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Design, Synthesis, Characterization and Biological Evaluation of Some Substituted Dihydropyrimidinone Derivatives

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ABSTRACT

Pyrimidine nucleus is an important pharmacophore in medicinal chemistry. The synthesis of novel pyrimidine derivatives remains a main focus of modern drug discovery. In the present study, eight hybridized pyrimidine derivatives were synthesized via Biginelli condensation and evaluated for their in-vitro antimicrobial activity. The antimicrobial results revealed that all the compound exhibited promising antibacterial activity and the compounds DP-3, DP-5, DP-7, DP-8 exhibited pronounced antifungal activity displaying minimal inhibitory concentration values of against bacterial strains and fungal strains compared with standard Ciprofloxacin (for bacteria) and Cotrimazole (for fungi).

Keywords: Dihydropyrimidines, Biginelli reaction, Antibacterial activity, Antifungal activity

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INTRODUCTION

An anti-microbial agent is a substance which kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans. Anti-microbial drugs either kill microbes or prevent the growth of the microbes. The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time the reason was one bacterium failed to grow, was that the other bacterium were producing an antibiotic. Of course, in today's common usage, the term antibiotic is used to almost any drug attempts to rid of your body of the bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.

However, with the development of antimicrobials, microorganisms has adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology were based either poisons or heavy metals, which may not have killed the microbes completely, allowing the microbes to survive, change, and become resistant to the poisons and/or heavy metals [1].

Pyrimidine nucleus is an important pharmacophore in medicinal chemistry. The synthesis of novel pyrimidine derivatives remains a main focus of modern drug discovery. The versatility of newer generation pyrimidines would represent a fruitful pharmacophore for further development of better medicinal agents. Since now, researchers have been attracted toward designing more potent pyrimidine derivatives having wide range of biological activity [2].

Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms. Purines, uric acid, alloxan, barbituric acid and a mixture of anti-malarial and antibacterials also contain the pyrimidine ring. In view of our observations and in continuance of our research work [3-9], we hereby report the synthesis of some pyrimidine Schiff bases. This work made us understand the antibacterial and antifungal potency of newly synthesized pyrimidines.

The synthetic pathway for the reported compounds is illustrated in Scheme. The key intermediate compound was biginelli compound, ethyl-

6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate. Biginelli compound [10] was synthesized by multi component reaction of condensation of urea, ethylacetoacetate and aromatic aldehyde in presence of ethanol using conc. hydrochloric acid as a catalyst. Reaction of ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate with hydrazine hydrate afforded 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide derivative. Condensation of the carbohydrazide derivatives with various substituted aromatic aldehydes yielded 6-methyl-2-oxo-4-phenyl-*N*'-[(*Z*)-substituted phenylmethylidene]-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide derivatives, the target compounds. The physicochemical data of the titled compounds are described in Table 1. Structures of the synthesized compounds were established based on the physico chemical and spectral data. (IR, ^{1}HNMR) [11-13]. In conclusion we have synthesized some potent schiff bases of pyrimidine derivatives.

Table 1: Physical data of target compounds

S. No.	Compound Code	Molecular formula	Molecular weight	%yield (w/w)	m.p (°c)
1.	DP-1	$C_{19}H_{18}N_4O_2$	334.87	75.68	215
2.	DP-2	$C_{19}H_{18}N_4O_3$	350.37	98.22	210
3.	DP-3	$C_{20}H_{20}N_4O_4$	380.39	96.14	212
4.	DP-4	$C_{20}H_{20}N_4O_3$	364.39	98.21	208
5.	DP-5	$C_{19}H_{17}ClN_4O_2$	368.81	52.25	210
6.	DP-6	$C_{20}H_{20}N_4O_2$	348.39	60.41	210
7.	DP-7	$C_{21}H_{22}N_4O_4$	394.42	81.17	212
8.	DP-8	$C_{19}H_{16}Cl_2N_4O_2$	403.26	71.77	210

MATERIALS AND METHODS

Appreciable number of five membered and six membered heterocycles containing nitrogen, oxygen and sulphur atoms has turned out to be potential chemotherapeutic and pharmacotherapeutic agents. Various useful synthetic analogues with improved therapeutic properties can be obtained from pyrimidine derivatives. Many classes of chemotherapeutic agents containing pyrimidine derivatives are in clinical use such as antimicrobials. Inspired from these observations, it was planned to synthesize some Schiff bases of dihydropyrimidinone derivatives and evaluate their antimicrobial activity. Dihydropyrimidinones were prepared by Biginelli reaction using various substituted aromatic aldehydes which was then treated with hydrazine hydrate to afford carbohydrazido derivatives and was finally condensed with substituted aromatic aldehydes to afford Schiff bases of dihydropyrimidinone derivatives. The compounds were characterized by means of spectral analysis.

EXPERIMENTAL WORK

Synthesis of biginelli compound

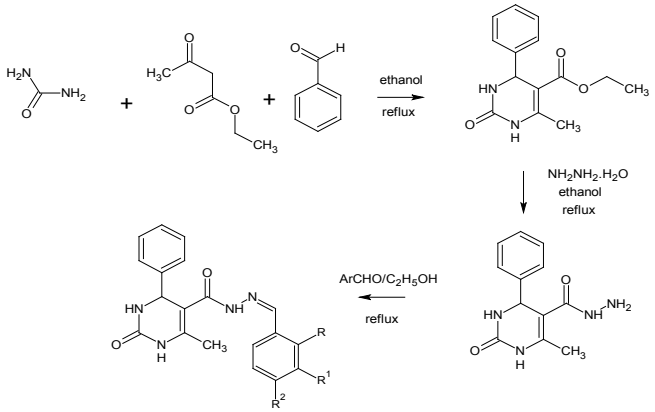
A mixture of 0.15 mole of urea, 0.1 mole of ethylacetoacetate and 0.1 mole of benzaldehyde were dissolved in 25 ml of ethanol along with 3 drops of conc. HCl and refluxed for one and half an hour. The reaction mixture was then poured into 100 ml ice cold water with stirring and left overnight at room 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. The purity of the compounds was determined by thin layer chromatography.

Synthesis of carbohydrazido derivative

A mixture of 0.1 mole of biginelli compound and 0.1 mole of hydrazine hydrate were dissolved in 20 ml of ethanol along with 4 drops of conc. sulphuric acid and refluxed for 3 h. The reaction mixture was then evaporated to obtain a residue which was further recrystallised from ethanol. The purity of the compounds was determined by thin layer chromatography.

Synthesis of schiff bases of dihydropyrimidinone derivatives

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



Compound Code	R	R1	R2
DP-1	H	H	H
DP-2	OH	H	H
DP-3	H	OCH_3	OH
DP-4	H	H	OCH_3
DP-5	H	H	Cl
DP-6	H	H	CH3
DP-7	H	OCH_3	OCH_3
DP-8	H	Cl	Cl

DP-1: 6-methyl-2-oxo-4-phenyl-*N*'-[(*Z*) phenylmethylidene]-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide: **IR Spectra KBr (cm^{-1}):** 3119.78(Ar-Hstretch), 1648.36(C=O stretch), 3414.35(-NH stretch), 3242.72 (-NH stretch in amide), 1701.39 (C=O stretch in amide), 2937.06 (C-H stretch in CH_3), **λ_{max}:** 284, **Rf:** 0.507

DP-2 *N*-(2-hydroxyphenyl)methylidene]-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro pyrimidine-5-carbohydrazide: **IR Spectra KBr (cm^{-1}):** 3119.78(Ar-Hstretch),1648.84(C=O stretch), 3473.65(-NH stretch), 3242.72 (-NH stretch in amide), 1700.91 (C=O stretch in amide), 2973.7 (C-H stretch in CH_3), 3411.46(OH stretch), **λ_{max}:** 289, **Rf:** 0.550

DP-3: *N*-(4-hydroxy-3-methoxyphenyl)methylidene]-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide: **IR Spectra KBr (cm^{-1})** 3124.6(Ar-Hstretch),1648.36(C=O stretch), 3551.75(-NH stretch), 3244.16(NH stretch in amide), 1700.91 (C=O stretch in amide), 2931.75 (C-H stretch in CH_3), 1091.99(C-O-C stretch), 3484.26(OH stretch), **λ_{max}:** 284, **Rf:** 0.616

DP-4: *N*-(4-methoxyphenyl)methylidene]-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetra hydro pyrimidine--5-carbohydrazide: **IR Spectra KBr (cm^{-1})** 3119.78(Ar-Hstretch),1648.84(C=O stretch), 3417.73(-NH stretch), 3245.13 (-NH stretch in amide), 1701.39 (C=O stretch in amide), 2926.93 (C-H stretch in CH_3), 1091.51(C-O-C stretch), **λ_{max}:** 203, **Rf:** 0.218

DP-5 *N*-(4-chlorophenyl)methylidene]-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide: **IR spectra KBr (cm^{-1}):** 3115.44(Ar-Hstretch),1648.36(C=O stretch), 3421.58(-NH stretch), 3254.77 (-NH stretch in amide), 1700.91 (C=O stretch in amide), 2916.32 (C-H stretch in CH_3), 781.99(C-Cl stretch), **λ_{max}:** 283, **Rf:** 0.532

DP-6 6-methyl-*N*-(4-methylphenyl)methylidene]-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide : **IR spectra KBr (cm^{-1}):** 3114.47(Ar-Hstretch),1648.36(C=O stretch), 3536.33(-NH stretch), 3245.13 (-NH stretch in amide), 1700.43 (C=O stretch in amide), 2931.75 (C-H stretch in CH_3 in pyrimidine ring), 2848.83 (C-H stretch in CH_3), **λ_{max}:** 203, **Rf:** 0.70

DP-7: *N*-(3,4-dimethoxyphenyl)methylidene]-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide: **IR Spectra KBr (cm^{-1}):** 3119.78(Ar-Hstretch),1648.84(C=O stretch), 3437.49(-NH stretch), 3246.57 (-NH stretch in amide), 1701.39 (C=O stretch in amide), 2979.00 (C-H stretch in CH_3), 1091.99(C-O-C stretch) **NMR Spectra in ppm** Ar-H(m,9H) -7.235-7.347, NH(s,1H) -9.163, C-CH_3(s,1H) -1.118, C-O-CH_3(s,3H) -2.254, C-O-CH_3(s,3H) - 4.017, N=CH(s,1H) -7.715, NH in a mide(s,1H) -5.158, **λ_{max}:** 284, **Rf:** 0.50

BPS-8: *N*'-[(*Z*)-(2,4-dichlorophenyl)methylidene]-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide: **IR Spectra KBr (cm^{-1}):** 3114.47(Ar-Hstretch),1648.84(C=O stretch), 3421.58(-NH stretch), 3244.16 (-NH stretch in amide), 1700.91 (C=O stretch in amide), 2937.06 (C-H stretch in CH_3), 781.99(C-Cl stretch), **λ_{max}:** 284, **Rf:** 0.54

Anti microbial studies

Filter paper disc of 6 mm diameter are impregnated with optimum amount of drug. The discs may be dried in incubator and stored in refrigerator. Liquid culture of the bacteria in broth is flooded on a solid medium in a plate (Muller Hinton agar (or) nutrient agar) and excess is thrown away. Alternatively, the culture plate may be sub cultured by the bacterial culture by swab. The medicated discs are then placed on the plate and incubated overnight. The zone of inhibition around the disc was noted in Figure 1.

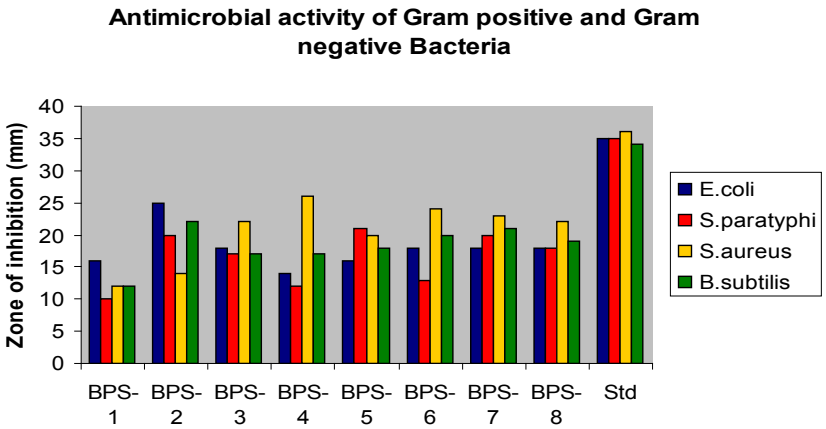


Figure 1: Antibacterial activity of synthesised compounds

Antibacterial activity

The standardized inoculums were inoculated in the plates prepared earlier (aseptically) by dipping a sterile cotton swab in the inoculums removing the excess of inoculums by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application [14]. Finally the swab was swabed round the edge of the agar surface. The inoculums were left to dry at room temperature with the lid closed. Each Petri dish was divided into parts, in each part samples disc such as DP-1 to DP-8 (100 µg) discs (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 (Table 2).

Table 2: Antibacterial activity of synthesised compounds

S. No.	Compound code	Zone of inhibition (mm)			
		Escherichia coli	*Staphylococcus paratyphi*	*Staphylococcus aureus*	*Bacillus subtilis*
1	**DP-1**	16	10	12	12
2	**DP-2**	25	20	14	22
3	**DP-3**	18	17	22	17
4	**DP-4**	14	12	26	17
5	**DP-5**	16	21	20	18
6	**DP-6**	18	13	24	20
7	**DP-7**	18	20	23	21
8	**DP-8**	18	18	22	19
9	**Standard Ciprofloxacin (10µg/disc)**	35	35	36	34

Anti-fungal activity

The standardized inoculums were inoculated in the plates prepared earlier (aseptically) by dipping a sterile cotton swab in the inoculums removing the excess of inoculums by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application [15]. Finally the swab was swabed round the edge of the agar surface [15]. The inoculums were left to dry at room temperature with the lid closed. Each Petri dish was divided into parts, in each part samples disc such as DP-1 to DP-8 (100 µg) discs (discs are soaked overnight in sample solution) and standard Clotrimazole 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 in Table 3 and Figure 2.

Table 3: Antifungal activity of synthesised compounds

S. No.	Compound code	Zone of inhibition (mm)	
		Candida albicans	*Aspergillus niger*
1	**DP-1**	19	8
2	**DP-2**	19	16
3	**DP-3**	14	18
4	**DP-4**	8	7
5	**DP-5**	21	18
6	**DP-6**	16	14
7	**DP-7**	20	17
8	**DP-8**	20	6
9	**Standard Clotrimazole (10 µg/disc)**	27	27

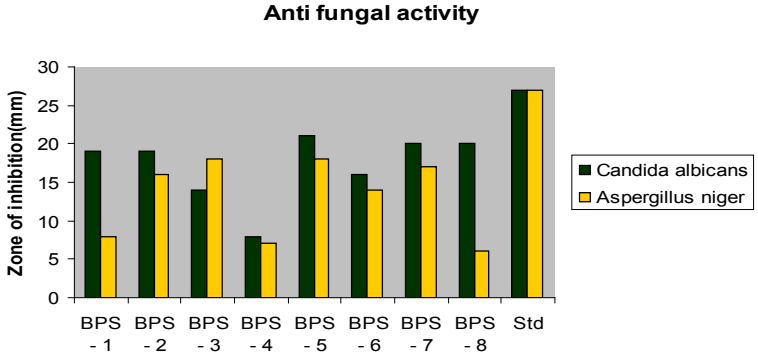


Figure 2: Antifungal activity of synthesised compounds

Determination of minimum inhibitory concentration (MIC) for fungi and bacteria

The minimum inhibitory concentration was found for all the test compounds against *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*.

Tube dilution method

This method depends upon the inhibition of growth of microbial culture in a uniform solution of the test sample in a fluid medium that is favorable to its rapid growth. The test sample were dissolved in dimethyl sulfoxide and diluted to highest concentration (1000 µg/ml) to be tested, and then fold serial dilutions were made in a concentration range from 1000 µg/ml to 15.6 µg/ml in sterile test tubes containing standardized inoculums. All the tubes were incubated at 37ºC for 24 h (Bacteria) and 24ºC for 48 h (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 (Table 4).

Table 4: MIC of synthesised compounds

Micro Organisms	Compound Code	1000 Mg/ml	500 Mg/ml	250 Mg/ml	125 Mg/ml	62.5 Mg/ml	31.25 Mg/ml	15.625 Mg/ml	Blank
Staphylococcus aureus	**DP-4**	–	–	–	+	+	+	+	+
Bacillus subtilis	**DP-7**	–	–	–	+	+	+	+	+
Candida albicans	**DP-5**	–	–	–	+	+	+	+	+
Candida albicans	**DP-7**	–	–	–	–	+	+	+	+
Candida albicans	**DP-8**	–	–	–	–	+	+	+	+
Aspergillus niger	**DP-3**	–	–	–	+	+	+	+	+
Aspergillus niger	**DP-5**	–	–	–	–	+	+	+	+

–: Inhibition, +: No inhibition

RESULTS AND DISCUSSION

The titled compounds were synthesized in a three step process. The first step was synthesis of substituted dihydropyrimidines by the condensation of urea and ethylacetoacetate with various substituted benzaldehydes in presence of an acid and ethanol, commonly known as Biginelli reaction. The advantage is that it is a useful intermediate to afford various medicinally important heterocyclic compounds. Dihydropyrimidines were further condensed with hydrazine hydrate to afford carbohydrazido derivatives. The carbohydrazido derivatives of substituted dihydropyrimidines were finally condensed with various aromatic aldehydes to afford the titled compounds. The melting points of all the titled compounds were reported. The melting points were determined in open capillary tubes with electrically heating melting point apparatus and are uncorrected.

The solubility of all the synthesized compounds was checked by using the following solvents: Water, Benzene, Chloroform, Alcohol and DMSO. The purity of the all the titled compounds were checked by thin layer chromatography using silica gel as stationary phase, employing Toluene: Ethyl acetate (8:2) as mobile phase, spots was visualized using Iodine vapours. The R_f values of the synthesized compounds were also reported.

The infrared spectra of all the synthesized compounds were elucidated and expressed as wave number in cm-1. The presence of N=CH bond stretch confirmed the formation of titled compounds. The nuclear magnetic resonance spectra of synthesized compound was elucidated. The presence of N=CH proton confirmed the formation of Schiff bases and spectral data were in correlation with the expected structure. The λ_{max} of the synthesized compounds were determined. The wavelength at which each compound has shown maximum absorbance has specific absorptivity.

The synthesized compounds were tested for antibacterial activity against gram positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria such as *Escherichia coli* and *Salmonella paratyphi*. The zone of inhibition and MIC were measured and compared against a standard. The synthesized compounds were tested for antifungal activity against *Candida albicans* and *Aspergillus niger*. The zone of inhibition and MIC were measured.

The antibacterial screening of all the compounds showed a moderate zone of inhibition than standard Ciprofloxacin. All the compounds exhibited better activity against *Staphylococcus aureus*.

Among the dihydropyrimidinone derivatives synthesized compound DP-3, DP-5, DP-7, DP-8 exhibited stronger inhibition in comparison with standard for antifungal activity. *Candida albicans* and *Aspergillus niger* were found to be highly inhibited by dihydropyrimidinone derivatives exhibiting high antifungal activity than antibacterial activity.

It was found that the dihydropyrimidinone derivatives exhibited a significant antifungal activity. The compounds were tested for minimum inhibitory concentration on bacterial strain and fungal strain.

CONCLUSION

Pyrimidines are therapeutically important class of compounds. The entitled work describes the synthesis of series of substituted dihydropyrimidinone derivatives via Biginelli reaction. The purity of the compounds was established as single spot by Thin Layer chromatography. The structures of the compounds were elucidated by UV, IR and NMR analysis.

Substituted dihydropyrimidine derivatives were synthesized and screened for their antimicrobial activity. 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 such as antitumor, anti HIV, anti-malarial, antihypertensive etc., which can broaden the therapeutic utility for the compounds synthesized that will form part of a future study.

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