SOLVENT-FREE RAPID MICROWAVE SYNTHESIS AND ANTIVIRAL ACTIVITY OF 2,4-(I H, 3H)PYRIMIDINEDIONE

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ABSTRACT: Microwave-assisted method has wide application in combinatorial chemistry. It has become a powerful tool in building the bioactive and pharmaceutical important compounds library within a short time. In view of a pronounced effect of Microwave-assisted methods in synthetic chemistry and their importance in drug discovery, a systematic work on the solvent-free synthesis of 2,4-(IH,3H)Pyrimidinedione(uracil) was carried out. Comparison was made between classic (conventional) and Novel (microwave-assisted) methods and it was found that compounds synthesized by microwave-assisted method showed better yield and reduced reaction time under safe and environment-friendly conditions, while conventional methods were tedious and time consuming. The structure elucidation of synthesized compounds was done by its melting/boiling points, solubility, thin layer chromatography (TLC) techniques and spectral analysis i.e ultraviolet (UV), Fourier transform infrared spectroscopy (FTIR) and Gas chromatography–mass spectrometry (GC-MS) were used to get the results. The pharmacological evaluation of synthesized compound was investigated by its antiviral activities against *Lasota* strain of *NDV (ranikhait disease virus)* and it showed the positive activity.

Keywords: Pyrimidinedione, Microwave, Solvent-free, Green Chemistry, *NDV.*

(Received 12-02-2014 accepted 12-02-2015).

INTRODUCTION

The solvent free synthesis plays a vital role in synthetic chemistry (Tanaka and Toda, 2000).The solvent-free methods in combination with microwave radiation is a forward step towards green chemistry. The absence of solvents and microwave heating are found to be useful and economical as well as provide rapid and safe methods by decreasing the chances of pollution. The main priorities for the chemical industry are the product safety and the environment- friendly conditions (Haggin, 1996). New techniques and methods for green chemistry are subject of intense activity now a days (Strauss, 2001).

Microwave irradiation is reported to be a quick and rapid approach for heating the contents than through conventional heating (Lew *et al*, 2002). In this technique electromagnetic energy is converted into heat by some liquids and solids. The conversion of microwave radiation in chemistry is useful for building the library of important bioactive compounds (Aysola *et al*, 2003)

Uracil is one of the five main nucleo bases found in both the nucleic acids i.e DNA and RNA. The others are adenine, cytosine guanine and thymine. Uracil is only found in RNA, replacing the thymine. It can be used as pharmaceutical drug with antiviral and anticancer properties (Garrette *et al*, 1997). It is also used in animal and plant studies to help carry out the synthesis of many enzymes necessary for cell function through bonding with ribose and phosphates.

MATERIALS AND METHODS

Chemicals: All chemicals used in the synthesis were of analytical grade from a Standard Company.

Sample Preparation: The sample solutions were prepared in Normal saline maintaining 0.1 µg/ml concentration.

Instrumentation: Melting points were determined using Gallenkamp melting apparatus. UV spectra were recorded within the range of 200-500 nm on spectrophotometer. FTIR spectra were recorded within the range of 400-4000 cm^{-1} as KBr pellets on a M-2000 Spectrometer, while Mass data was recorded on GC-MS QP- 2010 spectrometer. For Microwave-assisted synthesis, microwave oven DW-180, 2450 MHz, 950W was used.

Synthesis of 2, 4-(1H, 3H) Pyrimidinedione

Conventional (Uracil-1): Propiolic acid (5 g) was added to stirred solution of urea (4.3g) and anhydrous benzene (120 mL) at 25^{o}C. The solids underwent a change in crystal size shortly after the acid addition. Three drops of concentrated sulphuric acid were added and the solution was heated to reflux. After 6 hr water appeared to separate and was removed by Dean-Stark trap. After a reflux of18 hr , the reaction mixture was allowed to cool. The solid product (insoluble in benzene) was recovered by filtration. TLC of the solid (dissolved in water, eluted

with a 70:40:10 by volume mixture of ethyl acetate-acetone-water and visualized by UV) showed the presence of uracil as a major component (Subbaraman *et al*,1980).

Seventy percent yield (5.4g) was obtained with m.p 295^0C (decomposed), which was soluble in hot water. Maximum absorption occurred at $\lambda_{max.}$258 nm. The FTIR (KBr) showed (N-H)str.vibration frequency at 3212 cm^{-1}, the carbonyl(C=O) vibration frequency (amide group) appeared at 1623cm^{-1}, (C=C) absorption band at 1440 and (C_5-H) bending vibration appeared at 1114cm^{-1}, EIMS showed molecular ion peak at m/z=112

Microwave (Uracil-II): urea (1.1g) and anhydrous benzene (6.0 mL) were stirred at room temperature and propiolic acid (1.3mL) and concentrated sulphuric acid (1-2 drops) were added to it.The reaction mixture was radiated for 30seconds and then allowed to cool up to 25 ^{o}C. The solid product was insoluble in benzene which was decanted off. TLC of the solid (dissolved in water, eluted with a 70:40:10 by volume mixture of ethyl acetate-acetone-water and visualized by UV) showed uracil as a major component (Shyamaprosad *et al*, 2007)

Eighty two percent yield (2.2g) was obtained with m.p.293^0C (decomposed) which was soluble in hot water. The λ max. appeared at 258.5nm. FTIR (KBr) showed absorption peak of (N-H) str.frequency at 3134 cm^{-1}, carbonyl (C=O, amide) at 1662 cm^{-1} and (C_5-H) bending vibration appeared at 1199 cm^{-1}, EIMS showed molecular ion peak at m/z=112

Microwave Solvent- free Synthesis of 2, 4-(1H, 3H) Pyrimidinedione

(Uracil-III): The mixture of urea (1.1g), propiolic acid (1.5g) and conc.sulphuric acid (2-3 drops) was subjected to microwave radiation for 20 seconds. TLC of the solid product (dissolved in water, eluted with 70:40:10 by volume mixture of ethyl acetate-acetone-water and visualized by UV) showed uracil as a major component (Mojtahedi *et al* , 2002)

Eighty five percent yield (2.04g) was obtained with m.p 295^oC, which was soluble in hot water. Maximum absorption appeared at λ_{max} 257.5nm, FTIR (KBr) showing the vibrational frequency of (N-H)str. at 3275cm^{-1}, The (C=O,amide)str.vibration at 1680 cm^{-1} and (C_5-H) bending vibration at 904 $cm^{-1}$was achieved. EIMS showed molecular ion peak at m/z=112

Antiviral Activity: Infectious diseases are one of the most important factors confronting the expansion of poultry industry. It has been reported that in poultry, various infectious and non-infectious diseases could cause heavy economic loses (Qureshi, 1981and Lancaster, 1976). Many of such disasters were caused by viruses. The havoc created by the virus can be controlled by using either the bio security measures or vaccination (Spradbrow, 1997). Newcastle disease (*ND*) caused by NDV is an important viral disease of poultry worldwide especially in the developing countries causing up to 70-80% mortality in the unvaccinated chickens(Nawathe *et al*,1975). It is known to the farmer as a threat particular in rural poultry. To counter this deadly disease, proper control measures need to be adopted. Vaccination has been the fundamental method used to reduce the loss of susceptible birds (Demey and Pandey, 1991) .

Propagation of Virus

Source of Virus: A freeze-dried ampule of live attenuated vaccinal La Sota strain (Izovac) of Newcastle disease virus with EID_{50} 10^{-6} was obtained from a medical store in Lahore.

Re-Constitution of vaccine: The ampoule of freeze-dried vaccine was diluted in Physiological saline solution (1000ml) (NaCl 0.85 g,distt. H_2O 100 ml at pH-7.2) containing 1 ml antibiotic solution.

Source of Embryonated Eggs: Seven days old embryonated eggs of hen were procured from commercial Hatchery**.** The eggs were brought to the Microbiology Dept. University of Veterinary and Animal Sciences, Lahore and incubated at 37 ˚C for two days prior to inoculation in the laboratory.

Inoculation of Vaccinal Newcaslte Disease Virus (*La Sota*): Nine days-old embroyonated eggs of hen were candled to observe the viability of the embryo. The margin of the air sac and position of the head were marked with a lead pencil.

The egg shells were swabbed with pyodine for disinfection at inoculation site.

A hole was drilled through the shell at the upper extremity over the air shell, opposite to the site of head using 18 gauge 1"needle.

The ampoule of freeze-dried vaccine which was diluted in 1000 ml physiological saline solution containing 1 ml antibiotic solution was used in the experiment. A 0.1 ml of this diluted material was inoculated in each of the embryonated eggs through allantoic sac route with the help of a disposable syringe. The vaccinal virus suspension in physiological saline solution containing antibiotic solution was inoculated in a set of five embryonated eggs.

Site of inoculation was sealed with a drop of molten wax. All the inoculated and control eggs were incubated at 37^oC.The eggs were candled after 24 hours of inoculation for the evidence of any mechanical death (Amita *et al*, 2003)

The allantoic fluid was collected aseptically for harvesting NDV in a bio-hazard cabinet with the help of Pasteur pipette in a conical flask while the sediments in test tubes were discarded.

Spot Agglutination Test: A drop of allantoic fluid was placed on a glass slide and mixed with an equal volume of 5% freshly prepared washed chicken RBC's (Tiwari *et al*, 1996)

RESULT AND DISCUSSION

The compound synthesized by solvent-free microwave-assisted method showed remarkable reduction in heating time along with better yield as shown in Table-1

A large number of uracil derivatives were reported to be synthesized using such methods which may inhibit the human parainfluenza virus infection (Eric and Kelly, 2014).

This compound showed maximum absorption at 258 nm wavelength, which matched with standard value (258.5nm).The presence of amide group in uracil was found by stretching vibration frequency near 1680-1630 cm^{-1}.The absorption band of (N-H)str. appeared at 3218-2690 cm^{-1}, the (C =C) str. vibrational region appeared at 1510-1450cm^{-1},the bending vibration frequency of (C_5-H) lied within range of 1225-950 cm^{-1} (Ten and Baranov,2004)

The Mass spectral analysis of synthesized compounds was done by using GC-MS and result is depicted in Table- 2 and is shown by Fig- 1.

U-I and U-II which were synthesized by the condensation of propiolic acid and urea in presence of benzene (Subbaraman *et al* ,1980) showing molecular ion peak at m/z=112 corresponding to molecular formula $C_4H_4N_2O_2$. The same result was obtained in U-III which was the solvent free microwave synthesis of uracil (Mojtahedi *et al*, 2002)

Antiviral activity of synthesized compounds was determined against *Lasota* strain of *ND* (new castle disease) virus by spot agglutination test(Amita *et al*,2003) The clumping of erythrocytes occurring within 3 min was declared as positive for antiviral activity(Ganti and Shastri,1989) as is shown in Table-3

The Newcastle disease viruses were reported to be confirmed by Haemagglutination inhibition test using known specific *NDV* antiserum (Alexander and Chettle, 1977). The results of the present study are recorded in Table-3 showing the significant activity against *NDV*.

The result of present investigation showed that microwave assisted method gave the quick, rapid and accurate synthesis. The time required for conventional heating was greatly reduced from 18 hours to only 30 sec. Another novelty of this method was the solvent free synthesis of **U-III** (without the carcinogenic effect of benzene). It is concluded that by selecting such novel synthetic method, a large number of medicinally important compounds can be synthesized under safe, clean and environment-friendly conditions which may increase yield, saving time and energy.

Table-1. Conventional heating versus microwave radiation for the synthesis of Pyrimidinedione(uracil)

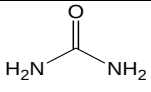
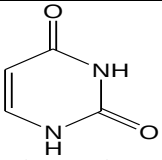
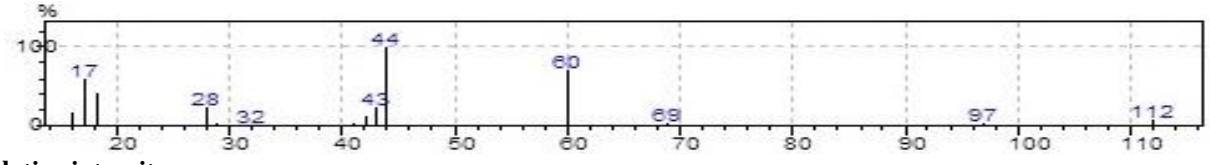
Compd-No.	Substrate	Product	Conv. Time (min)	Yield %	MW Time (sec.)	Yield %
U-I U-II	 Urea + HC≡C.COOH Propiolic Acid +Benzene		1080	70	30	82
U-III	Urea + propiolicacid without Benzene(solvent-free condition)	2, 4-(1H, 3H) Pyrimidinedione	1080	70	20	85

Table-2. GC- MS analysis of 2,4- (1H,3H) Pyrimidinedione

S. No.	Compd. No.	Formula	Base peak	Molecular ion M^+peak
1	U-I			
2	U-II	$C_4H_4N_2O_2$	44	112
3	U-III			



Relative intensity

Fig. Mass spectrum of 2,4- (1H,3H)pyrimidinedione **m/z**

Table-3. Antiviral Activity of pyrimidinedione against ND virus.

S. No	Compund No.	Conc. in µg/ml	Result
1	U-I	0.1	+ve
2	U-II	0.1	+ve
3	U-III	0.1	+ve

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