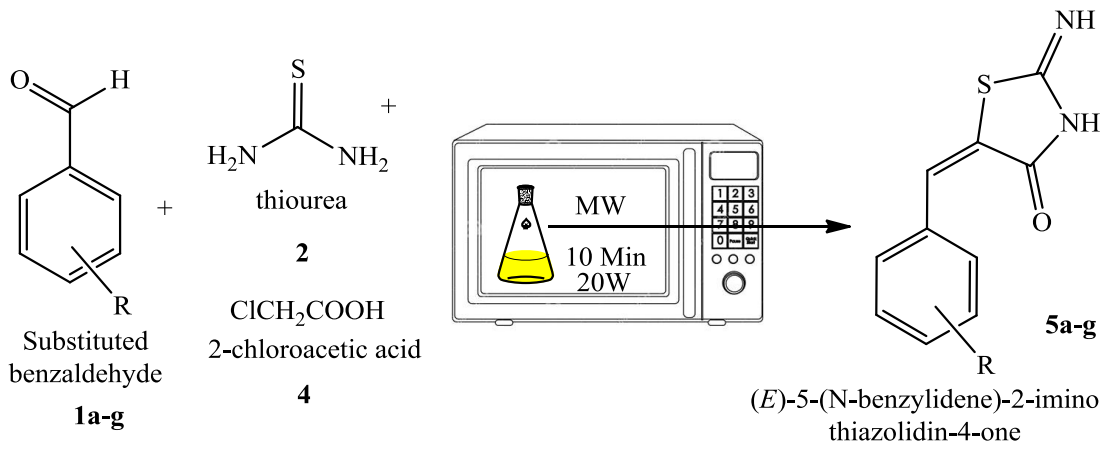
II. SYNTHESIS OF N-SUBSTITUTED IMINOTHIAZODIN-4-ONE DERIVATIVES

Material Methods:

Melting points were recorded in open glass capillaries and were uncorrected. IR spectra in (KBr pallets) were verified on Shimadzu FTIR-8400S and ATR Brucker alpha FT-IR spectrophotometer.^{13}C NMR and ^{1}H NMR spectra were recorded on400.13 MHz by Brucker spectrophotometer. The NMR prediction reference values were stated from the reference books [24-25]. The reaction was monitored by thin layer chromatography which was performed by using pre-coated silica gel aluminium plates run in the solvent benzene. All the compounds **5a-g** were synthesized in the domestic microwave oven Samsung in hours from the corresponding commercially purchased and available 4-hydroxy-3-methoxy benzaldehyde (vanillin), furfural, 4-chloro benzaldehyde, 4-bromo benzaldehyde, 4-nitro benzaldehyde, 4-hydroxy benzaldehyde, 4-methyl benzaldehyde, chloro acetic acid, thiourea, urea, citric acid and ethanol.

Experimental Procedure:

The substituted (E)-5-(4-N-benzylidene)-2-iminothiazolidin-4-one **(5a-g)** are synthesized by the mixture of 1 mole of substituted benzaldehyde **(1a-g)** and 1mole of thiourea**(2)** in 1.2 mole of 2-chloro acetic acid under microwave assisted solvent free conditions at 20W powers for 5-10 minutes. The synthesized compounds were recovered and recrystallized by ethanol **(Scheme–I)**



Scheme-I: Synthesis of Substituted iminothiazolidin-4-one

(E)-5-(4-chlorobenzylidene)-2-iminothiazolidin-4-one(5a):
Molecular Formula: $C_{10}H_7ClN_2OS$; Physical Appearance: Pale yellowish solid; Nature of Compound: Granular Powder; Molecular Weight: 238; Percent Yield: 66.90%; Melting Point (ºC): 194-196 ºC; Elemental Analysis: C, 49.80; H, 3.11; N, 12.16; FTIR (KBr):γ-lactam C=O: 1680 cm^{-1},stretching C=C in α,β ketone: 1590 cm^{-1}, tri-substituted bending for C=C: 820 cm^{-1}, cyclic aromatic ring C=C (3-peaks): 1425 cm^{-1}, 1490 cm^{-1} and 1509 cm^{-1}, aromatic compound C-H overtone: 2050 cm^{-1}, imine C=N stretching: 1715 cm^{-1}, aldehyde C-H: 1367 cm^{-1}, stretching of N-H: 2980 cm^{-1}, 2° amine bending N-H: 3190cm^{-1}, Ar-Cl: 594 cm^{-1}

(E)-5-(4-bromobenzylidene)-2-iminothiazolidin-4-one (5b):
Molecular Formula: $C_{10}H_7BrN_2OS$; Physical Appearance: Pale yellowish solid; Nature of Compound: Granular Powder; Molecular Weight: 283.14; Percent Yield: 52.75%; Melting Point (ºC): 215-217 ºC; Elemental Analysis: C, 50.21; H, 3.21; N, 11.26; FTIR (KBr):γ-lactam C=O: 1673 cm^{-1}, stretching C=C in α,β ketone: 1588 cm^{-1}, tri-substituted bending for C=C: 818 cm^{-1}, cyclic aromatic ring C=C (3-peaks): 1372 cm^{-1}, 1487 cm^{-1} and 1511 cm^{-1}, aromatic compound C-H overtone: 2050 cm^{-1}, imine C=N stretching: 1739 cm^{-1}, aldehyde C-H: 1372 cm^{-1}, stretching of N-H: 2980 cm^{-1}, 2° amine bending N-H: 3198 cm^{-1}, Ar-Br: 518 cm^{-1}

(E)-2-imino-5-(4-nitrobenzylidene)thiazolidin-4-one (5c):
Molecular Formula: $C_{10}H_7N_3O_3S$; Physical Appearance: Whitishyellowish solid; Nature of Compound: Granular Powder; Molecular Weight: 249.25; Percent Yield: 65.62%; Melting Point (ºC): 270-272 ºC; Elemental Analysis: C, 49.28; H, 3.05; N, 17.26; FTIR (KBr):γ-lactam C=O: 1675 cm^{-1}, stretching C=C in α,β ketone: 1579 cm^{-1}, tri-substituted bending for C=C: 846 cm^{-1}, cyclic aromatic ring C=C (3-peaks): 1488 cm^{-1}, 1528 cm^{-1} and 1579 cm^{-1}, aromatic compound C-H overtone: 2050 cm^{-1}, imine C=N stretching: 1711 cm^{-1}, aldehyde C-H: 1394 cm^{-1}, stretching of N-H: 2981 cm^{-1}, 2° amine bending N-H: 3486 cm^{-1}, Ar-NO_2: 1528 cm^{-1}; ^{1}H NMR (400.13 MHz, $CDCl_3$, δ ppm):δ (ppm): 8.42-8.08 (m, 4H, Ar), 10.17 (s, 1H, =CH), 4.14 (s, 2H, -NH)

(E)-5-(4-hydroxybenzylidene)-2-iminothiazolidin-4-one (5d):
Molecular Formula: $C_{10}H_8N_2O_2S$; Physical Appearance: Dark brown solid; Nature of Compound: Flakes; Molecular Weight: 220.25; Percent Yield: 47.22%; Melting Point (ºC): 231-233 ºC; Elemental Analysis: C, 52.98; H, 3.45; N, 12.02; FTIR (KBr):γ-lactam C=O: 1669 cm^{-1}, stretching C=C in α,β ketone: 1653 cm^{-1}, tri-substituted bending for C=C: 818 cm^{-1}, cyclic aromatic ring C=C (3-peaks): 1488 cm^{-1}, 1509 cm^{-1} and 1579 cm^{-1}, aromatic compound C-H overtone: 1816 cm^{-1}, imine C=N stretching: 1721 cm^{-1}, aldehyde C-H: 1376 cm^{-1}, stretching of N-H: 2981 cm^{-1}, 2° amine bending N-H: 3426 cm^{-1}, Ar-NO_2: 1528 cm^{-1}

(E)-2-imino-5-(4-methylbenzylidene)thiazolidin-4-one (5e):
Molecular Formula: $C_{11}H_{10}N_2OS$; Physical Appearance: Yellow solid; Nature of Compound: Granules; Molecular Weight: 218.27; Percent Yield: 25.51%; Melting Point (ºC): 211-213 ºC; Elemental Analysis: C, 59.99; H, 4.15; N, 12.42; FTIR (KBr):γ-lactam C=O: 1827 cm^{-1}, stretching C=C in α,β ketone: 1721 cm^{-1}, tri-substituted bending for C=C: 805 cm^{-1}, cyclic aromatic ring C=C (3-peaks): 1510 cm^{-1}, 1599 cm^{-1} and 1669 cm^{-1}, aromatic compound C-H overtone: 2050 cm^{-1}, imine C=N stretching: 1744 cm^{-1}, aldehyde C-H: 1392 cm^{-1}, stretching of N-H: 2980 cm^{-1}, 2° amine bending N-H: 3423 cm^{-1}, Ar-CH_3: 1446 cm^{-1}

(E)-5-(4-hydroxy-3-methoxybenzylidene)-2-iminothiazolidin-4-one (5f):
Molecular Formula: $C_{11}H_{10}N_2O_3S$; Physical Appearance: Brown solid; Nature of Compound: Granular Powder; Molecular Weight: 250.27; Percent Yield: 25.60%; Melting Point (ºC): 240-242 ºC; Elemental Analysis: C,

53.09; H, 4.19; N, 11.59; FTIR (KBr):γ-lactam C=O: 1827 cm^{-1}, stretching C=C in α,β ketone: 1722 cm^{-1} , tri-substituted bending for C=C: 804 cm^{-1}, cyclic aromatic ring C=C (3-peaks): 1447 cm^{-1}, 1515 cm^{-1} and 1580 cm^{-1}, aromatic compound C-H overtone: 2014 cm^{-1}, imine C=N stretching: 1668 cm^{-1}, aldehyde C-H: 1395 cm^{-1}, stretching of N-H: 2980 cm^{-1}, 2° amine bending N-H: 3462 cm^{-1}, Ar-OCH_3: 1286 cm^{-1}, Ar-OH: 2898 cm^{-1}

(E)-5-(furan-2-ylmethylene)-2-iminothiazolidin-4-one (5g):

Molecular Formula: $C_8H_6N_2O_2S$; Physical Appearance: Carbon black solid; Nature of Compound: glittery lumps; Molecular Weight: 194.21; Percent Yield: 25.86%; Melting Point (ºC):>320 ºC; Elemental Analysis: C, 49.09; H, 3.54; N, 14.79; FTIR (KBr):γ-lactam C=O: 1826 cm^{-1}, stretching C=C in α,β ketone: 1722 cm^{-1} , tri-substituted bending for C=C: 811 cm^{-1}, cyclic ring C=C (2-peaks): 1518 cm^{-1} and 1598 cm^{-1}, cyclic compound C-H overtone: 1980 cm^{-1}, imine C=N stretching: 1669 cm^{-1}, aldehyde C-H: 1393 cm^{-1}, stretching of N-H: 2981 cm^{-1}, 2° amine bending N-H: 3487 cm^{-1}

Figure 1: (E)-5-(4-N-benzylidene)-2-iminothiazolidin-4-one **(5a-g)** derivatives

III. ANTIOXIDANT ACTIVITIES OF THE SYNTHESIZED COMPOUNDS

Anti-oxidation Assay:

DPPH radical scavenging is a tool commonly used to test antioxidant function in relatively short time compared to other approaches. DPPH radical scavenging of synthesized compounds (A-M) was investigated using balanced free radical DPPH ethanolic solution. The freshly prepared DPPH solution exhibits a deep purple color generally convert them to a colorless/ bleached product when an antioxidant is present in the medium. Therefore, antioxidant compounds will slake DPPH free radical by supplying hydrogen atoms or by absorbing electron at 517nmand the faster the absorbance decreases, the more powerful the compounds ' antioxidant operation [42].

Method Used:

DPPH radical scavenging assay: DPPH inhibition in MPE was determined by using the protocol of Brand-Williams et al., [26] with a number of modifications [27]. The DPPH radical (Hi-media) is stable due to the delocalization of an auxiliary electron over the molecule, thus inhibiting dimer formation. This radical is used in the DPPH radical scavenging capacity assay to quantify the ability of antioxidants to quench the DPPH radical. The dark purple color of DPPH will be lost when it is concentrated to its non-radical form stable organic nitrogen centered free radical with a dark purple color which when reduced to its non-radical form by antioxidants turn out to be colorless. DPPH radicals are widely used in the model system to investigate the scavenging activities of a number of natural compounds. DPPH radical scavenging activity was performed using a reaction mixture containing 1 mL of DPPH solution (0.1 mmol/L, 95% ethanol v/v) with 3 ml extract was shaken and incubated at room temperature for 20 min, and absorbance was read at 517 nm against a blank [28]. The blank was 80% (v/v) methanol. Ascorbic acid (Vitamin C) was used for comparison. Radical scavenging behavior was determined as a decrease in DPPH absorbance in triplicate and mean absorbance was taken, and then scavenging percentage was estimated using the following equation:

Effect of scavenging (%) = [1-A sample (517nm) /A control (517nm)] ×100

Standard Used: Ascorbic acid (Vitamin C)

Reagent Used:2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH)

Concentration of compounds: Stock solution [600 µg/mL] of each compound was prepared in methanol. From the stock, various concentrations such as 200 µg/mL, 400 µg/mL and 600 µg/mL, were prepared.

Table 1: Antioxidant activities of 5a-g

Compound Code	Antioxidant (DPPHAssay) Mean Absorbance (A_{517} nm) Concentration of Extract (µg/mL)		
	200 µg/mL	400 µg/mL	600 µg/mL
5a	0.626±0.076	0.617±0.019	0.602±0.085
5b	0.698±0.052	0.645±0.042	0.618±0.056
5c	0.788±0.016	0.727±0.019	0.700±0.076
5d	0.590±0.027	0.567±0.078	0.523±0.083
5e	0.538±0.009	0.521±0.046	0.556±0.079
5f	0.637±0.013	0.581±0.085	0.533±0.035
5g	0.641±0.037	0.599±0.092	0.570±0.096
Std	0.589±0.067	0.500±0.078	0.402±0.099

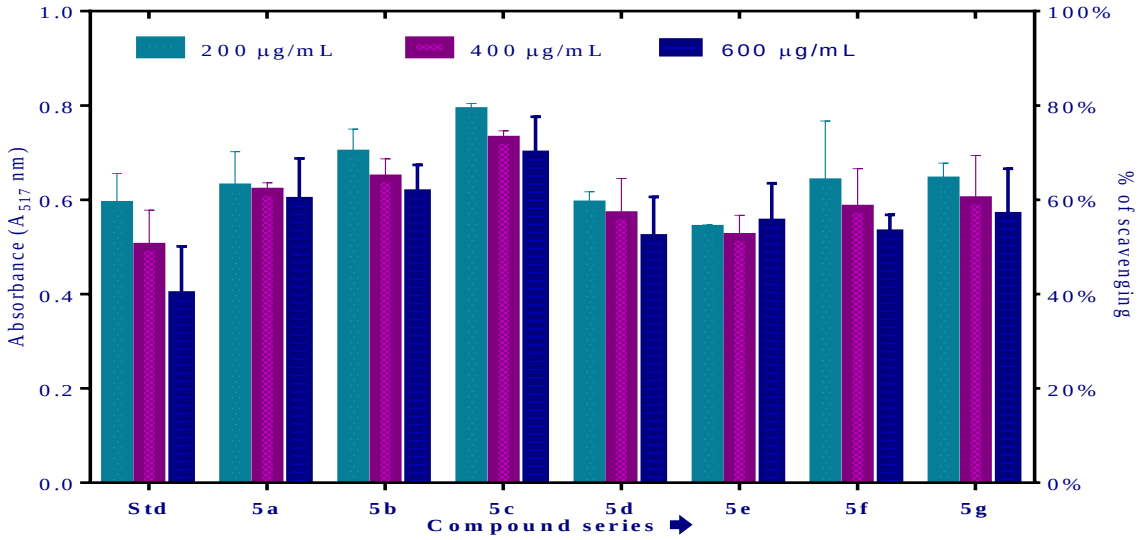


Chart 1: Antioxidant activities of 5a-g (Mean±SD)

Statistical Analysis:

The results of allthe synthesized compound series **5a-g** were carried out *in-vitro* anti-oxidant activities were calculated in the chart-3.5. The statistical tests were performed by using GraphPad prism trial version and GraphPadInStat 3.10 demo version software. The statistical significance was performed by Two-way ANOVA by Ordinary Test were performed by standard drug against synthesized compounds. P value < 0.05 was considered as statistically significant remarked by *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to standard groups. ('--' stands for no zone of inhibition)

IV. RESULT AND DISCUSSION

All the synthesized compounds **5a-g** from this chapter were evaluated *in-vitro* for anti-oxidant DPPH radical scavenging assay 1 mL of DPPH solution with 3 ml extract was shaken and incubated at room temperature for 20 min, and absorbance was read at 517 nm against a blank. The blank was 80% (v/v) methanol. Ascorbic acid (Vitamin C) was used for comparison. Radical scavenging behavior was determined as a decrease in DPPH absorbance in triplicate (100,200 and300 µg/mL) in the table-3.5 and table-3.6 and mean absorbance was taken, and then scavenging percentage calculated while the HRBC membrane stabilization system of in-vitro anti-inflammatory activities 2 mL human red blood cell (HRBC) membrane stabilization procedure used to test T anti-inflammatory function in vitro compared with the prepared1 mL of diclofenac sodium levels (200,400 and 600 µg/mL) with 1mL phosphate buffer, 2mL hypo saline and 0.5mL HRBC suspension incubated up to 30 min at 37°C and centrifuged 20 min at 3000 rpm. The supernatant water decanted and a 560 nm spectrophotometer measured the hemoglobin concentration in the supernatant solution. Percentage hemolysis was estimated by assuming the control hemolysis was 100% control and then all the prepared compounds were measured against standard drug diclofenac sodium. Almost all the synthesized compounds, **5a-g** showed excellent activity, some major compounds **5b, 5d** and **5g** showed excellent activity, and other compounds showed moderate to good anti-inflammatory activity against diclofenac sodium standard drug.

REFERENCES

[1] Verma, A., Shailendra, K. S., *Eur. J. Med. Chem*, 43: 897, 2008.
[2] Dalvie D. K., Kalgutkar A. S. and Khojasteh-Bakht*et al*., *Chem. Res. Toxicol.*, 2002, 15 (3), 269.
[3] Patani, G. A and La Voie, E. J. Bioisosterism: A rational approach in drug design. *Chem. Rev*. 1996, 96, 3147.
[4] Patil, T. S., Bhatt, P. A., *Int. J. Pharm & Tech*, 1: 674, 2004.
[5] Mistry, K. M., Desai, K. R., *E- J. Chem*, 4:189. 2004.
[6] Jubie, S., Gowramama, B., Muthal, N. K., Kalirajan, R., Gomathi, S., and Elango, K., *Int. J. Chem. Tech. Res*., 1(2): 153 ,2009.
[7] Yeriqeri, M. C., Murari, S. K., Thiammaiah, K. N., Math, S. K., Vishwanath, B. S., *J. Med. Chem*., 4(2): 190, 2008.
[8] Sayyad, M., Mokle, S., Bokhare, M., Mankar, A., Surwase, S., Bhusare, S., and Vibhute, Y., *Arkivoc* ,2,187, 2006.
[9] Colombo, A., Ferna`ndez, J. C., Ferna´ndez-Forner, D., de la Figuera, N., Albericio, F., and Forns, P., *Tetrahedron Lett.* 49(10), 1569, 2008.
[10] Murugesan, V., Prabhakar, Y. S., and Katti, S. B., *J. Mol. Graph Model*, 27,735, 2009.
[11] Patai S. The Chemistry of the carbonnitrogen double bond, John Wiley &Sonsltd., London,1970.
[12] Nimitsirwat, N.; Vernon, C. *J Am ChemSoc*2004, *32*,126.
[13] Arora K. and Harma K. P. *Synth. React. Inorg. Met.-Org. Chem.* 2003, *32*, 913.
[14] Mirkin, M.V. and Bard, A.J. *J. Anal. Chem*. 1991, *63*, 532.
[15] Saito, H.; Hoffman, A. S.; Ogawa, H. I. J. Bioact. Compat. Polym. 2007, 22, 589
[16] Kratz, F.; Beyer, U.; Schutte, M. T. Crit. Rev. Ther. Drug 1999, 16, 245.
[17] Belwal S., (2013), Green revolution in chemistry by microwave assisted synthesis: a review, *Modern Chemistry*, 1(3), 22-25
[18] Polshettiwar V. and Varma R. S., (2008), Aqueous Microwave Chemistry: A Clean and Green Synthetic Tool for Rapid Drug Discovery, *Chem. Soc. Rev*., 37, 1546-1557
[19] Leadbeater N. E. and McGowan C. B., (2006), Clean, Fast Organic Chemistry: Microwave-assisted laboratory experiments, CEM Publishing, Sample Edition, U.S.A
[20] Nerkar A. G., Pawale D., Ghante M. R. and Sawant S. D., (2013), Microwave assisted organic synthesis of some traditional and named reactions: a practical approach of green chemistry, *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3), 564-566
[21] Ravichandran S. and Kartikeyan E., (2011), Microwave synthesis- A potential tool for green chemistry, *International Journal of ChemTech Research*, 3(1), 466-470
[22] Das R., Mehta D. and Bhardawaj H., (2012), An Overview on Microwave Mediated Synthesis, *International Journal of Research and Development in Pharmacy and Life Sciences*, 1(2), 32-39
[23] Chawla A., Sharma A. and Sharma A. K., (2012), Review: A convenient approach for the synthesis of imidazole derivatives using microwaves, *Der PharmaChemica*, 4(1), 116-140
[24] Donald L. Pavia, Gary M. Lampman and George S. Kriz, Introduction to Spectroscopy, Thomson Learning, Third Edition, 2001
[25] Robert M. Silverstein, Francis X. Webster and David J. Kiemle, Spectrometric Identification of Organic compounds, John Wiley and Sons, Seventh Edition, 2005
[26] W. Brand-Williams, M. E. Cuvelier, and C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT—Food Science and Technology, vol. 28, no. 1, pp. 25–30, 1995
[27] L. L. Mensor, F. S. Menezes, G. G. Leitao et al., Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, Phytotherapy Research, vol. 15, pp. 127–130, 2001
[28] Kar et al, antioxidant and anti-inflammatory activity of mimusopselengi leaves, Asian Pacific Journal of Tropical Biomedicine S976-S980, (2012)