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Dihydropyrimidinone Derivatives: Green Synthesis and Effect of Electronic Factor on Their Antimicrobial Properties.

Tanay Pramanik[†*], Aadil Hussain Pathan[‡], Richa Gupta[‡], Joginder Singh[§] and Simranjeet Singh[§.]

[†]Department of Chemistry, Faculty of Technology and Sciences, School of Civil Engineering, Lovely Professional University, Phagwara, P.O.Box 144411, India.
[‡]Department of Chemistry, Faculty of Technology and Sciences, School of Science, Lovely Professional University, Phagwara, P.O.Box 144411, India.
[§]Department of Microbiology and Bio Process, School of Bio-Science and Bio-technology, Lovely Professional University, Phagwara, P.O.Box 144411, India.

ABSTRACT

A series of Dihydropyrimidinone (DHPM) derivatives were synthesized using urea, ethyl acetoacetate with electron rich as well as electron deficient aromatic aldehydes through Biginelli reaction in an eco friendly and green condition. Their antimicrobial evaluations were performed against some common pathogenic microorganisms. It was observed that the electronic factor of the phenyl ring of DHPM has significant influence on their antimicrobial properties.

Keywords: Green Synthesis, Dihydropyrimidinone, Antibacterial, Antifungal, Electron rich, Electron deficient.

Corresponding author

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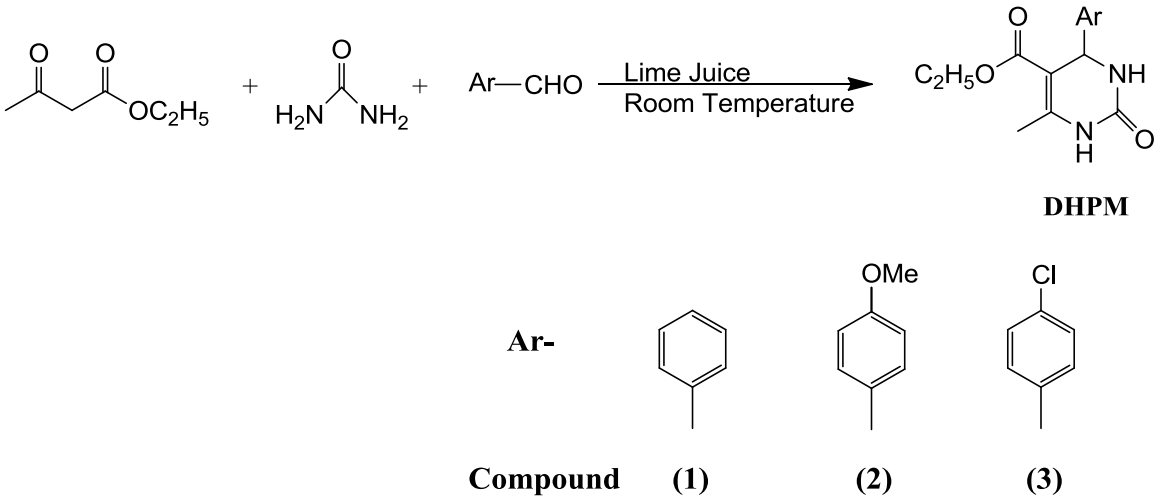
INTRODUCTION

Dihydropyrimidinone (DHPM) derivatives belong to an interesting class of heterocyclic molecules which has attracted huge attention from the researchers because of its interesting and multifaceted biological activities, such as antiviral [1], antitumor [2], and antibacterial [3] as well as calcium channel modulating activity [4]. Thus the synthesis of dihydropyrimidinone (DHPM) via conventional Biginelli reaction and evaluation of their biological properties is of recent interest by the researchers. As our growing concern for the environment demands the development of an eco-friendly and green procedure for synthesis of dihydropyrimidinone (DHPM), our group has recently reported a "solvent-free and catalyst-free" green methodology for synthesizing this DHPM [5]. Very recently a safe, eco-friendly, economical, efficient and green method has also been developed by our group for synthesizing DHPM via Biginelli reaction at room temperature in common fruit juice (amla, orange and lime juice) medium [6]. However Biginelli reaction using electron rich aromatic aldehydes in lime juice medium has not been reported in literature so far. So hereby we are reporting a green procedure for DHPM synthesis via Biginelli reaction with electron rich as well as with electron deficient aromatic aldehydes in lime juice medium at room temperature. [**Scheme-1**].

Scheme 1: Synthesis of DHPM with different aromatic aldehydes in lime juice medium.



Recently Bhatewara *et al.* have reported a microwave assisted green synthesis of DHPM and their antimicrobial properties [7]. Green synthesis and antimicrobial properties of DHPM has also been reported in literature by other researchers [8, 9]. However antimicrobial properties of DHPMs which have been synthesized in lime juice medium, is not reported in literature yet [6, 10, 11]. Although antimicrobial properties of DHPM is well known and well documented in literature, our extensive literature search has revealed that the influence of electronic factor of phenyl ring of DHPM on their antibacterial and antifungal properties is yet to be explored in literature.

So keeping this research gap stated above in mind, our main objective is to perform green synthesis of a series of DHPM containing electron rich as well as electron deficient phenyl rings, in lime juice medium at room temperature. Then a comparative study of antifungal and antibacterial activity of the synthesized compounds will help us to gain insight into the effect of electronic factor on their antimicrobial properties.

MATERIALS AND METHODS

The required lime juices were extracted directly from the naturally obtained lime (Citrus aurantifolia) and it was used straightaway for the reaction without adding any foreign chemicals or additives.

General Procedure for Synthesis of DHPMs

Compound **1** and compound **3** were synthesized following the same procedure as it was reported earlier from our group [6].

Procedure for Synthesis of Ethyl 4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (Compound 2)

The equimolar quantities of ethyl acetoacetate (0.02 mole, 2.6 gm), urea (0.02 mole, 1.2 gm) and 4-anisaldehyde (0.02 mole, 2.4 ml) were stirred together in 4 ml of lime juice at room temperature continuously for 12 h. Upon completion of reaction after 12 h, the solid product was precipitated out of the reaction medium. Upon filtration of the reaction mixture, the crude solid product was collected and crude product was recrystallized from hot ethanol to get the pure compound as pale yellow solid.

The obtained DHPMs were characterized by melting point, IR and NMR spectroscopy. The melting point, IR and NMR spectra of the synthesized compounds were identical to those of reported ones.

Method of media preparation for studying anti-microbial properties

The media was prepared by following standard literature reported procedure [12]. 40 grams of Luria Bertani agar was added to the 1 liter of double distilled water, it was mixed thoroughly and pH was adjusted at 7.5 ± 0.2. The solution was heated to dissolve the ingredients completely after which the media was autoclaved. After autoclaving, 15-20 ml of that media was poured into petri dish for studying antimicrobial activities.

Anti-microbial properties study

Four common pathogenic microorganisms namely *Escherichia coli*, *Salmonella typhimurium, Aspergillus niger* and *Penicillium chrysogenum* were chosen to evaluate the anti-microbial activities of our synthesized DHPM compound **1, 2** and **3**. *In vitro* study of anti-bacterial activities were performed against Gram -ve bacterium (*Escherichia coli*, *Salmonella Typhimurium*) and *in vitro* study of anti-fungal activities were performed against fungi (*Aspergillus niger*, *Penicillium chrysogenum*) by following standard literature reported procedure of disk-diffusion method [13]. Whattman no. 1 filter paper disks were sterilized by autoclaving at 160 ^{0}C for 1 h. Then the sterile disks were impregnated with the test compounds of different concentrations (125 ppm, 250 ppm and 500 ppm). The impregnated disks were placed on the medium suitably spaced apart, and the plates were incubated at 32 ^{0}C for 24 h. DMSO was used as solvent control. Finally the zones of inhibition were measured in mm scale. The results of anti-bacterial activities are summarized in **table-1** and that of anti-fungal properties are listed in **table-2**.

EXPERIMENTAL

Characterizations

Ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (Compound 1)

Melting point: 210 ^{0}C (Reported [14]: 209-210 ^{0}C)
IR (neat): 3242, 3113, 1724, 2958, 1703, 1487, 1321 cm^{-1};
^{1}H-NMR (400 MHz, DMSO–d^6): δ 1.17 (t, 3H), 2.27 (s, 3H), 4.02 (q, 2H), 5.20 (d, 1H), 7.19–7.31 (m, 5H), 7.63 (m, 1H), 9.09 (s, 1H)

Ethyl 4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (Compound 2)

Melting point: 203 ^{0}C. (Reported [16]: 199-201 ^{0}C)
IR (neat): 3242, 3111, 2955, 2835, 1708, 1649, 1612, 1512, 1460 cm^{-1}
^{1}H-NMR (400 MHz, DMSO–d^6): δ 1.15 (t, 3H), 2.25 (s, 3H), 3.73 (s, 3H), 4.02 (q, 2H), 5.13 (d, 1H), 6.83 (d, 2H), 7.18 (d, 2H), 7.57 (s, 1H), 9.06 (s, 1H).

Ethyl 4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (Compound 3)

Melting point: 215 ^{0}C (Reported [15]: 212-214 ^{0}C)
IR (neat): 3242, 3113, 2929, 1724, 1703, 1649, 1487, 1460 cm^{-1}

^{1}H-NMR (400 MHz, DMSO–d^{6}): δ 1.14 (t, 3H), 2.26 (s, 3H), 4.02 (q, 2H), 5.18 (d, 1H), 7.25–7.31 (m, 5H), 9.10 (s, 1H).

RESULTS AND DISCUSSION

An equimolar mixture of urea and ethyl acetoacetate was employed to react individually with benzaldehyde, 4-methoxy benzaldehyde and 4-chloro benzaldehyde for synthesizing our desired dihydropyrimidinone (DHPM) compound **1**, **2** and **3** respectively. Compound **1** is containing phenyl ring, **2** is containing electron rich 4-methoxy phenyl ring and compound **3** contains electron deficient 4-chloro phenyl ring. So compound-**2** is the most electron rich DHPM among these three compounds, followed by compound-**1** whereas compound-**3** is an electron deficient DHPM.

All the reactions were carried out at room temperature in lime juice medium. The natural acid present in the lime juice acted as the solvent cum catalyst for our biginelli reaction. It was interesting to note that at room temperature the biginelli reaction needs 12 h to complete in lime juice medium when electron rich 4-methoxy benzaldehyde was used whereas the same reaction (in the same medium) was completed in just 1 h when either benzaldehyde or 4-chloro benzaldehyde was used as reactant. So this is consistent with our previous findings [6] that biginelli reaction with electron rich aromatic aldehyde needs longer time for completion compared to that with electron deficient aromatic aldehyde in fruit juice medium at room temperature. So three desired DHPM **1**, **2** and **3** were synthesized successfully via biginelli reaction in lime juice medium at room temperature. After successful synthesis of these three compounds **1, 2** and **3** in an eco friendly, efficient, economic and green condition, the antibacterial and antifungal activities of these three compounds were evaluated to get an insight into the effect of electronic factor of phenyl ring of DHPM on their anti-microbial properties.

While performing *in vitro* study of antimicrobial activities it was observed that in most of the cases, with increase in concentration from 125 ppm to 500 ppm the antimicrobial activities of compound **1** and **2** were enhanced which is very obvious. Unfortunately compound-**3** did not show any antimicrobial activity even at 500 ppm concentration against any of the four selective pathogenic microorganisms namely *Escherichia coli, Salmonella typhimurium, Aspergillus niger* and *Penicillium chrysogenum*.

It's seen from **table-1** that during *in vitro* study of antibacterial properties, compound-**1** did not show any antibacterial activity against either Escherichia coli, Salmonella typhimurium at 125 ppm concentration. When zone of inhibition (in mm) of DHPM **1** and **2** (with concentration 500 ppm) were compared against Escherichia coli, it was observed that inhibition zone of compound **2** (4 mm) is double than that of compound **1 (**2 mm). At 500 ppm concentration, DHPM **2** has shown better inhibition than DHPM **1** against Salmonella typhimurium. So DHPM-**2** has shown better anti-bacterial property than DHPM-**1** against both of these two Gram -ve bacterium Escherichia coli, and Salmonella typhimurium. Moreover, inhibition property of DHPM-**1** is better against Salmonella typhimurium compared to its inhibition property against Escherichia coli at 500 ppm concentration whereas DHPM-**2** is almost equally active against both Escherichia coli, and Salmonella typhimurium at 500 ppm concentration. Comparative view of antibacterial properties of compound-**1** and **2** are shown by graphical representation in **figure-1.**

In vitro study of antifungal properties of DHPM **1**, **2** and **3** were evaluated against two selective fungi Aspergillus niger, Penicillium chrysogenum and the results are summarized in **table-2**. It was unfortunate to note that DHPM-**3** which contain electron deficient 4-chloro phenyl ring, did not show any antifungal activities against either of these two fungi Aspergillus niger and Penicillium chrysogenum even at 500 ppm concentration. When the antifungal properties of DHPM **1** and **2** (at 500 ppm concentration) were compared, it's worth noting that compound-**1** has shown better inhibition than compound-**2** not only against Aspergillus niger but also against Penicillium chrysogenum. So the DHPM-**1** which contains phenyl ring is showing better antifungal activity than DHPM-**2** which contains electron rich 4-methoxy phenyl ring. Comparative view of antifungal properties of compound-**1** and **2** are shown by graphical representation in **figure-2**.

It's also interesting to note that in case of DHPM-**1**, its antifungal activity (which is indicated by zone of inhibition) have increased significantly with increase in concentration against both of the fungi whereas DHPM-**2** has shown only marginal increase in antifungal activity with increase in concentration.

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Table 1: Anti-Bacterial Properties.

Bacteria	Compound 1			Compound 2		
	Zone of inhibition (in mm) with Concentration			Zone of inhibition (in mm) with Concentration		
	125 ppm	250 ppm	500 ppm	125 ppm	250 ppm	500 ppm
Escherichia coli	-	1 mm	2 mm	3 mm	4 mm	4 mm
Salmonella Typhimurium	-	2 mm	3 mm	2 mm	2 mm	4 mm

*Compound-**3** did not show any antibacterial activity against any of these two Gram -ve bacterium.

Table 2: Anti-Fungal Properties.

Fungi	Compound 1			Compound 2		
	Zone of inhibition (in mm) with Concentration			Zone of inhibition (in mm) with Concentration		
	125 ppm	250 ppm	500 ppm	125 ppm	250 ppm	500 ppm
Aspergillus niger	3 mm	6 mm	7 mm	4 mm	4 mm	5 mm
Penicillium chrysogenum	2 mm	4 mm	4 mm	2 mm	3 mm	3 mm

*Compound-**3** did not show any antifungal activity against any of these two fungi.

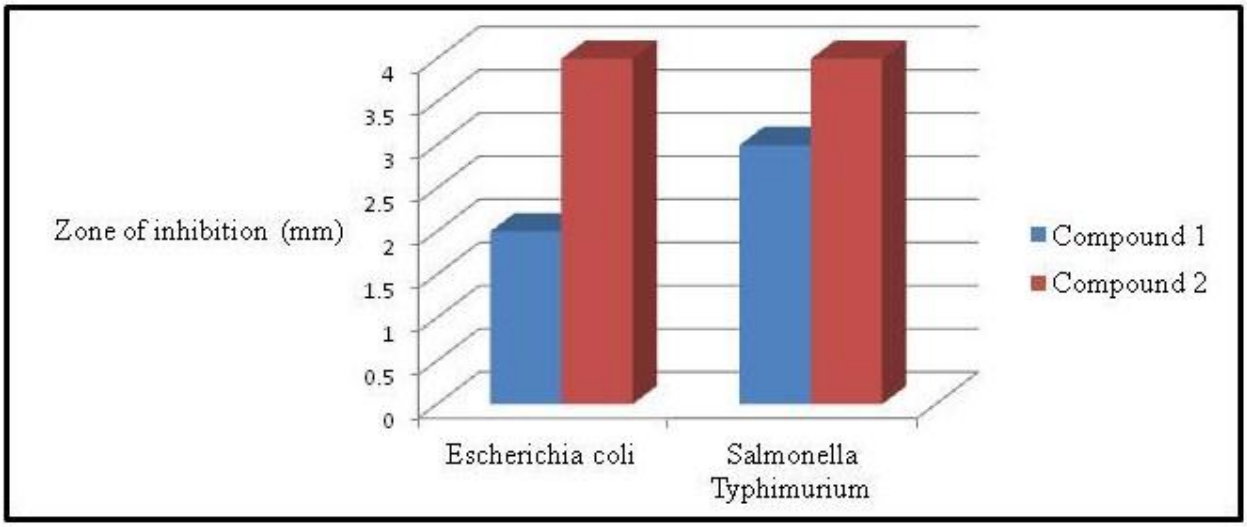

Figure 1: Comparison of antibacterial activities of compound-1 and 2.

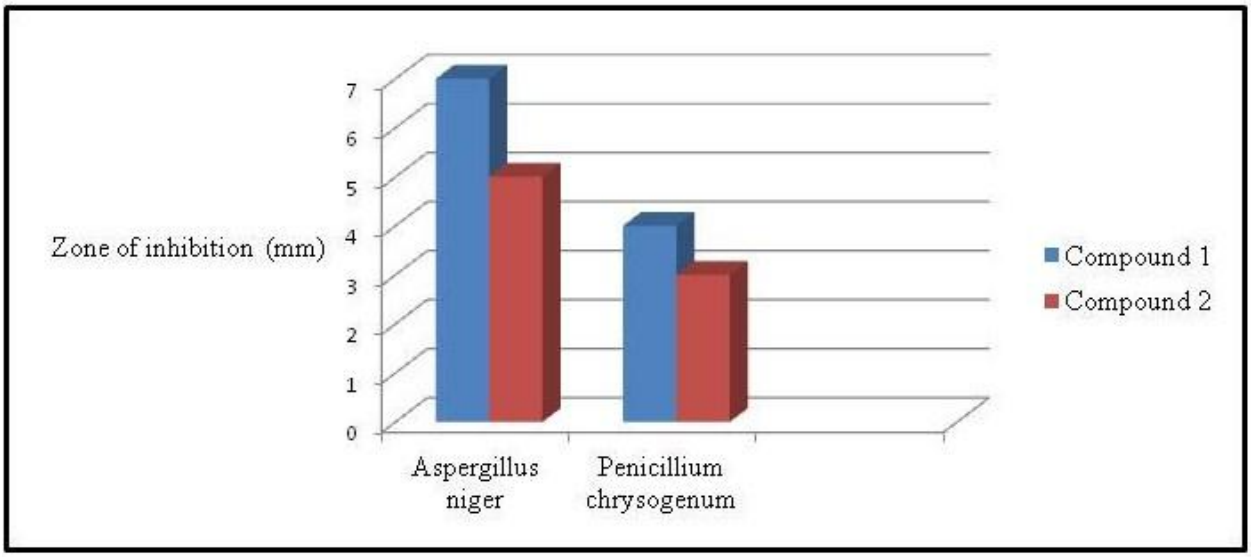

Figure 2: Comparison of antifungal activities of compound-1 and 2.

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CONCLUSION

Herein we have reported an eco-friendly, efficient and green methodology for the synthesis of a series of DHPM derivatives containing both electron rich as well as electron deficient aromatic ring, at room temperature in lime juice medium. It was observed that biginelli reaction with electron rich aromatic aldehyde requires longer time for completion than with electron deficient aromatic aldehyde at room temperature in lime juice medium.

After *in vitro* study of their antimicrobial properties against four selective pathogenic microorganisms namely Escherichia coli, Salmonella typhimurium, Aspergillus niger and Penicillium chrysogenum, it was concluded that DHPM containing electron rich phenyl ring is more effective antibacterial agent whereas DHPM containing neutral phenyl ring is more efficient antifungal agent. DHPM which contains electron deficient phenyl ring shows neither antibacterial nor antifungal properties against these four selective microorganisms. So our work has given an insight into "the effect of electronic factor of the phenyl ring of DHPM on their antimicrobial properties" which is first-of-its kind study. However the scope of this conclusion is limited to only against these selective microorganisms under specific experimental condition as mentioned earlier.

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