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Simple Method of Preparation and Characterization of New Antifungal Active Biginelli Type Heterocyclic Compounds

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A simple, efficient and cost effective method is described for the synthesis of Biginelli type heterocyclic compounds of dihydropyrimidinones analogous. They were prepared from a reaction mixture consisting of substituted benzaldehydes, thiourea and ethylacetoacetate using ammonium dihydrogenphosphate as catalyst. The procedure for the preparation of the compounds is environmentally benign and safe which is advantageous in terms of experimentation, catalyst reusability, yields of the products, shorter reaction times and preclusion of toxic solvents. The four new synthesised compounds were tested for their antifungal activity. They have good antifungal activity comparing to the standard (Fluconazole).

KEYWORDS : Antifungal, Biginelli reaction, Dihydropyrimidinones, Ethylacetoacetate, Heterocyclic compounds, Thiourea

Nowadays worldwide, the production of the materials from various plant kingdom such as cotton, sugar cane, potato, maize *etc.*, are affected by various plant pathogenic fungi which also decrease the quality of the materials. Global antifungal drug resistance by plant pathogenic fungi is becoming an increasing public health concern and the race to discover new antifungal drugs for new therapeutic agents with novel modes of action from heterocyclic compounds. These compounds act as 'synthetic nucleases' that mimic the action of pharmacological drugs. These compounds are seen in a solvent free condition. Therefore, they have high purity and there is no side effect in a plant. 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 (Kappe, 1993, 2000; Raman *et al.*, 2007). They serve as building units of many valuable chemotherapeutic agents (bleomycine), vitamins (vitamin B_1), drugs (hyprotic, antibacterial, antimalarial) and nucleic acids (cytosine and uracil). In 1893, Italian chemist Pietro Biginelli reported on the acid-catalyzed cyclocondensation reaction of ethylacetoacetate (**1**), benzaldehyde (**2**) and urea (**3**). 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(1*H*)-one (**4**) (Fig. 1), (Muniz and Juaristi, 2003).

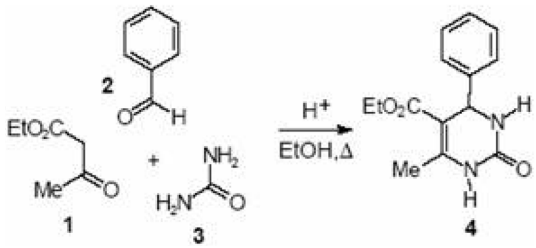

Fig. 1. The Biginelli dihydropyrimidine synthesis.

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 shown in Fig. 1 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 (Kumar *et al.*, 2005; Gangadasu *et al.*, 2006), solid assisted synthesis (Kumar *et al.*, 2005) and bismuth oxide perchlorate (Reddy *et al.*, 2006) have also been reported to overcome the drawback

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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 (Vaghasia and Shah, 2007). Keeping these facts in mind, we have been prompted to synthesize some dihydropyrimidinones analogous derived from substituted benzaldehyde, thiourea and ethylacetoacetate using the catalyst (ammonium dihydrogen phosphate) and also to study their antifungal efficiency. In this work we have synthesised these nuclease compounds within short duration (2 h). Low cost is enough for the preparation of these compounds.

Materials and Methods

Microanalytical data, ^{1}H-NMR and Mass spectra of the compounds were recorded at the Regional Sophisticated Instrumentation Center, Central Drug Research Institute (RSIC, CDRI), Lucknow. Microanalyses were done using a Carlo Erba 1108 CHN Elemental Analyzer. The FAB mass spectrum of the complex was recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using *m*-nitrobenzylalcohol (NBA) as the matrix. The IR spectra of the samples were recorded on a Perkin-Elmer 783 spectrophotometer in 4000~400 cm^{-1} range using KBr pellet.

General procedure for the synthesis of compounds. A mixture of *m*-nitrobenzaldehyde (L^1)/*p*-methoxybenzaldehyde (L^2)/benzaldehyde(L^3)/*o*-hydroxybenzaldehyde (L^4), thiourea and ethylacetoacetate in presence of ammonium dihydrogen phosphate was stirred for 2 h in ethanol. The solid product was filtered and washed with water and dried (Fig. 2).
where R-CH_2CH_3; X=-NO_2(L^1); -OCH_3(L^2); -H(L^3); -OH(L^4).

Culture isolation and maintenance. *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viride*, *Trichoderma harzianum* and *Sclerotium rolfsii* were isolated from the soil sample by serial dilution and pour plate techniques. The pure cultures were identified by their morphology and colony characteristics. The fungi were maintained in the potato dextrose agar plates (PDA) and stored at 4°C.

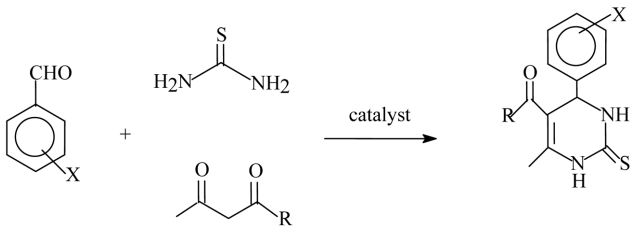

Fig. 2. A procedure for the synthesis of compounds.

Antifungal activity. *A. niger*, *A. flavus*, *T. viride*, *T. harzianum* and *S. rolfsii* were employed for the testing of the antifungal activity using the cup-plate method. The culture was maintained on Sabouraud's agar for 72 h, which gave the optimum growth of the test fungal spores. All the purified compounds were loaded with different concentrations ($\mu g/ml$) (Tables 2~5) and kept in a sterile petri dish. The inoculum consisted of an overnight-grown broth culture of different fungi diluted in such a manner that a 2 mm (internal diameter) loopful of the cultures containing 10^5 colony-forming units (CFU). Then the cultures were inoculated on Sabouraud's agar plates and incubated at 37°C for upto 48 h to determine the minimum inhibitory concentration (MIC).

Results and Discussion

In the present investigation, Biginelli type dihydropyrimidinones analogous were prepared from a reaction mixture consisting of substituted benzaldehydes, thiourea and ethylacetoacetate in presence of ammonium dihydrogen phosphate as catalyst. All the products were characterized by IR, ^{1}H-NMR, Mass spectral and elemental analytical data.

The yields, melting points and the molecular mass of the compounds obtained from the mass spectral study are given in Table 1. The elemental analytical data of the compounds are in good agreement with the theoretical values which are given as follows:

Data of L^1: m.p. 186~187°C; Anal. Calc. for $C_{14}H_{15}N_3O_4S$: C, 52.33; H, 4.67; N, 13.08%. Found: C, 52.28; H, 4.62; N, 13.05 %. **Data of L^2**: m.p. 208~ 210°C; Anal. Calc. for $C_{15}H_{18}N_2O_3S$: C, 58.82; H, 5.88; N, 9.15%. Found: C, 58.78; H, 5.83; N, 9.13 %. **Data of L^3**: m.p. 195~196°C; Anal. Calc. for $C_{14}H_{16}N_2O_2S$: C, 60.87; H, 5.80; N, 10.14%. Found: C, 60.83; H, 5.75; N, 10.10%. **Data of L^4**: m.p. 178~180°C; Anal. Calc. for $C_{14}H_{16}N_2O_3S$: C, 54.54; H, 5.19; N, 9.09%. Found: C, 54.50; H, 5.15; N, 9.04%.

IR spectra. The IR spectra provide some information regarding the skeleton of the compounds and were ana-

Table 1. Ammonium dihydrogen phosphate catalyzed synthesis of compounds

S. No	R	X	Yield (%)	Mass	mp
L^1	-CH_2CH_3	-NO_2	95	321	186~187
L^2	-CH_2CH_3	-OCH_3	90	306	208~210
L^3	-CH_2CH_3	-H	94	276	195~196
L^4	-CH_2CH_3	-OH	89	308	178~180

Table 2. Antifungal screening for the compound L^1

Name of the organisms	Diameter of the zone of inhibition (mm)							
	Compound L^1 (in $\mu g/ml$)				Fluconazole (in $\mu g/ml$)			
	289	28.9	5.78	3.85	326	32.6	6.52	4.65
A. niger	R	R	R	R	24	22	18	13
A. flavus	16	14	12	10	28	25	21	17
T. viride	14	11	10	8	28	24	22	12
T. harzianum	18	14	12	12	24	22	16	16
S. rolfsii	20	16	14	11	32	24	18	16

R, Resistant.

Table 3. Antifungal screening for the compound L^2

Name of the organisms	Diameter of the zone of inhibition (mm)							
	Compound L^2 (in $\mu g/ml$)				Fluconazole (in $\mu g/ml$)			
	274	27.4	5.4	3.6	326	32.6	6.52	4.65
A. niger	37	34	32	31	24	22	18	13
A. flavus	25	23	21	20	28	25	21	17
T. viride	24	22	20	15	28	24	22	12
T. harzianum	20	18	16	14	24	22	16	16
S. rolfsii	22	16	14	10	32	24	18	16

Table 4. Antifungal screening for the compound L^3

Name of the organisms	Diameter of the zone of inhibition (mm)							
	Compound L^3 (in $\mu g/ml$)				Fluconazole (in $\mu g/ml$)			
	244	24.4	4.88	3.25	326	32.6	6.52	4.65
A. niger	30	28	27	24	24	22	18	13
A. flavus	24	20	18	17	28	25	21	17
T. viride	18	16	13	12	28	24	22	12
T. harzianum	20	19	17	14	24	22	16	16
S. rolfsii	20	18	17	14	32	24	18	16

Table 5. Antifungal screening for the compound L^4

Name of the organism	Diameter of the Zone of inhibition (mm)							
	Compound L^4 (in $\mu g/ml$)				Fluconazole (in $\mu g/ml$)			
	276	27.6	5.52	3.68	326	32.6	6.52	4.65
A. niger	27	25	21	20	24	22	18	13
A. flavus	19	17	15	13	28	25	21	17
T. viride	17	14	14	12	28	24	22	12
T. harzianum	19	17	16	13	24	22	16	16
S. rolfsii	16	14	11	11	32	24	18	16

lyzed by a careful comparison with that of the parent compounds. The selected IR absorption bands are discussed here. The compounds show characteristic band for n(N-H) at 3320 cm^{-1}. The sharp bands in the 750~790 and 1520~1540 cm^{-1} regions are due to aromatic uC-H and uC=C, respectively. The band observed at 1165~1175 cm^{-1} is due to u C-N. The broad band in the 3000~2800 cm^{-1} region is due to an OH group. The band appearing at 1710 cm^{-1} is assigned to the carbonyl group of the ethylacetoacetate moiety n(C=O) of the compound.

^{1}H-NMR spectra. Comparison of the ^{1}H-NMR spectra of the compound (L^2), recorded in $CDCl_3$ (Fig. 3) at room temperature reinforces the conclusions drawn from the IR spectra. This compound (L^2) shows signals at 10.02 (-NH), 8.15 (-PhNH), 7.50 (-NH), 4.1 (-CH), 3.12 ($-OCH_3$), 2.44 ($-CH_2$), 1.98 ($-CH_3$), 1.50 ppm ($-CH_3$). Similarly, the other ^{1}H-NMR spectra support the proposed skeleton of the compounds. Based on the above data, the proposed structure of the compounds was given as shown in Fig. 4:

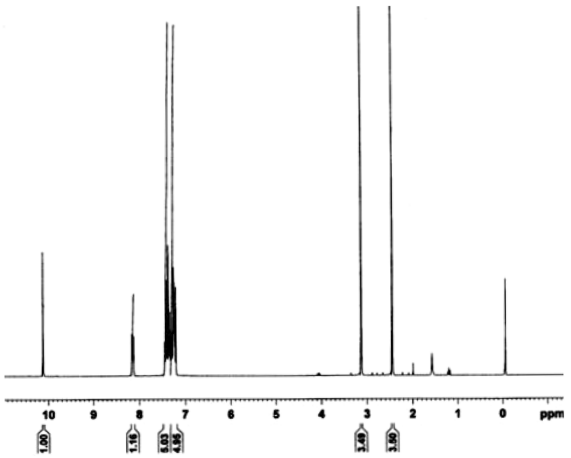

Fig. 3. ^{1}H-NMR spectrum of compound L^{2}.

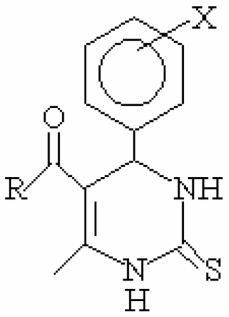

Fig. 4. Structure of the compounds.

Antifungal activity. The *in vitro* antifungal activity of the compounds was tested against filamentous fungi such as *A. flavus*, *A. niger*, *T. harzianum*, *T. viride* and *S. rolfsii* by cup-plate method. Antifungal activities of all the compounds (L^{1}~L^{4}) were compared with known chosen standard fungicide like fluconazole. The four compounds against the growth of microorganisms are summarized in Tables 2~5. A comparative study of the compounds indicates that in general, compound L^{2} has higher activity than the other compounds. *A. niger* had resistant to compound L^{1}.

Variety of subtituents introduced on the organic part using heterocycles and ether -O- atom (Kaim and Schwedersk, 1991) *etc*., increase antimicrobial activity because this increases basic strength and furnishes delocalization of ð-electrons over the whole rings. Such molecules possess higher activity in consistent with great stability of the compounds. This increased activity can also be explained on the basis of Overtone's concept (Anjaneyula and Rao, 1986). According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid-soluble materials due to which liposolubility is an important factor, which controls the antifungal activity.

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