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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL BIGINELLI TYPE HETEROCYCLIC COMPOUNDS: A STRUCTURE-REACTIVITY STUDY

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ABSTRACT
A novel series of Biginelli type dihydropyrimidinones derivatives were synthesized and investigated for their antibacterial activity against gram positive and gram negative bacteria. The recorded data of zone of inhibition showed significant broad activity when compared with standard. The structure reactivity correlation of the compounds has been studied.

KEYWORDS: Biginelli type compounds, dihydropyrimidinones derivatives, antibacterial activity, correlation studies.

INTRODUCTION

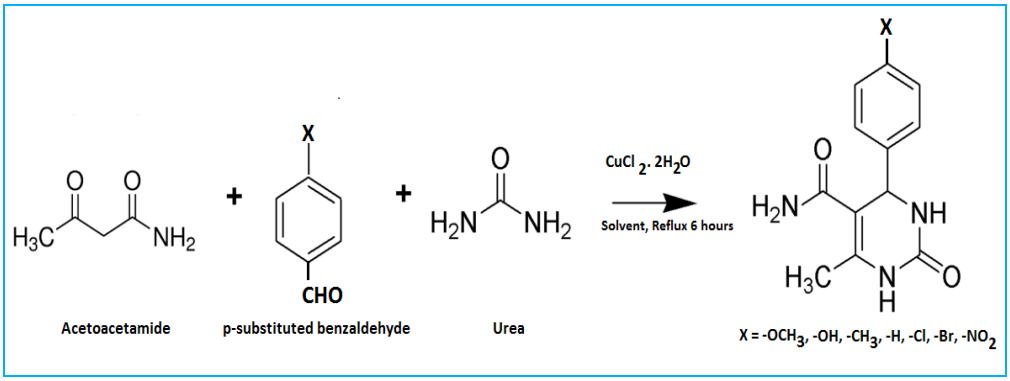
The most spectacular advances in medicinal chemistry have been made when heterocyclic compounds played an important role in regulating biological activities. Nitrogen and oxygen containing heterocyclic compounds have received considerable attention due to their wide range of pharmacological activity. The most thoroughly studied ring system amongst the heterocyclic compounds is that of pyrimidine.[1,2] They serve as building units of many valuable chemotherapeutic agents (bleomycine), vitamins (vitamin B1), drugs (hyprotic, antibacterial and antimalarial) and nucleic acids (cytosine and uracil). In 1893, Italian chemist Pietro Biginelli reported on the acid-catalyzed cyclocondensation reaction of ethylacetoacetate, benzaldehyde and urea. The reaction was carried out simply by heating a mixture of the three components dissolved in ethanol with a catalytic amount of HCl at reflux temperature. The product of this novel one-pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3,4-dihydropyrimidin-2(1H)-one (4).[3]

The synthetic potential of this new heterocyclic synthesis (now known as Biginelli reaction) remained unexplored for quite some time. In the 1970's and 1980's interest slowly increased and the scope of the original cyclocondensation reaction was gradually extended by variation of all three building blocks, allowing access to a large number of multifunctionalized dihydropyrimidines. Dihydropyrimidinones (DHPMs, Biginelli compounds) are an important class of compounds which are becoming interesting due to their therapeutic and pharmacological activities. Because of the importance of the DHPMs, much work on improving their synthesis has been actively pursued for several decades.

Recently, several other methods including the use of lanthanide compounds, several other Lewis acids, $AlCl_3$, Co or Ca or Mn or Sn compounds, solid assisted synthesis[4] and bismuth oxide perchlorate[5] have also been reported to overcome the drawback of the classical Biginelli reaction. Currently it was reported that the Biginelli reaction can occur more smoothly upon irradiation by microwaves in the presence of ferric chloride as the catalyst.[6,7] Keeping these facts in mind, we have been prompted to synthesize some dihydropyrimidinones analogous derived from substituted benzaldehyde, urea and acetoacetamide using the catalyst ($CuCl_2.2H_2O$). In this work we have synthesised these nuclease compounds with in short duration (Scheme I). Low cost is enough for the preparation of these compounds. Studies of substituent effects on zone of inhibition against the growth of microorganisms in various substituted N-(1-piperidino benzyl) nicotinamide[8] and 2-benzylidene-1,3-indandiones[9] have been reported. As a part of our interest in the structure-reactivity study, we have studied the antibacterial activity to find out the substituents effect on 6-aryl-4-methyl-2-oxo-1, 2, 3, 6 - tetrahydropyrimidine-5- carboxamides.

MATERIALS AND METHODS

An aromatic aldehyde (10 mmol), acetoacetamide (10 mmol), urea (20 mmol) and $CuCl_2.2H_2O$ (5 mmol) were mixed in R.B flask and the mixture was magnetically stirred at 70°C for the time needed to complete the reaction (as monitored by TLC). The initial syrupy reaction mixture solidifies within 25-30 minutes. The solid was poured onto crushed ice, filtered and recrystallized by using either ethanol or the mixture ethyl acetate and petroleum ether (1:3) (**Scheme I**).



Scheme I

Methods to Detect Antibacterial Activity

The antibacterial activities may be determined using three main methods, agar dilution, broth micro dilution, and disc diffusion which are standard methods recommended by Clinical and Laboratories Standards Institute (CLSI) for measuring in vitro susceptibility of bacteria to antimicrobial agents used in clinical settings. Since these methods apply different principles, the results obtained may differ. Besides methods, antibacterial susceptibility testing results can also be affected by many other factors, such as the microorganisms tested and the degree of solubility of each test-compound. Among the three methods, disc diffusion has been the most popular one used to examine the antibacterial activity. Below is an overview of the disc diffusion method.

Disc Preparation

The 6 mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C.Then discs were mixed with chemical compounds separately and control discs were prepared.

Collection of test microorganisms

The Bacterial strains of *Bacillus subtilis (MTCC 2057)* and *Escherichia coli (MTCC 1556),* obtained from Microbial Type culture Collection Centre (MTCC), Chandigarh.

Assay of Antibacterial Activity

Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.,* (1966).[10] Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each petri plates and also placed control and standard (*Ampicillin*) discs. The plates were incubated at 37°C for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

RESULTS AND DISCUSSION

In the present investigation, Biginelli type dihydropyrimidinones analogous were prepared from a reaction mixture consisting of substituted benzaldehydes, urea and acetoacetamide in presence of copper chloride as catalyst. All the products were screened for antibacterial activities.

The in vitro antibacterial activities of the compounds were tested against Bacillus *subtilis and Escherichia coli*, compared with standard *Ampicillin.* The seven compounds against the growth of microorganisms are summarized in Table 1. A comparative study of the compounds indicates that in general, compound 7($-NO_2$ substituted) has higher activity than the other compounds. Halogens pulls electrons toward itself and positively polarizes the C to which it is bonded, it is called an inductive electron withdrawing group (EWG). The halogen atoms, as well as the NO_2 group, are also inductive EWGs. However, the effect of the nitro group (NO_2) is greater than that of halogen atoms. This is a result of the combined effect of the three relatively electronegative atoms in NO_2 and the high electron deficiency on nitrogen in this group. It is expected that electron withdrawing substituents enhance the nucleophilic reaction, whereas electron donating ones reduce it. These factors, as well as, the combined inductive and conjugative effects of the substituents (X) result in differences in activity according to the following order:

$-OCH_3 < -OH < -CH_3 < -H < -Cl < -Br < -NO_2$

Table – 1: Antibacterial activity

S. No.	Bacteria	Standard Antibiotic Disc*	Zone of inhibition (mm in diameter)							
			Control	$-OCH_3$	-OH	$-CH_3$	H	-Cl	-Br	$-NO_2$
1	*Bacillus subtilis*	24	-	14	16	17	19	21	22	25
2	*Escherichia coli*	34	-	27	28	29	31	33	34	36

*Ampicillin.

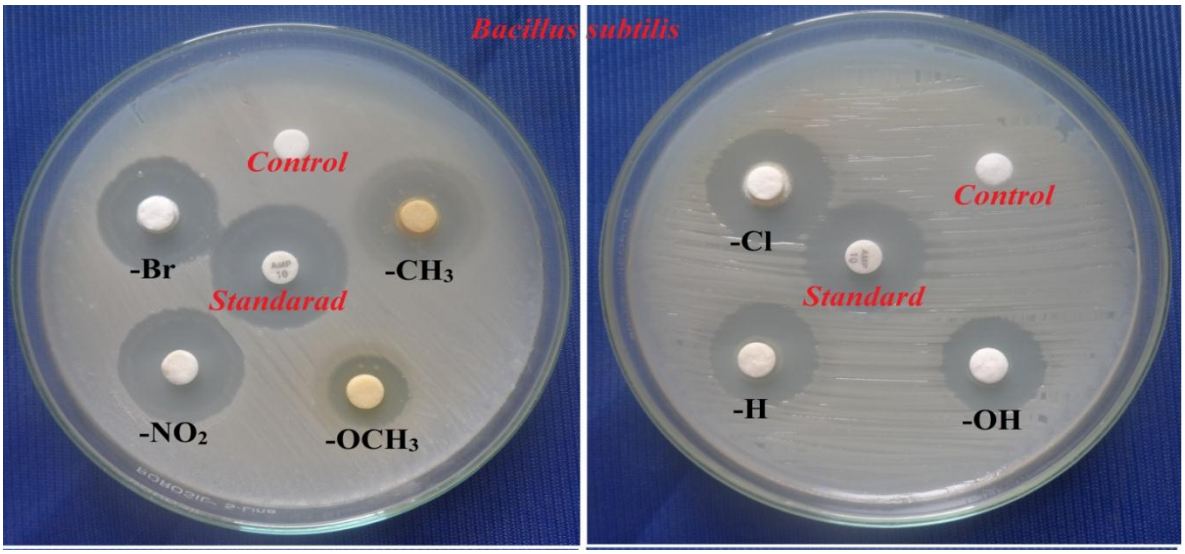


Figure 1: Antibacterial activity of compounds against *Bacillus subtilis*

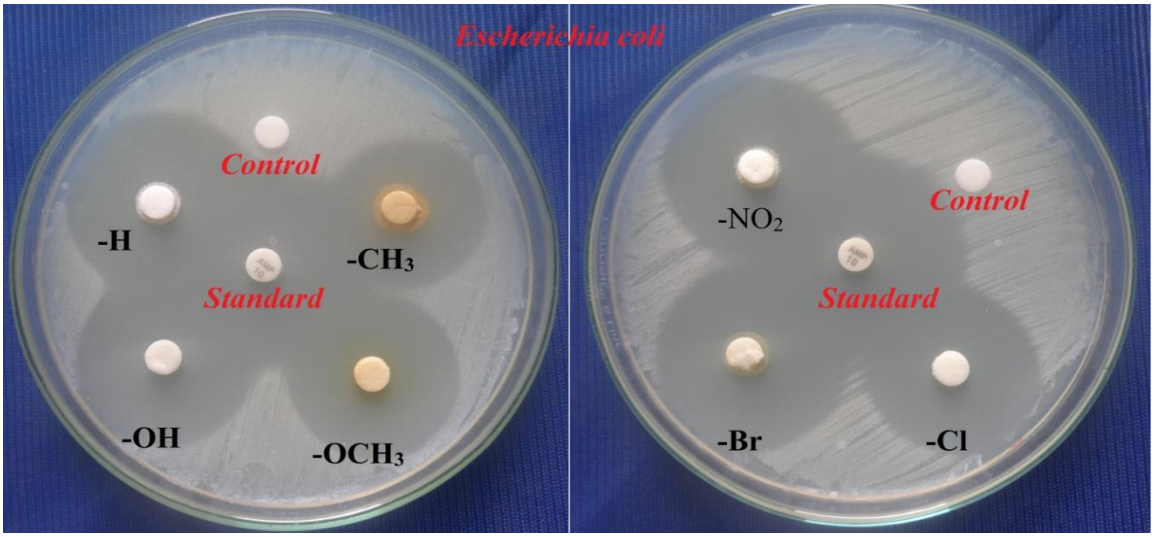


Figure 2: Antibacterial activity of compounds against *Escherichia coli*

In order to express the effect of substituents quantitatively it was considered worthwhile to correlate the logarithm of inhibition zone diameter (IZD) of 6-aryl-4-methyl-2-oxo-1, 2, 3, 6- tetrahydropyrimidine-5-carboxamides at the same concentration with the Hammett substituent constants for all the microorganism. The results of statistical SSP analysis are given in Table 2. The corresponding Hammett plot for *Bacillus Subtilis* is shown in Figure 3**.**

The positive value of the reaction constant (ρ) equation (1)

$$\log IZD=(0.17\pm0.01)\sigma_P^{+}/\sigma_P + (1.283\pm0.005) \quad (1)$$

$$(r=0.992, n=6, F=258.42)$$

indicates that electron withdrawing substituents increase the antibacterial activity and electron releasing substituents retard it.

Table 2: Results of statistical treatment of log (IZD) mm with σ_P, σ_P^o, σ_P^+, σ_P^+/σ_P, σ_P^+/σ_P^-, $\sigma_P^+/\sigma_P/\sigma_P^-$ substituent constants using single parameter equation 1

S. No	Name of the microorganism	Scale	ρ	r	s	F	Log(IZD)^o	n
1	*Bacillus subtilis*	σ_P	0.23± 0.04	0.932	0.03	26.51	1.257±0.016	6
		σ_P^o	0.22±0.06	0.870	0.04	12.41	1.247±0.023	6
		σ_P^+	0.17±0.02	0.976	0.02	81.93	1.287±0.009	6
		σ_P^+/σ_P	0.17±0.01	0.992	0.01	258.42	1.283±0.005	6
		σ_P^+/σ_P^-	0.12±0.02	0.925	0.04	23.69	1.277±0.016	6
		$\sigma_P^+/\sigma_P/\sigma_P^-$	0.12±0.02	0.941	0.03	31.20	1.270±0.014	6
2	*Escherichia coli*	σ_P	0.13±0.02	0.934	0.02	33.90	1.499±0.008	7
		σ_P^o	0.12±0.04	0.838	0.03	9.45	1.494±0.014	6
		σ_P^+	0.09±0.01	0.980	0.01	122.31	1.518±0.004	7
		σ_P^+/σ_P	0.09±0.01	0.985	0.01	163.04	1.516±0.004	7
		σ_P^+/σ_P^-	0.06±0.01	0.928	0.02	30.83	1.495±0.007	7
		$\sigma_P^+/\sigma_P/\sigma_P^-$	0.06±0.01	0.935	0.02	34.60	1.492±0.007	7

" n=6 means calculated without –OH group"

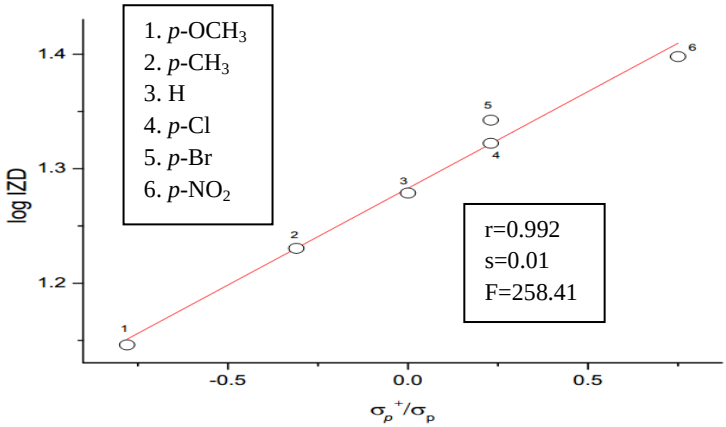


Figure 3: Hammett plot for *Bacillus Subtilis*

DSP analysis has been performed for each of the resonance scale (σ_R, σ_R^+ σ_R^-, σ_R^o). The best fit of DSP analysis for *Bacillus Subtilis* is taken from good correlation coefficient and least standard error (SE) of the regression equations (2) and (3) and the result obtained given in Table 3.

log IZD=(0.18±0.05) σ_I + (0.25±0.06) σ_R + (1.27±0.02) (2)

(R=0.961, SE=0.03, n=6, F=18.05)

log IZD=(0.16±0.07)F + (0.26±0.07) R + (1.27±0.03) (3)

(R=0.945, SE=0.04, n=6, F=12.56)

The sign of ρ_I and ρ_R are positive, reveals that the normal substituent effects operates on IZD, ie, an electron releasing substituents decrease the IZD and electron withdrawing substituents increase the IZD. The ρ_I values are rather smaller than ρ_R values and this reveals the importance of resonance component.

Table 3: DSP analysis of log IZD (mm) with dual parameter equations 2 and 3.

S. No	Name of the microorganism	scale	ρ_I	ρ_R	R	SE	F	Log(IZD)^o	n	$\lambda = \rho_R/\rho_I$
1	*Bacillus subtilis*	σ_I, σ_R	0.18±0.05	0.25±0.06	0.961	0.03	18.05	1.27±0.02	6	1.38
		σ_I, σ_R^+	0.09±0.12	0.10±0.06	0.854	0.06	4.05	1.31±0.06	6	1.11
		σ_I, σ_R^o	0.21±0.13	0.11±0.12	0.742	0.08	1.84	1.24±0.05	6	0.52
		σ_I, σ_R^-	0.17±0.11	0.13±0.09	0.810	0.07	2.85	1.25±0.05	6	0.76
		F, R	0.16±0.07	0.26±0.07	0.945	0.04	12.56	1.27±0.03	6	1.62
2	*Escherichia coli*	σ_I, σ_R	0.11±0.03	0.11±0.03	0.957	0.02	16.47	1.49±0.01	6	1.00
		σ_I,σ_R^+	0.06±0.06	0.05±0.03	0.818	0.03	4.03	1.50±0.03	7	0.83
		σ_I, σ_R^o	0.12±0.06	0.05±0.06	0.773	0.04	2.22	1.47±0.03	6	0.42
		σ_I, σ_R^-	0.10±0.06	0.06±0.05	0.829	0.03	3.29	1.48±0.02	6	0.60
		F, R	0.10±0.03	0.11±0.02	0.949	0.02	18.01	1.48±0.01	7	1.10

The Yukava-Tsuno equation 4 and Table 4 for *Bacillus Subtilis* proved the less contribution of polar component. log IZD =(0.16±0.03) σ_P^o + (0.18±0.05) (σ_P^+-σ_P^o) + (1.29±0.02)

(R=0.977, SE=0.02, n=6, F=32.06) (4)

Table 4: Results of Multiple regression analysis of log IZD (mm) with σ_P, (σ_P^+-σ_P) and σ_P^o, (σ_P^+-σ_P^o) constants using Yukava-Tsuno equation 4

S. No	Name of the microorganism	scale	ρ	r	R	SE	F	n
1	*Bacillus subtilis*	σ_P, (σ_P^+-σ_P)	0.16±0.04	0.18±0.08	0.977	0.02	31.30	6
		σ_P^o, (σ_P^+-σ_P^o)	0.16±0.03	0.18±0.05	0.977	0.02	32.06	6
2	*Escherichia coli*	σ_P, (σ_P^+-σ_P)	0.09±0.03	0.04±0.04	0.960	0.02	23.82	7
		σ_P^o, (σ_P^+-σ_P^o)	0.09±0.02	0.08±0.03	0.964	0.02	19.98	6

CONCLUSIONS

The direct preparation of a series of DHPM by the Biginelli multicomponent reaction starting from acetoacetamide has been optimized with respect to the use of an adequate catalyst. The antibacterial searching suggests that all the synthesized Biginelli compounds showed moderate to very good activity against the tested organisms. Among the compounds, NO_2 substituted compound showed the most promising antibacterial activity, suggesting further work with similar analogues. The inhibition zone diameters of these compounds have been correlated with Hammett substituent constants, F and R parameters. From the results of statistical analysis, the effects of substituent on the antibacterial activity of compounds have been studied.

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