INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL EVALUATION OF 4-(SUBSTITUTED PHENYL)-N-(SUBSTITUTED PHENYL)-6-METHYL-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOHYDRAZIDE DERIVATIVES

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Article Received on: 13/09/17 Approved for publication: 27/10/17

DOI: 10.7897/2230-8407.0810197

ABSTRACT

Dihydropyrimidinones are therapeutically important class of compounds. The reported work reveals the synthesis of some novel substituted dihydropyrimidinones and their evaluation for in vitro antibacterial activity and in vitro antifungal activity. Dihydropyrimidinones were synthesized through Biginelli reaction and tested against gram positive bacteria such as Staphylococcus aureus, Bacillus subtilis and gram negative bacteria such as Escherichia coli and Salmonella paratyphi. All the compounds were found to exhibit a moderate antibacterial activity and antifungal activity against the tested microorganisms. Compound DC-1 and DC-3 exhibited significant activity against S.paratyphi and B.subtilis respectively. Compound DC-4 exhibited stronger inhibition against A.niger.

Key words: Dihydropyrimidinones, Biginelli reaction, Antibacterial activity, Antifungal activity

INTRODUCTION

In medicinal chemistry pyrimidine derivatives have been very well known for their therapeutic applications. The presence of a pyrimidine base in thymine, cytosine and uracil, which are the essential building blocks of nucleic acids, DNA and RNA is one of the possible reasons for their activities. Vitamins are essential for body. Pyrimidine ring is found in vitamins like riboflavin, thiamine and folic acid. Pyrimidine nucleus is also present in barbituric acid and its several derivatives which are used as hypnotics. As pyrimidine is a basic nucleus in DNA & RNA, it has been found to be associated with diverse biological activities. Pyrimidines and their derivatives are considered to be important for drugs and agricultural chemicals. Pyrimidine derivatives possess several interesting biological activities such as antimicrobial, antitumour and antifungal activities[1-7]. Many pyrimidine derivatives are used for thyroid drugs and leukemia. The biological significance of the pyrimidine derivatives has promoted us to synthesize some new substituted dihydropyrimidinones and evaluate them for their antimicrobial activity.

MATERIALS AND METHODS

All the chemicals and solvents were of analytical grade and used without further purification. Melting points of the compounds were determined using open end capillaries in an electrical melting point apparatus and are uncorrected. Purity of the compounds were checked by thin layer chromatography using ethyl acteate:toluene as solvent and the spots were visualized using Iodine vapours. IR spectra were recorded on JASCO 4100 FT IR using KBr pellet disc technique. ^{1}H NMR and ^{13}CNMR were recorded on Brucker 500MHz spectrometer using DMSO-d6 as solvent and tetramethylsilane as internal standard. Mass spectra were recorded on MS 2020 mass spectrometer[8-10]. The physiochemical parameters of the titled compounds are shown in table no.1 and the spectral data in table no.2.

GENERAL PROCEDURE FOR THE SYNTHESIS OF THE COMPOUNDS

Synthesis of biginelli compound

A mixture of 0.15mole of urea, 0.1mole of ethylacetoacetate and 0.1mole of benzaldehyde were dissolved in 25ml of ethanol along with 3 drops of conc.hydrochloric acid and refluxed for one and half an hour. The reaction mixture was then poured into 100ml ice cold water with stirring and left overnight at room temperature, filtered and dried. The products were recrystallised using ethanol. Similar procedure was followed for various substituted aromatic aldehydes. The precipitate was then recrystallised from ethanol.

Synthesis of carbohydrazido derivative

A mixture of 0.1mole of biginelli compound and 0.1mole of hydrazine hydrate were dissolved in 20ml of ethanol along with 4 drops of conc.H_2SO_4 and refluxed for 3 hrs. The reaction mixture was then evaporated to obtain a residue which was further recrystallised from ethanol.

Synthesis of Schiff bases of dihydropyrimidinone derivatives

About 0.01mole of hydrazido product and 0.01mole of substituted aromatic aldehydes dissolved in ethanol along with 5ml of glacial acetic acid were refluxed for 4-5 hours. The reaction mixture was then poured into ice cold water in a beaker, filtered and dried. The precipitate was then recrystallised from ethanol.

ANTIMICROBIAL ACTIVITY
In vitro Antibacterial Activity[11]

The synthesized compounds were evaluated for in vitro antifungal activity against gram positive bacteria S.aureus, B.subtilis and gram negative bacteria E.coli, S.paratyphi.

The standardized inoculums were inoculated in the plates prepared earlier (aseptically) by dipping a sterile cotton swab in the inoculums and streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application. Finally the swab was swabbed round the edge of the agar surface. Each Petri dish was divided into parts, in each part samples discs of 100 µg (discs are soaked overnight in sample solution) and standard Ciprofloxacin 10 µg were placed with the help of sterile forceps. The petri dishes were placed in the refrigerator at 4ºC or at room temperature for 1 h for diffusion and incubated at 37ºC for 24 h. The zone of inhibition produced by different samples was measured and tabulated in table 3.

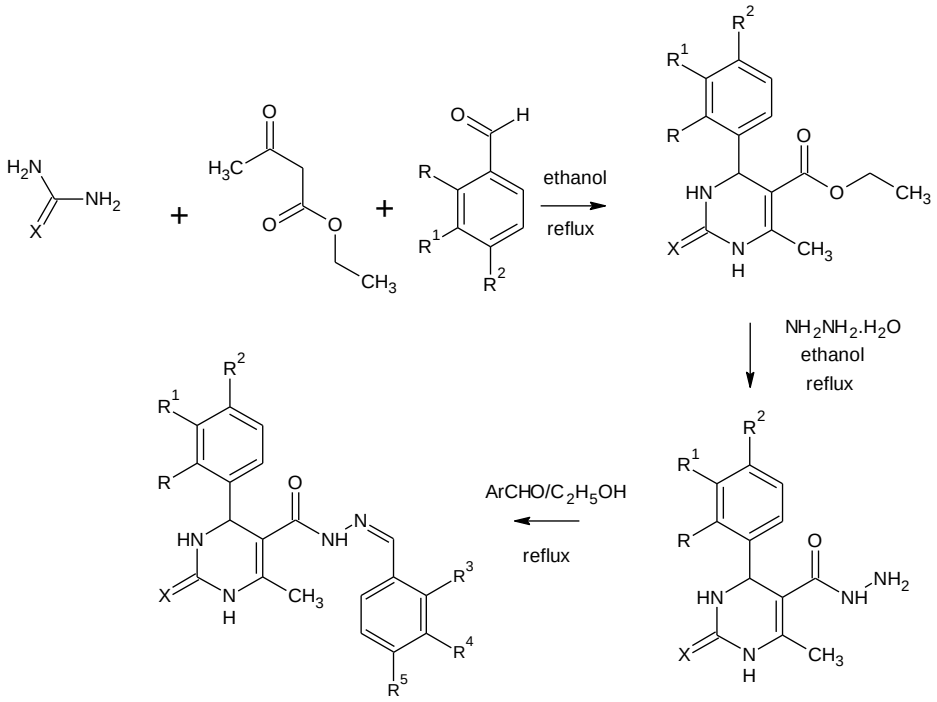
In vitro Antifungal Activity[12]

The synthesized compounds were evaluated for in vitro antifungal activity against Candida albicans and Aspergillus niger.

The standardized inoculums were inoculated in the plates prepared earlier (aseptically) by dipping a sterile cotton swab in the inoculums and streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application. Finally the swab was swabbed round the edge of the agar surface. Each Petri dish was divided into parts, in each part samples discs of 100 µg (discs are soaked overnight in sample solution) and standard Fluconazole 10 µg were placed with the help of sterile forceps. The petri dishes were placed in the refrigerator at 4ºC or at room temperature for 1 h for diffusion and incubated at 28ºC for 48 h. The zone of inhibition produced by different samples was measured and tabulated in table 4.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration was determined for the most active compound against S.paratyphi, B.subtilis and A.niger. The test samples were dissolved in dimethyl sulfoxide and diluted to highest concentration (1000µg/ml) to be tested, and then fold serial dilutions were made in a concentration range from 1000µg/ml to 15.625µg/ml in sterile test tubes containing standardized inoculums. All the tubes were incubated at 37°C for 24 hours (Bacteria) and 25ºC for 24 hours (Fungi). After incubation, minimum inhibitory concentration values were determined. The highest dilution of extract that shows no turbidity was observed and recorded. This dilution was considered to have the concentration of the drug equivalent to MIC. The results are shown in table 5.



Compound code	X	R	R^1	R^2	R^3	R^4	R^5
DC-1	O	Cl	H	H	H	OH	H
DC-2	S	Cl	H	H	OH	H	H
DC-3	S	Cl	H	H	H	OCH_3	OH
DC-4	O	H	Cl	H	H	H	H
DC-5	O	H	Cl	H	OH	H	H

Table 1 Physicochemical parameters of the synthesized compounds

S.No	Compound code	% yield	Melting Point	R_f Value	Solubility
1.	DC-1	52%	152^0C	0.78	Ethanol
2.	DC-2	71%	124^0C	0.82	Ethanol
3.	DC-3	83%	169^0C	0.65	Ethanol
4.	DC-4	69%	115^0C	0.69	Ethanol
5.	DC-5	82%	145^0C	0.61	Ethanol

Table 2 Spectral data of synthesized compounds

Compound code	IR(KBr)cm^{-1}	^{1}H NMR (δppm)	^{13}C NMR (δppm)	m/z
DC-1	3234.40(Ar-H stretching), 2923.88(NH stretching), 1703.03(C=O stretching), 1276.29(C-N stretching), 1644.20 (C=O stretching in amide), 799.44(C-Cl stretching),	10.104 (s, 1H, OH), 9.277 (s, 1H, NH), 8.989 (s, 1H, NH), 7.397 (s, 1H, O=CNH), 1.007 (s, 3H, CH_3), 6.854-7.712 (m, 10H, Ar-H)	160.77 (C-OH), 149.79(C=O), 14.40(CH_3), 158.79(C-CH_3), 165.46(O=C-NH), 132.17(C-Cl), 135.19(C-phenyl ring),	386.81
DC-2	2923.88(OH stretching), 1703.99(C=O stretching), 1226.64(C-N stretching), 769.54(C-Cl stretching)	9.773 (s, 1H, OH), 9.271 (s, 1H, NH), 8.718 (s, 1H, NH), 1.112 (s, 3H, CH_3), 7.249-7.931 (m, 10H, Ar-H)	152.43(C-OH), 161.13(C=O), 14.54(CH_3), 165.67(O=C-NH), 131.37(C-Cl), 191.49(C=S)	400.82
DC-3	2923.88(NH stretching), 1700.13(C=O stretching), 1226.64(C-N stretching), 1090.67(C-O-C stretching), 782.08 (C-Cl stretching), 677.93 (C-S stretching)	10.378 (s, 1H, OH), 9.850 (s, 1H, NH), 9.614 (s, 1H, NH), 8.648 (s, 1H, NH), 8.179(s,1H,O=CNH), 1.028 (s, 3H, CH_3), 7.339-7.631 (m, 9H, Ar-H), 2.509 (s, 3H, OCH_3)	158.75(C-OH), 40.48(O-CH_3), 133.69(C-Cl)	428.90
DC-4	2926.78(NH stretching), 1703.03(C=O stretching), 1226.64(C-N stretching), 769.54 (C-Cl stretching)	9.275 (s, 1H, NH), 8.719 (s, 1H, NH), 1.114 (s, 3H, CH_3), 7.199-7.961 (m, 11H, Ar-H)	149.46(C=O), 14.53(CH_3), 165.65(O=C-NH), 134.21(C-Cl)	366.80
DC-5	2939.31(OH stretching), 1708.81(C=O stretching), 1226.64(C-N stretching), 751.22 (C-Cl stretching), 1644.20 (C=O stretching in amide)	9.996 (s, 1H, OH), 9.768 (s, 1H, NH), 9.277 (s, 1H, NH), 1.107 (s, 3H, CH_3), 6.877-7.847 (m, 10H, Ar-H)	150.89(C=O), 14.54(CH_3), 165.67(O=C-NH), 134.22(C-Cl), 152.41(C-OH)	384.64

Table 3 In vitro antibacterial activity of the synthesized compounds

S.No	Compound Code	Zone of Inhibition in mm			
		E.coli	S.paratyphi	S.aureus	B.subtilis
1.	DC-1	16	21	20	18
2.	DC-2	16	10	12	12
3.	DC-3	25	20	14	22
4.	DC-4	18	17	22	17
5.	DC-5	14	12	26	17
6.	Standard	35	25	36	24

Table 4 In vitro antifungal activity of the synthesized compounds

S.No	Compound Code	Zone of Inhibition in mm	
		C.albicans	A.niger
1.	DC-1	16	14
2.	DC-2	19	8
3.	DC-3	19	16
4.	DC-4	14	18
5.	DC-5	14	12
6.	Standard	35	25

Table 5 MIC of synthesized compounds

Micro organisms	Compound code	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.625 µg/ml	Solvent
S.paratyphi	DC-1	–	–	–	+	+	+	+	+
B.subtilis	DC-3	–	–	–	+	+	+	+	+
A.niger	DC-4	–	–	–	+	+	+	+	+

\- No inhibition
\+ Inhibition

RESULTS AND DISCUSSION

The titled compounds were synthesized in a three step process. Substituted dihydropyrimidines were prepared by the condensation of urea and ethylacetoacetate with different substituted aromatic aldehydes in presence of an acid and ethanol. This method of preparing dihydropyrimidinones is commonly known as Biginelli reaction. It is a functional intermediate to grant medicinally important heterocyclic compounds. Dihydropyrimidinones were further condensed with hydrazine hydrate and finally condensed with various substituted aromatic aldehydes to form the target compounds. The presence of N=CH bond and N=CH proton confirmed the formation of Schiff bases and spectral data were in correlation with the expected structure.

The titled compounds were screened for antibacterial activity against gram positive bacteria Staphylococcus aureus, Bacillus subtilis and gram negative bacteria Escherichia coli and Salmonella paratyphi. The zone of inhibition and MIC were measured and compared against the standard antibiotic, Ciprofloxacin. The target compounds were also screened for antifungal activity against Candida albicans and Aspergillus niger. The zone of inhibition and MIC were measured.

The antibacterial screening of most of the compounds showed a moderate zone of inhibition than standard Ciprofloxacin. Compound DC-1 and DC-3 showed remarkable activity against S.paratyphi and B.subtilis respectively. The antifungal screening revealed a moderate zone of inhibition than the standard antibiotic, Fluconazole. Compound DC-4 exhibited stronger inhibition against A.niger.

SUMMARY AND CONCLUSION

Pyrimidines are therapeutically important class of compounds. The entitled work describes the synthesis of series of substituted dihydropyrimidinone derivatives via Biginelli reaction. The synthesized compounds were found to have a moderate antibacterial activity and a more pronounced antifungal activity. The present work details on the broad spectrum of antibacterial and antifungal activity in comparison with a standard antibiotic. It will be worthwhile to investigate the effect of titled compounds on other biological activities which can broaden the therapeutic utility for the compounds synthesized that will form part of a future study.

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Cite this article as:

Beena K.P *et al*. Synthesis, characterisation and antimicrobial evaluation of 4-(substituted phenyl)-n-(substituted phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide derivatives. Int. Res. J. Pharm. 2017;8(10):142-145 http://dx.doi.org/10.7897/2230-8407.0810197

Source of support: Nil, Conflict of interest: None Declared