SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME PYRIMIDINE DERIVATIVES

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A B S T R A C T

Synthesis of 4-substituted -6-methyl -2-oxo-N- (4- (3-oxobutanamido) phenyl) -1,2,3,4-tetrahydropyrimidine-5-carboxamide4(a-d) from 1,4-Bis(acetoacetylamino)benzene 4(a-e) have been achieved using both conventional as well as through green chemistry method. Compounds have been characterized using diverse spectroscopic tools such as FTIR, ^{1}H & ^{13}C NMR and Mass spectrometry. All the compounds gave adequate elemental analysis. Furthermore, these compounds shows moderate to good antibacterial properties when evaluated for invitro antibacterial properties against certain gram negative and gram positive species. High percentage yield, less reaction time, solvent free condition and compounds with potential antibacterial properties at a low concentration of 10 µl are the major features of the current research work.

Keywords: Pyrimidine Derivatives; Gram Negative; Biological Properties; Antibacterial; FTIR; Green Chemistry

1. Introduction

During recent decades pyrimidines derivative have drawn the major attention especially, dihydropyrimidinones (DHPMs) and their derivatives , which displayed a captivating assortment in natural, synthetic, pharmacological, therapeutic and bioorganic chemistry mainly due to their wide range of biological activities (Kappe, C. , 2000; Canto S., Bernardi A., Battastini O., Russowsky, D. 2011; Zhang Y., Wang B., Zhang X.,Huang J., Liu C., 2015). They are found as core units in many marine alkaloids (Batzelladine, Crambine), which have been found to be potent HIV, gp-120-CD4 inhibitors (Tajbakhsh M., Mohajerani B., Heravi M., Ahmadi A., 2005).. In the past few decades, interest in this reaction has increased dramatically since DHPMs and their derivatives were found to exhibit antibacterial l and antifungal, antiviral activities as well as anti-inflammatory, and antioxidative properties(Karde T., Rao S., Bhatewara A., Paliwal P., Jain S.,2012). In addition, being potent calcium channels modulators, they display coronary dilation and anti-hypertensive effects. These compounds are medicinally important as calcium channel blockers, α1a-antagonists, and neuropeptide Y (NPY) antagonists (Zhang Y., Wang B., Zhang X.,Huang J., Liu C.,2015). The original one pot synthesis of 3,4-dihydropyrimidine-2-(1H)-ones was firstly reported by Pietro Biginelli in 1893 performing the three component cyclocondensation reaction of ethyl acetoacetate, benzaldehyde and urea under Bronsted acid catalysis Sonawane R., 2014). The scope of Biginelli reaction was explored by varying all components, as well as the catalysts. In recent years, several protocols for preparing of dihydropyrimidinones have been reported using Lewis acids as well as protic acids as catalysts, such as concentrated HCI, $BF_3.OEt_2$, $LaCl_3$, $InCl_3$, H_2SO_4, $Mn(OAC)_3$, $BiCl_3$, $LiClO_4$, $FeCl_3$, $ZrCl_4$, $Bi(OTf)_3$, dry acetic acid under microwave irradiation, and so forth (KappeC.,2000; Heravi M., Bakhtiari K., BamoharramF., 2006; Brindaban R., Alakananda H., Dey S., 2002; Al-Bogami A., Saleh T., Moussa T.,2018; Guonan C., Jing J., Hualong C., Ran C., Xiaoguang C., Baling X., 2018; Mughal E.,Sadiq A., Hamayun M., Zafar M., Nighat F., Yameen A., Syed M., Amara M.,

Ahmed I., Fatima T.,2018). Most of these catalysts are harmful, toxic, dangerous as chemicals, and dangerous for the environment, too. Therefore, attempts were made for the development of new methods using less harmful catalysts.
derivatives extensively to evaluate their various pharmacological activities.

In view of the above findings some new pyrimidine derivatives has been synthesized under microwave irradiation and characterize them on the basis of various physiochemical and spectral analysis. To enhance the percentage yield environmental friendly procedure has been adapted for the synthesis of DHPMs. The use of microwave has enhanced the percentage yield as well as reaction time has been reduced from hours to minutes. The synthesized compounds have been screened for the antimicrobial studies and later by molecular docking we have tried to investigate the details of biological properties.. All the synthesized compounds were characterized using FTIR, ESI-MS, ^{1}H and ^{13}C NMR and they were also screened for the invitro antibacterial properties and were found to be of possess potential pharmaceutical interest.

2. Experimental

The progress of reaction was monitored by thin layer chromatography (TLC) using Silica Coated Aluminium TLC plates (TLC Silica Gel 60F254, Merck, Germany) by different eluent systems. The spots were visualized by keeping the dry plates in iodine vapors and in UV light. IR spectra were recorded on Perkin-Elmer Spectrometer (RX-IFTIR) scanned in KBr discs and wave numbers were expressed in cm-1.^{1}H NMR spectra were recorded in DMSO-d6 on a Bruker Avance II 400 MHz NMR spectrometer and chemical shift (δ) values are given in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard. ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avance II 100 MHz NMR spectrometer and chemical shift (δ) values are given in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on JMS-T100 LC and Expression CMS (ESI) mass spectrometer. Elemental analyses were carried out with Elementar Vario EL III elemental analyzer. All the melting points were determined by open capillary method and are uncorrected. Microwave reactions were performed at 300 W in the CEM Focused Microwave™ Synthesis System.

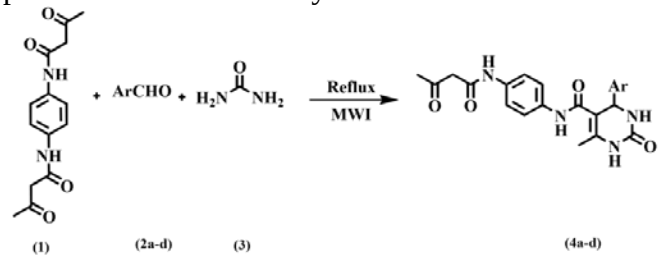
General Procedure:

2.1 Synthesis of 4-substituted-6-methyl-2-oxo-N-(4-(3-oxobutanamido)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide4(a-d) from 1,4-Bis(acetoacetylamino)benzenehas been done in two different parts(1):

Part A: Conventional Method (Reflux):- A mixture of 1,4-Bis(acetoacetylamino)benzene (1.35g; 5 mmol), urea (2.5g;5 mmol), p-Chlorobenzaldehyde (1 g; 1 mmol)in the presence of 3 drops conc.HCl using ethanol(20 mL)as solvent was refluxed for 5 h. The progress of reaction was monitored by TLC on silica gel-G using suitable solvent system. The reaction mixture was then poured into crushed ice and the solid product separated was filtered and residue was washed with hot water. Obtained product was further recrystallized in ethanol.

Part B: Microwave irradiation (MWI):

A mixture of 1,4-Bis(acetoacetylamino)benzene (0.27g;1mmol), urea (0.06g;1mmol), p-Chlorobenzaldehyde (1 g; 1 mmol) in the presence of 1 drops of conc. HCl using minimum amount of ethanol(to make slurry) was irradiated at 300 W at 130 ℃for 40 min through intermittent cooling. The progress of the reaction was monitored by TLCon silica gel-G using suitable solvent system. The reaction mixture was then poured into crushed ice and the solid product was filtered and residue was washed with hot water. Obtained product was further recrystallized in ethanol.



Compound	Ar
4a	- 4-MeO-C_6H_5
4b	- C_6H_5
4c	- 4-NO_2-C_6H_5
4d	- 4-Cl -C_6H_5

Scheme-1

3. Results and Discussion

Physicochemical analysis of the synthesized compounds **Table 1** indicates that the MWI (microwave irradiation) method has higher

yield and shorter reaction time as compare to conventional method. The simple experiment procedures, no use of any toxic chemicals and high percentage yield make the current method highly beneficial and environment friendly. Further, FTIR spectral data of 4-(4-chlorophenyl)-6-methyl-2-oxo-N-(4-(3-oxobutanamido)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide **4d** clearly show the presence of secondary amine with single peak around 3250, 3165.25 cm^{-1}. Similarly other peaks of ketonic observed at 1676.14(esters), 1564.24(amide) cm^{-1}. Peak observed at 806.25 cm^{-1}.gives the presence of para substitution in aromatic ring. Mass spectrum of 4-(4-chlorophenyl)-6-methyl-2-oxo-N-(4-(3-oxobutanamido)phenyl)-1,2,3,4 tetrahydropyrimidine-5-carboxamide **4d** give us two prominent peaks of M+1(87%) and M+2(28%) gives the credence to the structure along with existence of chlorine in the ratio of nearly 3:1. Some other peaks like 354.3(C18H18N4O4), 273.1(C14H15N3O3), 167.5(C8H6ClNO) and 101.8(C6H6Cl) has been obtained which give us information about the complete formation of the desired compound. The ^{1}H NMR spectrum recorded in DMSO-d6 showed a singlet at δ1.11, 2.09 and 2.26 ppm confirming the presence of $–CH_3$, $–CH_3$ (pyrimidine) and –CH2 groups. Also prominent peaks of –NH were observed at δ 5.15 and 6.5 ppm. Aromatic Hydrogen was observed between δ 7.2- 7.3 ppm. Similarly in ^{13}CNMR aromatic carbons were observed between δ 128.15- 128.35 and δ 143.73-148.66 ppm. Peaks observed at 17.76, 50.6 and 14.5 ppm were assigned for $–CH_3$, active methylation. All the physicochemical data has been reported in **Table No1**.

Table No1: Physicochemical Analysis of 4-substituted-6-methyl-2-oxo-N-(4-(3-oxobutanamido) phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide4(a-d):

Product	Ar	Reaction Time (hr)		Yield (%)		Melting point (℃)
		Reflux(h)	MWI(Min)	Reflux	MWI	
4a	4-MeO-C_6H_5	4	40	65	85	163-165
4b	-C_6H_5	5	50	50	90	123-125
4c	4-NO_2-C_6H_5	6	35	65	95	190-192
4d	4-Cl-C_6H_5	5	40	59	90	148-150

4. Biological Activities

4.1 InvitroAntimicrobial Study

The microbial strains are identified strains and were obtained from the microbiology laboratory. The studied bacterial strains were S. aureus, B. Subtilis (gram positive) and E. coli, K. aerogenes (gram negative). They were sub-cultured on nutrient agar after every 15 days and maintained on nutrient agar slants at 4 ^{o}C. MH agar (Mueller-Hinton Agar) was selected as a microbiological growth medium that is commonly used for antibiotic susceptibility testing was procured from Hi-media Mumbai, India. The media was prepared according to the instructions given by the manufacturer. The screening for the antimicrobial activity of different synthesized heterocyclic compounds were evaluated by agar well diffusion method using MH agar and zone of inhibition was measured in mm. Amoxicillin was used as standard drug and DMSO as control solvent. The compounds were tested at 1mg/ml concentration. A loopful of culture was inoculated from the stock slant culture in 5 ml of MH broth and the broth was incubated at 35 ^{o}C in incubator for 6-8 hr. After incubation, this culture was used for the inoculation of MH test agar plates. MH test agar medium was prepared as per instructions of manufacturer. Required amount of agar medium was melted and 25 ml of molten medium was distributed in test tubes. Medium was autoclaved at 15 lb for 20 min. After autoclaving, medium was maintained at 45-50 ^{o}C in constant temperature water bath. Inoculation has been done using 0.5 ml of 6-8 h old test organism is transferred to petridish of 100 mm size (Sterilized in oven at 180 ^{o}C for 1 h) using sterile micropipette. MH test agar medium maintained at 45-50 ^{o}C was poured and mixed properly to ensure uniform

distribution of organisms with medium. Seeded plates are allowed to set at room temperature. Using 10 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made. A 10 µl of the test sample was transferred by micropipette per well. Plates were immediately kept at 4 ^{o}C in refrigerator for 1hr for diffusion of the samples and then shifted to 35 ^{o}C in incubator. Zone of inhibition was measured by zone scale after 24 h of incubation. Ciprofloxacin has been used as standard drug and DMSO was used as control.

Table-3: Invitro Antibacterial activity of 4-substituted-6-methyl-2-oxo-N-(4-(3-oxo-butanamidophenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide 4(a-d):

Compound	Ar	S.aureus	B.subtilis	E.coli	K.aerogenes
4(a)	4-MeO-C_6H_5	18	20	18	19
4(b)	-C_6H_5	15	08	12	10
4(c)	4-NO_2-C_6H_5	20	20	18	10
4(d)	4-Cl-C_6H_5	20	20	20	18
Ciprofloxacin	-	22	22	20	22
DMSO	-	00	00	00	00

4.2 Molecular Docking Study

Molecular docking studies have been done using the Auto Dock Tools (ADT) version 1.5.6 and Auto Dock version 4.2.5.1 docking program. The crystal structure of CDK2 with 2-aminopyrimidine as ligand was obtained from the Protein Data Bank Structure (PDB ID: 3S2P) as structure checked for missing atoms, bonds, contacts and the ligand was removed. Then, the polar hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT. Further, ADT was used to remove crystal water, Gasteiger charges were added to each atom, and merged the non-polar hydrogen atoms to the protein structure. The structures were then saved in PDB file format, for further studies in ADT. Ligand 2D structures were drawn using Chemdraw Ultra 8.0.6. Chem3D Pro 8.0.3 was used to convert 2D structure into 3D and the energy minimized using semi empirical AM1 method. Minimize energy to minimum RMS gradient of 0.100 was set in each iteration. All structures were saved as .PDB file format for input to ADT. Further all these ligand structures were then saved in PDBQT file format in ADT, to carry out docking. A grid box with dimension of 74 × 76 × 78 with 1.0 Å spacing and centered on 24.559, 24.347, and 14.739 was created around the binding site of the ligand on Transferase inhibitor (CDK2)-3S2P using ADT. The centre of the box was set at ligand centre and grid energy calculations were carried out. The docking results of the synthesized compounds have been reported in **Table 3** and **Fig.1**. Docking of compound of **4c** and **4d** into CDK active site revealed that several molecular interactions (hydrogen bond, π-π interaction) were considered to be responsible for the observed antibacterial properties of the compounds. The docking results revealed that compound **4c** and **4d** exhibited better binding interaction with binding energy of -4.371 kcal/mol, -4.77 kcal/mol with docking energy of -15.39 kcal/mol and -10.09 kcal/mol respectively which reveals that these compounds interacts better with the enzyme.

Table 3. Molecular Docking of Studies of Synthesized Compounds 4(a-d)

Compound	Docking Energy(kcalmol^{-1})	Free energy of Binding (kcalmol^{-1})
4a	-8.67	-4.76
4b	-9.10	-5.42
4c	-9.59	-5.49
4d	-9.73	-5.93

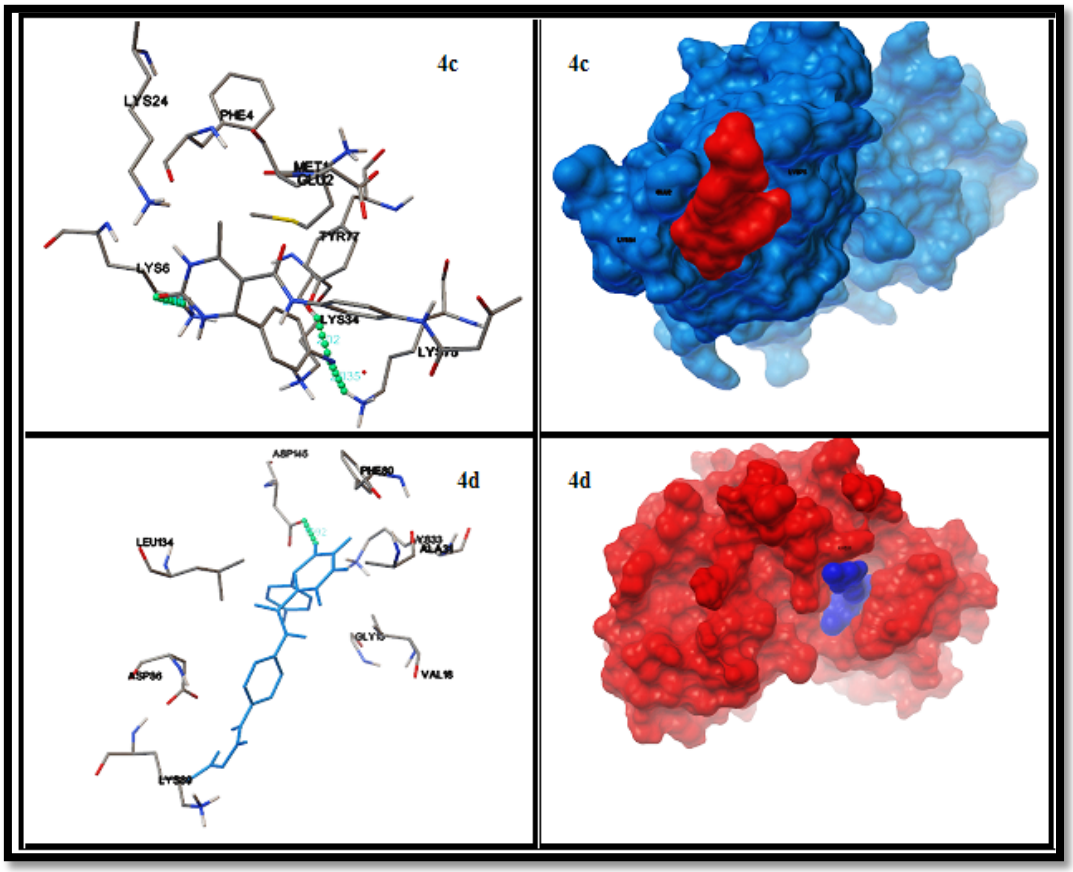


Figure 1 Hydrogen bonding and Molecular Surface diagram of compound 4(c) and 4(d)

Conclusion

In this work we have successfully synthesized some new pyrimidine derivatives using conventional as well as environment friendly method (MWI). Use of MWI for the synthesis of DHPMs gives us high yields in relatively shorter reaction time. Also the simple experiment procedures without use of any toxic chemicals make the current work to be fully under the agreement with green chemistry protocol. Furthermore, synthesized compounds shows moderate to good antibacterial activities. Molecular Docking give us insight view and logical explanations about various interaction such hydrogen bonding, docking energy and binding energy.

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