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Green Synthesis of Some Novel Bioactive Five-membered Heterocycles

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Abstract:- Chemical and pharmacological properties of five- membered heterocycles have gained interest in synthetic chemistry recently. They cover a wider spectrum of antimicrobial activities. Among them, Oxazoles have been proved as potent chemotherapeutics. A series of novel amido-oxazoles are synthesized from carbonyls, carboxylic acids and isocyanides under microwave irradiations in solid-support of alumina. Microwave-coupled organic synthesis has been proved as an efficient and eco-friendly tool in modern synthetic chemistry. It avoids the usage of hazardous chemicals in various steps of synthesis and accompanied by very short reaction time, high yield with extra degree of purity of the products and easy work-up. Synthesis of amido-oxazoles involves condensation of the reactants followed by cyclisation. A Comparative study has been made between conventional and microwave synthetic routes. The compounds have been found to possess moderate to excellent antifungal and antibacterial potentials.

Keywords: Microwave, solid support, heterocycles, chemotherapeutics, antimicrobial activities.

I. INTRODUCTION

The chemistry of heterocycles lies at the heart of drug discovery (Tempest, 2005). Plenty of natural and synthetic drugs possess heterocyclic moieties. These moieties have been indispensable elements of bioactivities (Hu, et. al, 2014). Chemical and pharmacological properties of five membered heterocycles have drawn interest in modern synthetic chemistry (Srivastava, et al, 2020). These drugs have been reported to cover a wider spectrum of antimicrobial activities (Tajudeen, & Khan, 2007). Heterocyclic compounds with oxygen, nitrogen and Sulphur remain in the soul of pharmaceutical chemistry (Mishra, 2010). Among them oxazoles have been proved as potent antifungal and antibacterial agents. Several oxazoles are reported to possess anti-inflammatory and analgesic properties (Rajanarendar, Shaik, & Reddy, 2008). Structural modifications in oxazole moiety can enhance their drug efficacy (Smith, et al, 2011). Several oxazoles with diverse substitution have been reported in the arena of heterocyclic drugs coupled with wider therapeutic applications (Varma, & Saini, 1997). Yet the functional and substitution diversity finds space in the designing are discovery of new drugs with eco-friendly routes (Nandi, et. al, 2009).

Present work intends the synthesis of a series of novel amido-substituted oxazoles from simple carbonyls, carboxylic acids and isocyanides under microware irradiations in solid supports of alumina. This synthetic procedure has got several benefits over the conventional methods as it avoids the usage of excess of reagents, harmful chemicals in various initial and post operational stages (Caddick, 1995). Various hazardous inorganic and organic acids, bases, solvents and other media have been either excluded or limited in the synthetic operations (Kidwai, Sapra, & Dave, 2000). Microwave coupled organic synthesis has been proved to be an efficient and eco-friendly tool in drug designing and development as it completes in shorter reaction time, high yield, easy workup with extra degree of purity (Mishra, 2012). Synthesis of amido-oxazoles completes in two stages, i.e., condensation of the reactants follows by subsequent cyclisation. A comparative study on the conventional and microwave methods has been conducted and the products are evaluated for their antimicrobial activities.

II. METHODS AND MATERIALS

Conventional reactions were carried out in R.B. flask fitted with air condenser on a calibrated magnetic stirrer. Microwave reactions were conducted in Kenstar microwave oven model no. OM 9925E at the frequency 2450 MHz and 800W. IR spectra were recorded on Nicolet 5PC FT-IR spectrometer using KBr pellets and the absorption frequency was measured in cm^{-1}, ^1H NMR spectra were recorded by using $CDCl_3$ solvent on a Brucker 300 MHz spectrometer with tetramethyl silane as an internal standard and the chemical shifts were measured in ppm. Elemental analysis was performed by means of Heracus CHN Rapid Analyzer. Similarly, temperature was measured on AZ Minigun non-contact IR thermometer model no. 8868 and melting points were determined on Thomas Hoover melting point apparatus. The purity of compounds was checked on silica gel plates using iodine vapour as visualizing agent. Oxytetracycline and Salicylic acid were used as reference drugs for the study of antibacterial and antifungal activities respectively. All the chemicals used were purchases from SD Find Chemicals Co. Ltd.

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Scheme 1

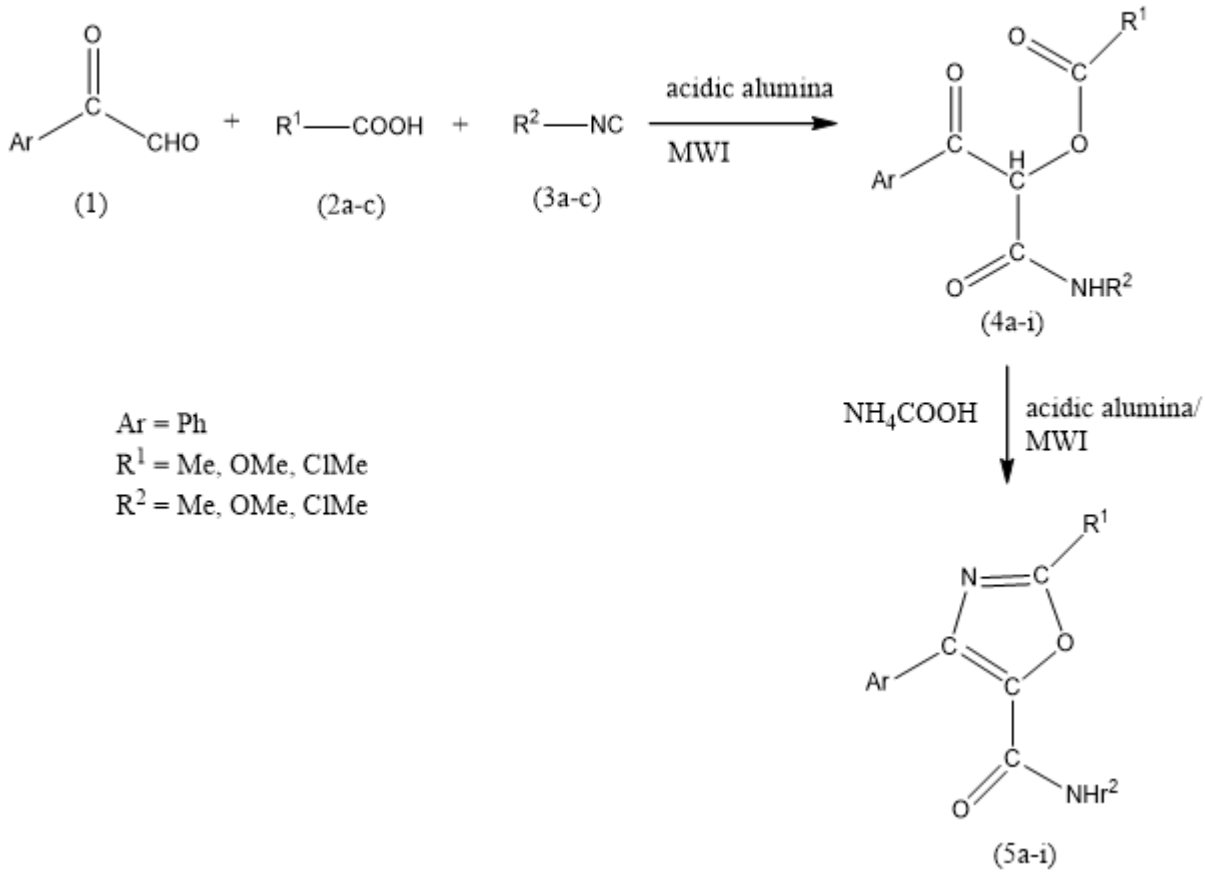

Fig 1 General Procedure for the synthesis of 2-alkyl-5-amido-4-aryl oxazole's, 5a-i

III. MICROWAVE METHOD

An equimolar amount of 0.01 mole each of arylglyoxal (1), alkyl carboxylic acids (2a-c) and alkyl isocyanides (3a-c) was dissolved in 20 ml of ethanol and adsorbed in 10 gms of acidic alumina in an Erlenmeyer flask with constant stirring homogeneously. Then the flask containing reaction mixture was put in alumina bath and irradiated with microwaves in the microwave oven for 7-8 minutes at an interval of 30 seconds. Reaction progress was monitored by thin layar chromatography. When the reaction was completed, the products 4a-i were cooled and eluted with 20 ml of ethanol for 4 times. The solvent was then recovered through distillation under reduced pressure by using rotaryevaporator leaving behind acetoxy amido-ketones 4a-i, in solid state. The products were then washed with cold water followed by recrystallization from ethanol. Pure compounds 4a-i, were reacted with ammonium formate in the solid support of acidic alumina under microwave irradiation for 4-6 minutes intermittently. Cyclisation takes place to yield 2-alkyl-4-aryl-5-amido oxazoles 5a-i. The completion of reaction was monitored by TLC. The products were then eluted with 20 ml of ethanol for 4 times as in the previous step followed by distillation under reduced pressure to get products in solid state along with the recovery of ethanol which can be reused in the process. Oxazoles 5a-I, thus obtained were washed with ice-cold water and purified by recrystallization from ethanol. The compounds were dried and characterized by determining melting points and spectroscopic analysis. The yield was found to be 80-97%.

IV. CONVENTIONAL METHOD

The equimolar mixture (0.01 mole) of the reactants mentioned above was dissolved in 100 ml of ethanol and refluxed for about 4-6 hours. After the completion of the reaction as monitored by thin layar chromatography, the products 4a-i was plunged into ice-cold water to get them in solid state. The products were filtered, dried and purified by re-crystallization in ethanol. Similarly, the compounds 4a-i were reacted with ammonium formate by dissolving in 20 ml of ethanol followed by refluxing for 3-5 hours. The products 5a-i were then plunged into ice-cold water and filtered, dried and purified by re-crystallization with ethanol. The yield was found to be 65-70%.

The synthesized oxazole derivatives 5a-i were tested for their anti-fungal activities against Aspergillus niger and Aspergillus flavus by disc diffusion method (Metwali, and Dosoki, 2007). Sabouraud Dextrose agar medium was prepared by dissolving peptose (1 gm), D-glucose (4 gm) and agar (2 gm) in distilled water (100 ml) and adjusted at pH 5.7. Normal saline was used to make a suspension of spores of fungal strains for lawning. A loop full of particular fungal strain was transferred to 3 ml saline to get a

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suspension of corresponding species. Agar medium (20 ml) was poured into each Petridis. Then the plates were dried by placing in an incubator at 35^0C for 1 hour and wells were made by using Agar punch and labeled. A control was also prepared and maintained at 35^0C for 72 hours. Salicylic acid was used as reference drug to observe antifungal activities of the oxazole derivatives under experiment. The zone of inhibition caused by the compounds was compared with that by reference drug.

Similarly, the compounds were screened for their antibacterial activities against E. coli, Rhizobium japonicum, Enterobactor aerogenes, and Bacillus mojavensis bacterial strains by disc diffusion method (Pedro and Juyan, 2006). A standard inoculum (1.2×10^7 c.f.u./ ml 0.5 Mcfarland standards) was introduced on the surface of sterile agar plates and a glass spreader was used for homogeneous distribution of the inoculums. The discs measuring 6 mm in diameter were prepared from Whatmann no. 1 filter paper and dried at 140^0C for 1 hour. The discs were soaked in a known concentration of the test compounds and placed in nutrient agar medium along with solvent and growth control. Oxytetracycline was used as reference drug for antibacterial activities. Thus prepared plates were incubated for 24 hours at 37^0C. Zone of inhibition were measured and compared with reference drug to evaluate the antibacterial activities of the compounds (Table 1).

V. RESULTS AND DISCUSSION

A series of novel five-membered heterocycles 2-alkyl-5-amido-4-aryl oxazoles (5a-i) have been synthesized from aryl glyoxal (1), carboxylic acids (2a-c) and alkyl isocyanides (3a-c) in two steps by conventional and microwave irradiation methods. Condensation of the reactants gave acetoxy amido-ketones (4a-i) in 7-8 minutes with about 85% yield in microwave reactions whereas conventional reactions took 4-6 hours with about 65% yield. These intermediates were reacted with ammonium format in the solid support of acidic alumina under microwave irradiation to furnish amido-oxazoles (5a-i) within 4-6 minutes accompanied by 80-97% yield. Conventional reactions took comparatively longer reaction time of 3-5 hours with 65-70% yield. Melting point of synthesized amido-oxazole derivatives were leveled up within 128^0C-167^0C. Solid supported microwave reactions have been proved efficient synthetic method in terms of reagent economy, saving of time, elimination of hazardous chemicals, recovery of organic solvents and inorganic solid support. Reaction time has been reduced from 5 hours to 6 minutes with an excellent yield of 97%.

Table 1: *In vitro* antibacterial and antifungal activities of Oxazoles (5a-i)

Comp.no.	R^1/R^2	Antibacterial activities				Antifungal activities	
		E. coli	Rhizobium japonicum	Enterobacter aerogenes	Bacillus mojavensis	Aspergillus niger	Aspergillus flavus
5a	Me/Me	+	+	+	++	+	+
5b	Me/OMe	+	++	+	++	++	+++
5c	Me/ClMe	++	++	++	+++	++	++
5d	OMe/Me	+	++	++	++	+++	+++
5e	OMe/OMe	+	+++	+++	++++	+++	++
5f	OMe/ClMe	++	++	+++	++	+++	+++
5g	ClMe/Me	+	++	++	+++	++	++
5h	ClMe/OMe	++	+++	+++	+++	++	+++
5i	ClMe/ClMe	+++	++	++++	+++	+++	++++
OTC*/SA**		++++	++++	+++++	++++	++++	+++++

*Oxytetracycline (OTC) - Reference drug for antibacterial activity:
+: 2-4 mm; ++: 5-8 mm; +++: 9-12 mm; ++++:13-15 mm; +++++: 16-18 mm.
** Salicylic acid (SA) - Reference drug for mm. antifungal activity:
+: 2-5 mm; ++: 6-10 mm; +++: 11-13; ++++: 14-18 mm; +++++: 19-21 mm.

Spectral analysis has supported the projected synthetic outputs. Cyclic C-O bond shows absorption at 1262-1295 cm^{-1} and C=N linkage in the oxazole rings showed absorption at 1645-1665 cm^{-1} with variations in substitution. Similarly, C=O of amide group appears at 1730-1760 cm^{-1} in IR analysis. The N-H absorption band was observed at 3350-3442 cm^{-1} followed by attachment of polar group on nitrogen atom. Similarly, in ^{1}H NMR analysis, C-CH_3, C-OCH_3 and C-CH_2C1 protons appear at δ 1.0-1.2, 3.6-3.7 and 3.8-4.2 ppm as singlet. On the other hand N-CH_3, N-OCH_3 and N-CH_2C1 protons appear slightly down field at δ 2.0-2.6, 3.5-3.7 and 4.0-4.3 ppm respectively. Sole proton of N-H group shows the signal at 5.0-5.6 ppm as broad singlet. All the aromatic protons present in aryl group of oxazole ring gave multiplet at 6.0-6.5 ppm (table 2). Observation of these spectral data supports the structural elucidation of newly synthesized oxazole derivatives (5a-i).

Amido-oxazole derivatives (5a-i) showed moderate to excellent antifungal and antibacterial activities. Compounds 5e, 5f, 5h and 5i showed excellent antimicrobial activities whereas, compounds 5a, 5b and 5c showed moderate antimicrobial properties. It means that the compounds with oxygen and chlorine in substituted alkyl groups possess higher potential against various microbes. On the top of this, the presence of polar groups on the oxazole moiety slightly increases melting point.

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VI. CONCLUSION

Amido-oxazole derivatives (5a-i) have been synthesized from carbonyls, carboxylic acids and isocyanides by adopting microwave irradiation method efficiently within 6 minutes accompanied by 97% yield. Work-up has been done in cold water and ethanol used as solvent was completely recovered for reagent economy. Inorganic solid support alumina and its bath can be reused in the synthetic procedure. The novel oxazole derivatives synthesized showed moderate to excellent antibacterial and antifungal activities for which they can be used as diverse chemotherapeutics.

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