International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.1, pp 435-441, Jan-Mar 2011

Synthesis and Characterizations of Diphenyl Imidazolylpyrimidines -5-Carboxylates (DPIPC) Derivatives and their Antifungal and Antibacterial activity under Conventional and Microwave Irradiation Method

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Abstract : The compound substituted ethyl-1,2,3,6-tetrahydro-4-methyl-2-oxo/thioxo-6-phenyl-1-(4,6-diphenyl-1H-imidazolyl-2-yl)pyrimidine-5-carboxylates (3a-g) have been synthesized by condensing substituted Benzil and enthyl-1-formyl-1,2,3,6-tetrahydro-4-methyl-6-phenyl-2-oxo/thioxo-pyrimidine-5-carboxylates (2a-g) in the presence of ammonium acetate were dissolved in glacial acetic acid was refluxed for 12 hrs in conventional method and 8 minutes for microwave irradiation method. All the compounds have been performed by IR, ^{1}HNMR, ^{13}CNMR, and spectral analysis, antifungal and antibacterial activity. All the synthesized compound carried out under conventional and microwave irradiation method.

Keywords : Conventional and microwave technique, imidazolylpyrimidine derivatives, spectral analysis and antimicrobial activity.

INTRODUCTION :

In continuation of our earlier on the synthesis of heterocyclic compound containing O, S and N as one of the heterocyclic moiety and in view of the biological activity of imidazole and pyrimidine derivatives, the synthesis of antifungal and antibacterial activity of diphenyl imidazolyl - pyrimidine-5-carboxylate (DPIPC) derivatives has been reported in this paper.[1-4]

The reaction of substituted Benzil and enthyl-1-formyl-1,2,3,6-tetrahydro-4-methyl-6-phenyl-2-oxo/ thioxo-pyrimidine-5-carboxylates (2a-g) in presence of ammonium acetate using different reaction solvent media conditions such as ethanol, DMF and DMSO and glacial acetic acid, the reaction were carried out for 12 hrs in conventional method and 8 min. for microwave digestion technique for getting the maximum yields of the products.[5-6]

In recent years, microwave irradiation using commercial domestic ovens has been rapidly increased for optimization and acceleration of organic synthesis under solvent free conditions[7-8]. It has been reported for the variety of reactions such synthesis of heterocyclic and more recently for synthesis of polymers because of advantages such as reduction in reaction time, improved energy utilization, potential for lower processing temperature and improved product uniformity.

In connection with our interest in the use of microwave, we report herein the synthesis of several imidazolylpyrimidines in minimum solvent and minimum time under microwave irradiation (Scheme-I).

Candida albicans is the most prevalent opportunistic fungal pathogen in human that causes various forms of candidiasis ranging from superficial mucosal infection to life threatening systemic diseases in immunocompromised patients[9]. Many azoles inhibiting 14 α-lanosterol demcthylase in ergosterol biosynthesis pathway are known to exhibit interesting antibacterial activity and antifungal activities. However, reported drug class having azoles ring system[10] suffers major shortcomings i.e. a rapid development of resistance against *Candida albieans*. This has highlighted the need to discover new effective antibiotic preferably with new modes of action against both bacteria and fungi.

It has been reported that a large number of imidazole-pyrimidine derivatives possess diverse pharmacological effects including anti-inflammatory, antimicrobial, antmalerial and antitumor activities.[11-12]

The products were characterized on the basis of their MP, Elemental analysis, TLC, IR, ^{1}HNMR , ^{13}CNMR and evaluated for their antifungal and antibacterial activity.

In conclusion we have described a novel and highly efficient rapid microwave digestions induced modification of the synthesis of diphenyl imidazolylpyrimidine-5-carboxylates (DPIPC) derivatives MORE chemistry reactions are highly accelerated, they are cleaner than conventional heating reactions and lead to higher atom economy (less chemical waste) and follow the environmentally friendly protocol include a reactions set-up not requiring specialized equipment high product yields, short reaction times and the elimination of usages of excess of solvent in some reactions.[13-20]

EXPERIMENTAL SECTION

All solvents were distilled prior to use. TLC was performed on silica gel G. Melting points were determined by open capillary method and are not correct. ^{1}HNMR and ^{13}CNMR spectra were recorded from $CDCl_3$/DMSO-d6 solution on a Brucker Avance-II 400(400 MHz) NMR Spectrometer. Chemical shifts are reported in ppm using TMS as an internal, standard. IR spectra were obtained on a Shimadzu FTIR spectrophotometer, using KBr discs. Mass spectra were recorded by using Shimadzu gas chromatograph coupled with QP5050 Spectrometer at 1-1.5 ev.

General procedure for synthesis of substituted ethyl 1, 2, 3, 6-tetrahydro-4-methyl-2-oxo/thioxo-6-phenyl-1- (4, 5-diphenyl-1-H-imidazol-2-yl) pyrimi - dine-5-carboxylate (3a-g)

METHOD-A (Conventional)

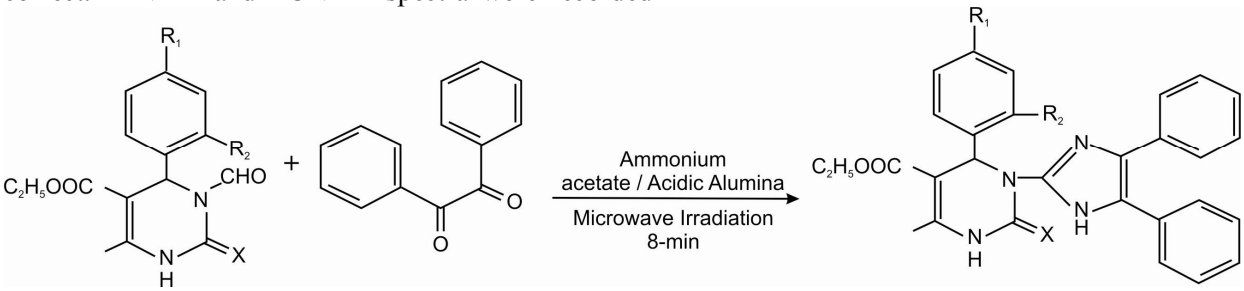
Benzil (2.5m mole; 5.25g), ethyl-1-formyl-1,,2,3,6-tetrahydro-4-methy-6-phenyl-2-oxo-pyrimidine-5-carboxylate (2.5m Mole; 11.95g) and ammonium acetate (0.12 mole; 10g) were dissolved in glacial acetic acid. The reaction mixture was refluxed for 10-12 hours. It was then cooled and poured in cold water then the precipitate was formed, filtered, washed with ammonium hydroxide and dried. The product was recrystallization from ethanol.

Yield : 64% **M.P.** 160ºC

METHOD-B (Microwave Irradiation)

Benzil (2.5m mole; 5.25g), ethyl-1-formyl-1,,2,3,6-tetrahydro-4-methy-6-phenyl-2-thioxopyrimidine-5-carboxylate (2.5m Mole; 11.95g) and ammonium acetate (0.12 mole; 10g) were dissolved in glacial acetic acid. The contents were thoroughly mixed. The reaction mixture was subjected to microwave irradiation in a commercially available IFB domestic microwave oven having a maximum power output of 110w operating at 2450 MHz intermittently at 30 sec. intervals for 8 min. on completion of reaction as monitored by TLC, the product was diluted with water then the precipitate was formed, filtered, washed with ammonium hydroxide and dried. The product was recrystallization from ethanol. The purity of the compounds was checked with TLC

Yield : 79% **M.P.** 160ºC



RESULT AND DISCUSSION

Substituted ethyl 1, 2, 3, 6-tetrahydro-4-methyl-2-oxo/thioxo-6-phenyl-l- (4, 5-diphenylI-H-imidazol-2-yl) pyrimidine-5-carboxylates (3a-g) were synthesized by condensing substituted ethyl-1-formyl-l, 2, 3, 6-tetrahydro-4-methyl-6-phenyl-2-oxo/thioxo pyrimidine-5-carboxylates (la-g) and Benzil with ammonium acetate by using acidic alumina, and four drops of glacial acetic acid under solvent free microwave irradiation for 8 minutes (Table 1.1).

Table 1.1 : Molecular structures of diphenyl imidazolylpyrimidine-5-carboxylates (DPIPC) derivatives used in the present study.

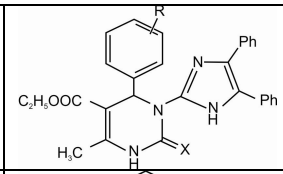
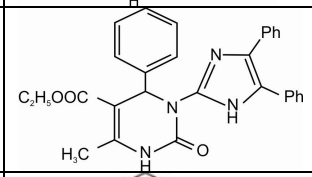
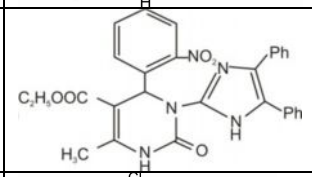
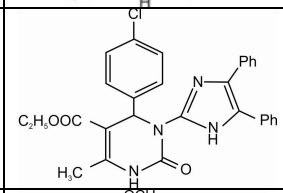
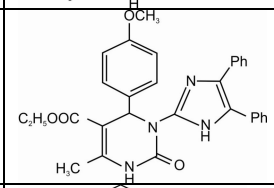
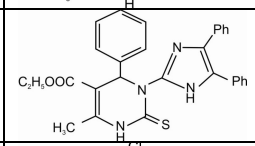
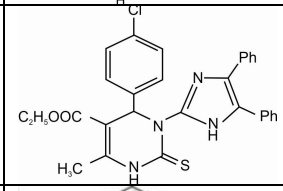
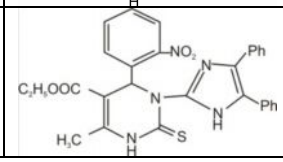
General Structure		
3a	Ethyl 1,2,3,6-tetrahydro-4-methyl-2-oxo-6-phenyl-1-(4,5-diphenyl-1H-imidazol-2yl) pyrimidine-5-carboxylate	
3b	Ethyl 1,2,3,6-tetrahydro-4-methyl-6-(2-nitrophenyl)-2-oxo-1-(4,5-diphenyl-1H-imidazol-2yl) pyrimidine-5-carboxylate	
3c	Ethyl 1,2,3,6-tetrahydro-4-methyl-6-(4-chlorophenyl) -2-oxo-1-(4,5-diphenyl-1H-imidazol-2-yl) pyrimidine-5-carboxylate	
3d	Ethyl 1,2,3,6-tetrahydro-4-methyl-6 (-4-methoxyphenyl) -2-oxo-1-(4,5-diphenyl-1H-midazol-2-yl)2-pyrimidine-5-carboxylate	
3e	Ethyl 1,2,3,6-tetrahydro-4-methyl-6-phenyl-1-(4,5-diphenyl-1H-imidazol-2-yl)-2-thioxopyrimidine-5-carboxylate	
3f	Ethyl 1,2,3,6-tetrahydro-4-methyl-6-(4-chlorophenyl)-1-(4,5-diphenyl-1H-imidazol-2-yl) -2-thioxopyrimidine-5-carboxylate	
3g	Ethyl 1,2,3,6-tetrahydro-4-methyl-6-(2-nitrophenyl)-1-(4,5-diphenyl-1H-imidazol-2-yl) -2-thioxopyrimidine-5-carboxylate	

Table 1.2: Characteristic data for diphenyl imidazolylpyrimidine-5-carboxylate (DPIPC) of conventional method-A (3a-g)

Compounds	X	R1	R2	Mol.Formula	MP(ºC)	Method-A Yield/Time %/hr
3a	O	H	U	$C_{29}H_{26}N_4O_3$	160	64/12
3b	O	H	NO2	$C_{29}H_{25}N_5O_5Cl$	210	64/12
3c	O	Cl	H	$C_{29}H_{25}N_4O_9Cl$	180	62/12
3d	O	OCH3	H	$C_{30}H_{28}N_4O_4$	240	65/12
3e	S	U	H	$C_{29}H_{26}N_4O_2S$	190	65/12
3f	S	Cl	H	$C_{29}H_{25}N_4O_2SCl$	195	63/12
3g	S	H	NO2	$C_{29}H_{25}N_5O_4S$	220	60/12

Table 1.3: Characteristic data for diphenyl imidazolylpyrimidine-5-carboxylate (DPIPC) of microwave irradiation method-B (3a-g)

Compounds	X	R1	R2	Mol.Formula	MP(ºC)	Method-B Yield/Time %/min
3a	O	H	U	$C_{29}H_{26}N_4O_3$	160	79/8
3b	O	H	NO2	$C_{29}H_{25}N_5O_5Cl$	210	78/8
3c	O	Cl	H	$C_{29}H_{25}N_4O_9Cl$	180	69/8
3d	O	OCH3	H	$C_{30}H_{28}N_4O_4$	240	72/8
3e	S	U	H	$C_{29}H_{26}N_4O_2S$	190	78/8
3f	S	Cl	H	$C_{29}H_{25}N_4O_2SCl$	195	75/8
3g	S	H	NO2	$C_{29}H_{25}N_5O_4S$	220	67/8

The structure of ethyl 1, 2, 3, 6-tetrahydro-4:"methyl-2-oxo-6-phenyl-1- (4, 5-diphenyl-lH-imidazol-2-yl) pyrimidine-5-carboxylate (3a) was supported by IR, IHNMR, 13_{CNMR}, MASS spectral data. IR spectra exhibited the N-H absorption band at 3198cm-[1], ester carbonyl group at 1750cm-[1], C=C stretch at 1495 em-I, C=N stretch at 1435cm-[1] were observed. IHNMR of (3a) in ($CDC1_3$/DMSO) was nicely resolved and showed the appearance of N-H proton as a characteristic singlet at 8 8.9 and the aromatic protons as a multiplet at 8 7.1-7.9 (Table 1.4).

Table 1.4: Characteristic spectral data for diphenyl imidazolylpyrimidine-5-carboxylate (DPIPC) (3a-g)

Compounds	IR (KBr disc) cm^{-1}	^{1}HNMR (DMSO)□, ppm	^{13}CNMR	MASS
3a	3198 (N-H), 1499 (C=C), 1435 (C=N)	1.1 (t,3H,CH_3, *J*=8), 2.3 (s,3H,CH_3), 4.2 (q, 2H, CH_2, *J*=4.8), 5.1 (s,1H,Ar-H), 8.9 (s,1H,N-H)	13.7, 17.7, 54.4, 59.0, 99.7, 127.2, 128.6, 129.5, 129.3, 132.7, 147.1, 184.2, 158.8, 165.3, 167.1, 194.1	478
3b	3190 (N-H)1493 (C=C), 1430 (C=N)	1.3 (t,3H,CH_3, *J*=8), 2.1 (s,3H,CH_3), 4.1 (q, 2H, CH_2, *J*=4.8), 5.4 (s,1H,Ar-H), 8.7 (s,1H,N-H)	13.5, 17.9, 54.2, 59.0, 99.6, 127.1, 128.6, 129.5, 129.3, 132.3, 134.0, 147.8, 148.5, 158.8, 165.7, 167.1, 194.3	523

3c	3198 (N-H) 1549 (C=C), 1435(C=N)	1.4 (t,3H,CH_3, *J*=7.6), 2.4 (s,3H,CH_3), 4.0 (q, 2H, CH_2, *J*=4.2), 5.2 (s,1H,Ar-H), 9.2 (s,1H,N-H)	13.4, 17.6, 54.3, 59.2, 99.7, 127.2, 128.4, 129.5, 129.6, 132.7, 134.6, 147.1, 184.2, 158.53, 167.1, 194.8	512
3d	3195 (N-H)1489 (C=C), 1469 (C=N)	1.1 (t,3H,CH_3, *J*=8.2), 2.3 (s,3H,CH_3), 3.7 (s, 3H, OCH_3) 4.0 (q, 2H, CH_2, *J*=4.8), 5.3 (s,1H,Ar-H), 8.9 (s,1H,N-H)	13.2, 17.6, 54.1, 59.3, 99.7, 127.2, 128.6, 129.5, 129.3, 132.7, 134.1, 147.1, 148.2, 158.8, 165.3, 167.1, 194.5	508
3e	3198 (N-H)1494 (C=C), 1430 (C=N)	1.3 (t,3H,CH_3, *J*=8), 2.2 (s,3H,CH_3), 4.2 (q, 2H, CH_2, *J*=4.8), 5.5 (s,1H,Ar-H), 9.5 (s,1H,N-H)	13.3, 17.7, 54.4, 59.0, 99.2, 127.4, 128.6, 129.5, 129.3, 132.7, 134.2, 147.1, 148.2, 158.8, 165.3, 167.1, 194.3	494
3f	3200 (N-H)1493 (C=C), 1433 (C=N)	1.2 (t,3H,CH_3, *J*=8), 2.4 (s,3H,CH_3), 4.1 (q, 2H, CH_2, *J*=4.8), 5.3 (s,1H,Ar-H), 9.3 (s,1H,N-H)	13.7, 17.7, 54.4, 59.3, 99.7, 127.2, 128.6, 129.5, 129.3, 132.7, 134.0, 147.1, 148.2, 158.8, 165.3, 167.1, 194.4	528
3g	3205 (N-H)1495 (C=C), 1435 (C=N)	1.3 (t,3H,CH_3, *J*=8), 2.3 (s,3H,CH_3), 4.0 (q, 2H, CH_2, *J*=4.6), 5.1 (s,1H,Ar-H), 9.6 (s,1H,N-H)	13.4, 17.6, 54.4, 59.0, 99.3, 127.2, 128.6, 129.5, 129.3, 132.7, 134.0, 147.1, 148.2, 158.8, 165.3, 167.1, 194.7	539

The appearance of multip1ets of 14 protons at 8 7.2-8.5 confirmed the presence of two more phenyl rings attached to the basic moiety .The disappearance of the -CHO peak at 8 10.2-10.4 supported the formation of product 3a. ^{13}CNMR showed the disappearance of peak of H-C=O in compound 3a and the appearance of peak of N-C-N at 8 134.0 which confirmed its structure.

Antifungal Activity:-

The antifungal activities of compounds (3 a-g) have been assayed in vitro at a concentration 100 g disc-^{1} against *C.albicans*. Griesofulvin was used as

standard fungicide for the antifungal test. Muller-Hinton agar was used as basal medium for test fungi. Glass Petri dishes were sterilized and 10ml of sterilized melted MH agar medium (4S°C) was poured into each Petri dish. After solidification of the medium small portion of mycelium of *C.albicans* was spread carefully over the centre of each MH agar plate with the help of spreader. Thus fungus was transferred to each plate. The plates were then incubated at (27°C) and after half an hour of incubation they were ready for use. The prepared discs of test sample were placed gently on the solidified agar plate, freshly seeded with the test organisms with sterile forceps. The plates were then incubated at 37.5ºC for 24hr. Dimethyl formamide (DMF) was used as a solvent to prepare desire solutions of the compounds initially[17-18].

The antifungal studies revealed that the compounds 3b and 3c having chloro and nitro groups respectively along with oxopyrimidine moiety were found to be most active amongst the entire tested compounds. 3a and 3g exhibited moderate activity in comparison with other compounds. 3f showed less activity where as 3d and 3e were found to be inactive against the *C.albicans* (Table 1.5).

Antibacterial Activity:-

The antibacterial activity of compounds (3a-g), which has been, assayed at concentration of 100 g disc^{-1} against strains of gram +ve and gram -ve pathogenic bacteria *(S. typhi, Paltrogenosa, K Pneumonae and S.aureus)*. Initially, susceptibility testing was carried out by measuring the inhibitory zone diameter on Muller-Hinton agar with conventional paper disc diffusion method, the inhibitory zone diameter was recorded and rounded off to the nearest whole numbers (mm) for further QSAR analysis[17-18]. The sensitivity of compounds (3a-g) against these organisms is depicted in (Table 1.5). The results were compared with standard drug i.e. Norfloxacin.

The screening results revealed that in addition to (3a-c), the compound 3d were found to be the most active against S.aureus amongst all the tested compounds. S. typhi is highly sensitive to the compound 3b and (3e-g) and moderately sensitive to Compounds 3a and 3c. It has been observed that *P.aurogenosa and K. Pneumonae* are highly resistant to the synthesized compounds.

As a result good recoveries were obtained for all the elements investigated in the microwave oven with less time and reagent consumption than conventional acid digestion techniques. The other important aspects are the minimization of the acid concentration required for dissolution of the sample. Thus the applied microwave digestion technique is an environmentally benign rapid and suitable technique for the preparation of heterocyclic compound.

The synthesized compounds were tested against *S.aureus, S.typhi, P.aurogenosa, K.pneumonae* in comparison with Norfloxacin.

Table 1.5:Antimicrobial screening results of compound synthesized

Sr.No.	Compounds	Zone of inhibition in mm for conc. for 100 g/ml	Logarithm of zone of inhibition in mm
C.albicans			
1.	3a	9.0	2.197
2.	3b	12.0	2.485
3.	3c	13.0	2.565
4.	3f	6.0	1.791
5.	3g	9.0	2.197
K.pneumonae			
1.	3a	12.0	2.485
2.	3b	12.0	2.485
3.	3c	10.0	2.303
4.	3d	15.0	2.708
5.	3e	9.0	2.197
6.	3f	18.0	2.890
7.	3g	8.0	2.079
P.aurogenosa			
1.	3a	9.0	2.197
2.	3c	12.0	2.485
3.	3d	10.0	2.303

4.	3e	12.0	2.485
5.	3f	6.0	1.791
6.	3g	10.0	2.303
S.typhi			
1.	3a	9.0	2.197
2.	3b	12.0	2.485
3.	3c	9.0	2.197
4.	3e	13.0	2.565
5.	3f	11.0	2.398
6.	3g	13.0	2.565
S.aureus			
1.	3a	10.0	2.303
2.	3b	10.0	2.303
3.	3c	9.0	2.197
4.	3d	12.0	2.485
5.	3e	6.0	1.791
6.	3f	6.0	1.791
7.	3g	7.0	1.946

ACKNOWLEDGEMENT

I am thankful to Dr. Pradipkumar Deo, Registrar and Dr. Anil Bhandari, Dean, Faculty of Applied Science (Chemistry), Jodhpur National University, Jodhpur, for his encouragement and inspiring me towards research work.

I am equally thankful to Dr. P. N. Charde, Principal, Servalal Mahila Mahavidhyalaya, Nagpur for his inspiration and providing facilities for screening antimicrobial activities.

I am also thankful to Dr. S. Z. Jadhao, Govt. Institute of Science, Nagpur for his valuable guidance during research work.

Authors hereby humbly acknowledge to Department of Pharmacy, Nagpur for IR spectral analysis, SAIF, Chandigarh for, ^{1}HNMR, 1CNMR spectral analysis, Pune for the Mass analysis.

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