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Preparation, Characterization and Biological Activity of New Derivatives 1,3- Diazines

Auhood kadhim zaid*

***Department of Chemistry, College of Science, University of Thi-Qar, Iraq.**

***corresponding Author Email: ohoud.k_mschem@sci.utq.edu.iq**
auhoodkadhim2019@gmail.com

Abstract

1,3-diazines and its derivatives , an important class of heterocyclic compounds, are of both biological and chemical interest, This study is concerned with the synthesis and characterization of 3,4-dihydropyrimidin-2-ones was prepared using three-component condensation reaction of an aromatic aldehyde, urea, and ester and identified these compounds using techniques IR, 1HNMR.All the prepared compounds showed various biological activities toward two kinds of bacteria gram negative (*E coli*) and gram positive bacteria(*staphylococcus aureus).*

Key words: Heterocyclic compounds, diazines, Biginelli reaction, IR, H-NMR.

Introduction

Heterocycles represent the largest diversity of organic compounds with signifcant chemical, biomedical, and industrial applications. They exist in numerous natural products, dyes, and as scaffolds in diverse drugs and related pharmaceutically active substances. 3,4-Dihydropyrimidin-2-(1H)-ones (DHPMs) are considered as effective compounds,play sessential role in several biological processes and have very important chemical and pharmacological properties such as potent calcium channel blockers, antihypertensive, antiviral, antiproliferative effect, stand- ing against cancer cell lines, and potent HIV gp-120-CD4 inhibitors [1-5]. coronaryheart disease [6] , and diabetic retinotherapy [7].

Substantial attention has been paid to develop various elegant methods to synthesize heterocycles.One such MCR that belongs in the latter category is the venerable Biginelli dihydropyrimidine synthesis. In1893, one pot cyclocondensation of aldehyde, 1,3-ketoester and urea or thiourea, is inarguably one of the most useful MCRs [8].

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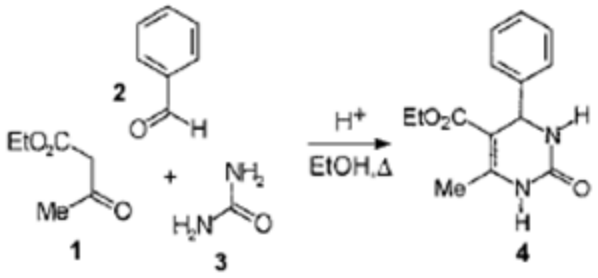

Scheme(1): Biginelli Dihydropyrimidine synthesis

interest slowly increased, and the scope of the original cyclocondensation reaction shown in Scheme 1 was gradually extended by variation of all three building blocks (Figure 1), allowing access to a large number of multifunctionalized dihydropyrimidines [9] .

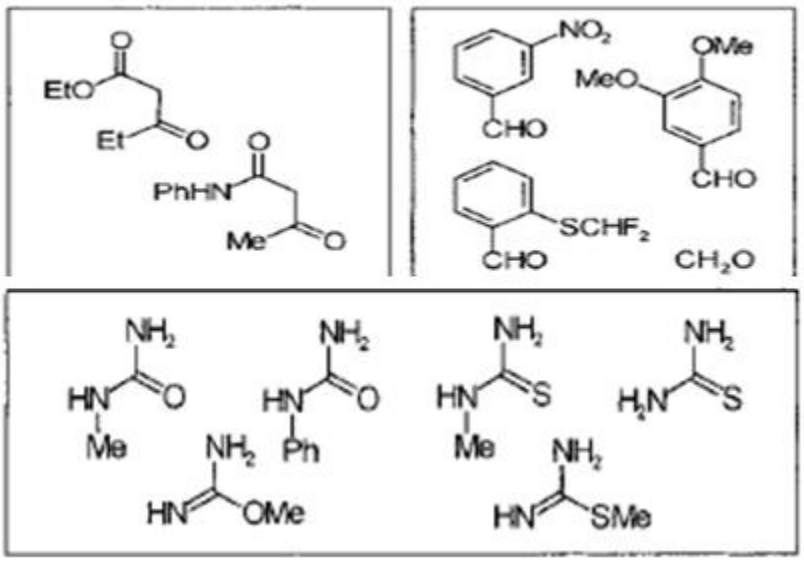

Figure (1):Building blocks in Biginelli MCRS.

Experimental Synthesis

General Method for the Synthesis of 3,4-dihydropyrimidin-2-ones(a-d). A mixture of aromatic aldehyde (0.01 mol), methyl-2-cyanoacetate or methyl-2-chloro (0.01 mol) and urea/thiourea (0.015 mol) were stirred overnight in absolute alcohol (20.00 ml) using HCl as catalyst (Scheme 2). The reaction mixture was cooled by adding ice water; the formed precipitate was filtered off, washed with water, and crystallized from ethanol to obtain the pure product [10,11]. Table (1). The FT-IR spectra were recorded using Shimadzu FT-IR affinity spectrophotometer . 1H NMR spectra were recorded on Bruker DRX. system (500 MHz) using

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DMSO-d6 and chloroform as solvents and TMS as an internal standard. All solvents and reagents were purchased from Sigma-Aldrich Chemicals Pvt. Ltd.

Scheme (2):General reaction of synthesis of compounds

1- Synthesis(6S,6´S)-6,6-(1,4-phenylene)bis(5-methoxy-2-oxo-1,2,3,6-tetrahydropyrimidine-4-carbonitrile) of :terephthaldehyde (0.67g,0.005mol), methyl-2-cyanoacetate (0.88ml ,0.01mol),urea (0.9g ,0.015mol).
2- Synthesis of (6S,6'S)-6,6'-(1,3-phenylene)bis(5-methoxy-2-oxo-1,2,3,6-tetrahydropyrimidine-4-carbonitrile): isophthaldehyde (0.67g,0.005mol), methyl-2-cyanoacetate (0.88ml ,0.01mol),urea (0.9g ,0.015mol).
3- Synthesis of (4S,4'S)-4,4'-(1,3-phenylene)bis(6-chloro-5-methoxy-3,4-dihydropyrimidin-2(1H)-one): isophthaldehyde (0.67g,0.005mol), methyl-2-chloroacetate(1.085ml ,0.01mol),urea (0.9g ,0.015mol).

Biological Activities [12]

The synthesized compounds were studied as antibacterial by the agar cup plate method. The organisms used were staphylococcus aureus as gram positive bacteria and Escherichia coli as gram negative bacteria and dimethyl sulphoxide was used as control. Using antiseptic cork borer cups were scooped out of agar medium contained in a Petri dish which was once upon a time injected with the bacteria. The test compound solution (0.1 mL) was added in the cups and the Petri dishes were after that incubated at 37^0C for 48 hrs. Table (4) mentioned the results of biological activities of the synthesized compounds.

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Cytotoxicity testing[13]

Different amounts of solution DHPMs , (0.1, 0.3, 0.5) mg/ml were prepared. serial dilutions of the compounds were made in phosphatebuffered saline. A total volume of 0.8 ml for each dilution was placed in an Eppendorf tube. A negative control tube (containing saline only) and a positive control tube (containing tap water) were also included in the analysis. human erythrocytes were added to each tube, to give a final volume of 1 ml. Solutions were incubated at 37°C for 30 min. The tubes were then examined for red blood cell decomposition, the experiment was repeated twice.

Results and Discussion

Biginelli reaction used for synthesis 3,4-Dihydropyrimidin-2(1H)-ones/thios can be prepared by one-pot condensation of an aldehyde, b-ketoester and urea/thiourea under strongly acidic conditions. The multicomponent Biginelli reaction can proceed in the presence of FeCl3.6H2O .

Table (1). Compounds of DHPM (a-d)

No.	Comp.	R1	R2	x	
1	a	CN		O	
2	b	CN		O	
3	c	Cl		O	

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the mechanism this reaction was reinvestigated by Kappe[14]. He proposed and established that the first step in this reaction, the acid catalyzed formation of acyl imine intermediate 4 formed by reaction of the aldehyde 1with urea 2. Interception of the iminium ion by β-ketoester 3 produces an open-chain ureide 6 which subsequently cyclizes to the dihydropyrimidinone

Scheme(3):Mechanism of the Biginelli reaction

FT-IR spectrum data of 3,4-Dihydropyrimidin-2(1H)-ones/thiones, different functional groups present in the molecule were identified by distinguishing frequency obtained by their functional groups. Presence of two carbonyl groups is confirmed by IR spectra as two different carbonyl stretching frequencies were observed. Cyclic C=O amide group peak was observed between 1643-1693 cm-1 while another C=O group was observed between 1674-1749 cm-1. Two N-H groups gave peaks between 3163-3410 cm-1. the aliphatic (C-H) and aromatic (C-H) appeared as per their characteristics between 2900-2985 cm-1 and 3062-3120 respectively. The FT-IR spectra of DHPMs (a-d) are included in Table(2).

Table (2): FT-IR spectra of Dihydropyrimidine-2(1H)-ones/thiones

Comp.	*Aliphatic C-H cm-1*	*Aromatic C-H cm-1*	*N1-H, N2-H cm-1*	*C=O cm-1*	*C=O Amide cm-1*
a	2985	3039	3200,3317	1666	1604
b	2962	3010	3226,3373	1717	1671
c	2993	3047	3101,3250	1728	1674

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^{1}H-NMR spectra of 3,4-dihydropyrimidin-2(1H)-ones/thiones, Numbers of proton identified from NMR spectrum and their chemical shift (δ ppm) were in agreement of structure of molecule. methyl group (-CH3) protons gave a singlet peak in the range of 1.24-1.67 ppm . The proton on C4 carbon atom gave a singlet and doublet in range of 4.38-5.28 δ ppm. Aromatic protons were observed between 6.57-8.08 δ ppm. Singlet observed for both cyclic (–NH) were gave at 8.22-9.94 δ ppm.The ^{1}H-NMR spectra of DHPMs(a-d) are included in Table(3).

Table(3): 1H-NMR spectra of Dihydropyrimidine-2-(1H)-ones/thiones

Comp.	*-CH3- aliphatic ppm*	*C4-H ring ppm*	*Aromatic protons ppm*	*-N1H-CO-N2H Or -N1H-CS-N2Hppm*
a	1.4	4.40	8.06-8.08	8.22, 8.24
b	1.35	4.38	7.90-8.2	8.2, 8.47
c	1.24	5.28	7.51-7.4	8.42

Biological Activity [12]

the prepared compounds(1,2 and 3) showed various biological activities toward two kinds of bacteria gram negative (*E .coli*) and gram positive bacteria(*staphylococcus aureus*). The finding observed that compounds (1 and 2) are active against *staphylococcus aureus* while compound (3) showed moderately active against this bacteria. Compounds (1and 2) showed moderate activity against *E coli* . All these results are shown in the table below.

Table (4):Biological activites for Dihydropyrimidine-2-(1H)-ones/thiones

NO.	**Comp.**	**Gram positive bacteria *staph.aureas***	**Gram negative bacteria *E.coli***
1	a	19	12
2	b	18	13
3	c	12	14

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Cytotoxicity testing[13]

Red blood cells have been used to detect the toxicity of prepared compounds because this method is inexpensive, easy to apply and quick results. This test is the first step to determine whether to continue or stop working. Red blood cell decomposition depends on the concentration of the material, incubation period and temperature.The results of cytotoxicity of the prepared compoundsin the direction of human red blood cells showed do not carry any toxicity at the concentrations (0.1, 0.3, 0.5) mg/ml.

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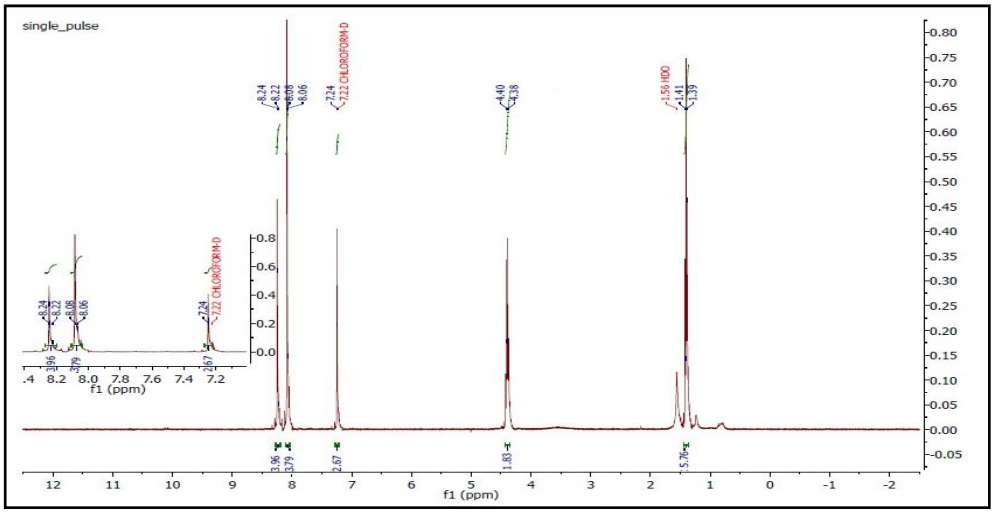
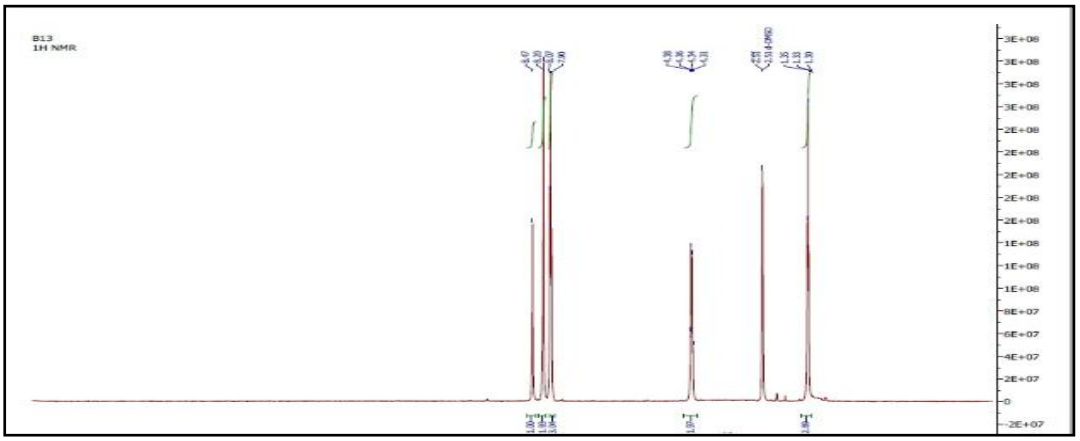


Figure(1):1H-NMR spectrum of (6S,6´S)-6,6-(1,4-phenylene)bis(5-methoxy-2-oxo-1,2,3,6-tetrahydropyrimidine-4-carbonitrile)



Figure(2):1H-NMR spectrum of (6S,6'S)-6,6'-(1,3-phenylene)bis(5-methoxy-2-oxo-1,2,3,6-tetrahydropyrimidine-4-carbonitrile)

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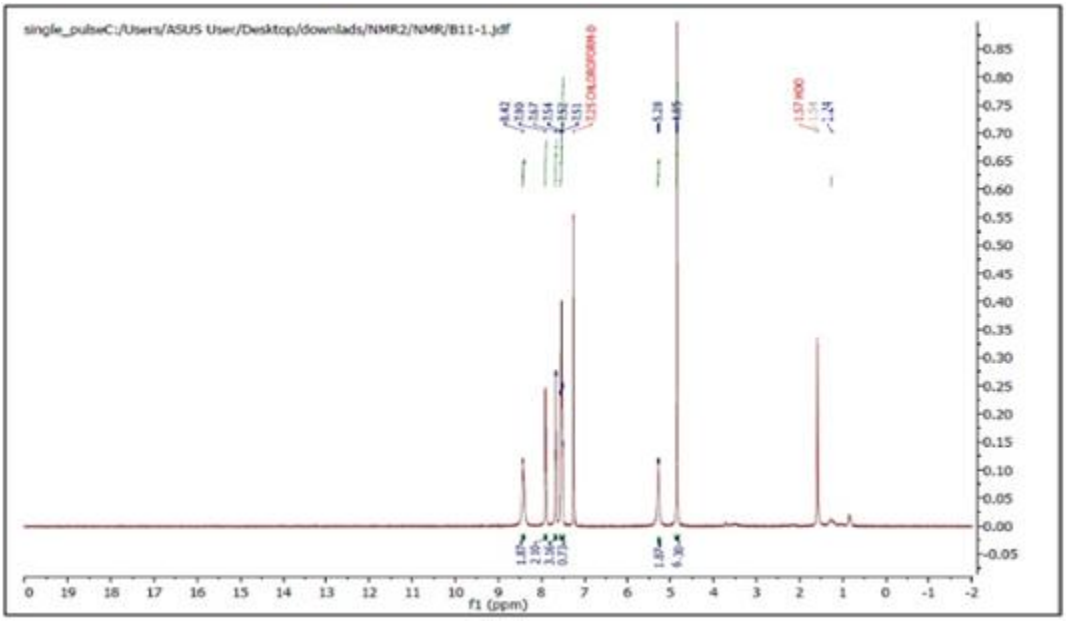
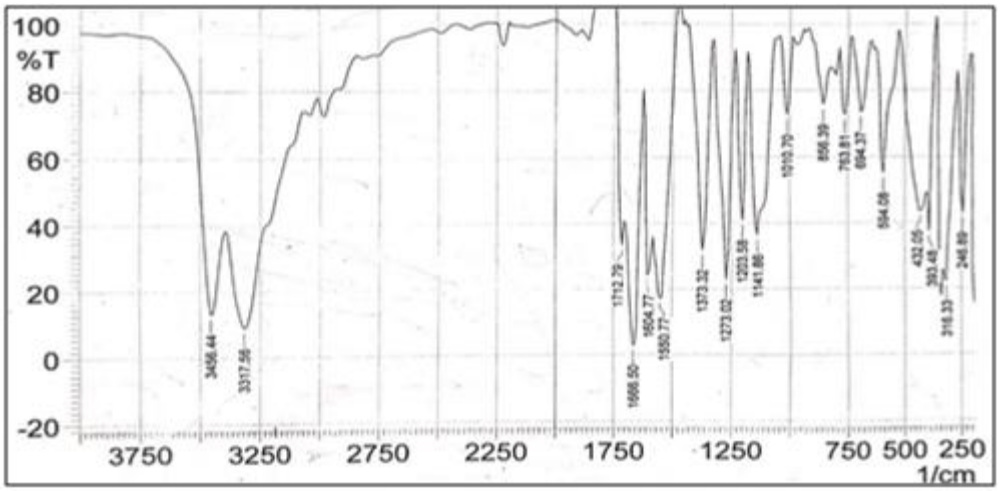


Figure(3):1H-NMR spectrum of (4S,4'S)-4,4'-(1,3-phenylene)bis(6-chloro-5-methoxy-3,4-dihydropyrimidin-2(1H)-one)



Figure(4):FT-IR spectrum of (6S,6´S)-6,6-(1,4-phenylene)bis(5-methoxy-2-oxo-1,2,3,6-tetrahydropyrimidine-4-carbonitrile)

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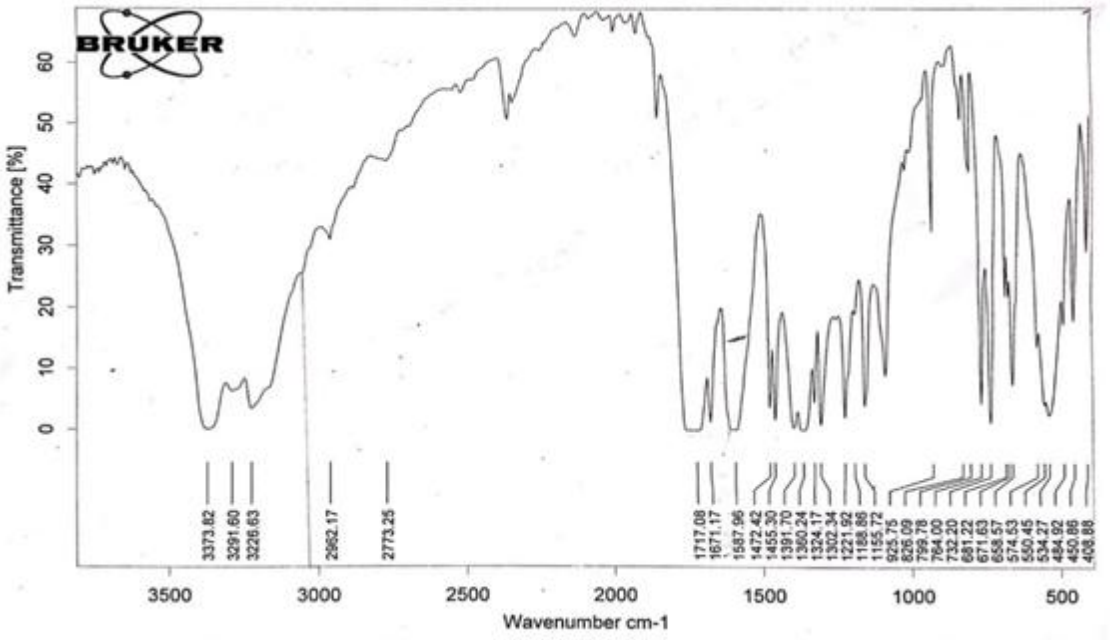
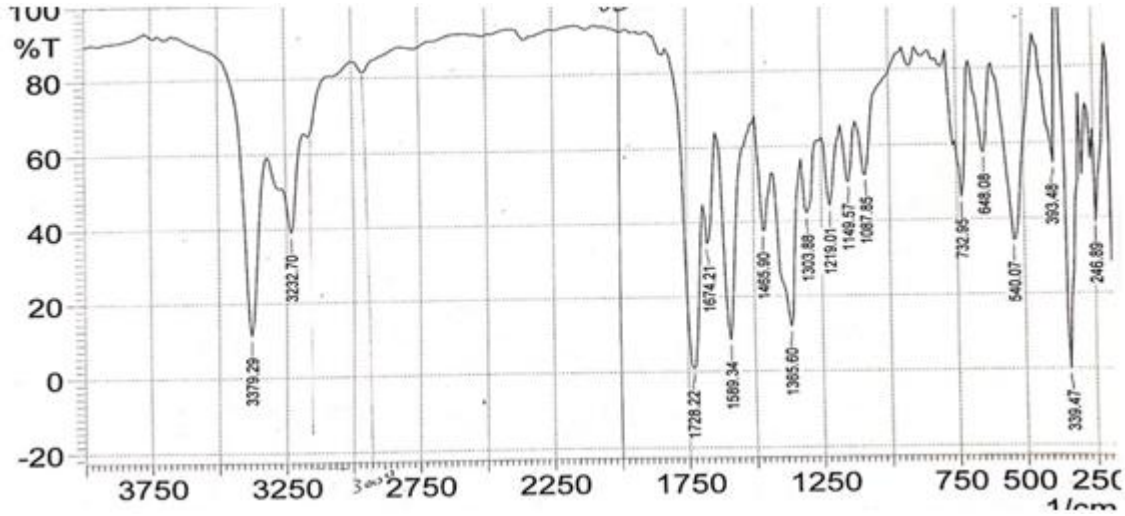


Figure(5):FT-IR spectrum of (6S,6'S)-6,6'-(1,3-phenylene)bis(5-methoxy-2-oxo-1,2,3,6-tetrahydropyrimidine-4-carbonitrile)



Figure(7):FT-IR spectrum of (4S,4'S)-4,4'-(1,3-phenylene)bis(6-chloro-5-methoxy-3,4-dihydropyrimidin-2(1H)-one)